# A novel approach to control stored sorghum beetle *Tribolium castaneum* (Coleoptera: Tenebrionidae) in small-scale farmers' storerooms in Kebbi- Nigeria

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**A thesis submitted in partial fulfillment of the requirements of the University of Greenwich for the Degree of Doctor of Philosophy**

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# DECLARATION

I certify that this work has not been accepted in substance for any degree, and is not currently submitted for any degree other than that of Doctor of Philosophy (PhD) being studied at the University of Greenwich. I also declare that this work is the result of my own investigations except where otherwise identified by references and that I have not plagiarized the work of others.

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# ABSTRACT

The aim was to develop a novel method to reduce infestations of the most common stored-product pest, *Tribolium castaneum* beetles, in bags of sorghum stored by small-scale farmers of Kebbi state. A survey of 240 farmers found greater quantities of sorghum than other grains (4,000 kilos/household, $p<0.001$) were stored and a majority in south Kebbi stored sorghum threshed ($p<0.001$), even though this form is more vulnerable to infestation. Inconsistencies in farmers' perceptions of the efficacy of repellent plants were apparent. A more efficient and effective bioassay (Thigmotactic assay) was developed to identify a plant product highly repellent to *T. castaneum,* 'Lem-ocimum,' which is composed of *Cymbopogon nardus* (Lemongrass) plus *Ocimum basilicum (Sweet basil),* 0.5%w/w each ($p<0.001$). A paste of Lem-ocimum was applied between layers of 5kg double-bags to prevent contamination of grain within inner bag. Treated double-bags provided better protection from *T. castaneum* infestation than untreated single or double-bags ($p<0.001$) and were most effective when a high number (9-18) were placed on top of untreated bags (~1% weight loss after 5 months, $p<0.01$, n=150 store-rooms of 42 farmers). A survey indicated participants were satisfied with outcome of Lem-ocimum treatment for trials using high numbers of treated bags. Male and female farmers differed in plant species they collected and their plant-drying methods. Chemical analysis showed plant species and drying methods affected repellency; cultivated *O. basilicum* had higher repellent compound content and repelled more beetles (0.88±0.015) than *O. africanum* (0.62±0.020, $p<0.001$), and shade-drying repelled more beetles (0.76±0.039) than sun-drying (0.61±0.034, $p<0.001$). Therefore, it is recommended that double-bags treated with cultivated shade-dried *Ocimum* (as normally prepared by women) should be tested further in the field. Application of the Lem-ocimum treated double bags method should ensure farmers have a proportion of high quality grain to sell to the market, thereby increasing their financial status.

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# CHAPTER 1

## Introduction

### 1.1 Overall objectives

This study describes the evaluation of the potential for improving the efficacy of using repellent plant species (*Ocimum basilicum* and *Cymbopogon nardus*), which are already used by farmers of Kebbi, to improve the protection of their stored sorghum from pest infestation. The main focus is the development of a novel method of reducing grain damage by insect pests by incorporating a paste of repellent plants into double bags without contaminating the grain, as against their current method of direct mixing grain with plant materials. This research is not aimed at killing pest insects, but to prevent them from becoming established in bags of grain (i.e., the use of plant protectants to repel pest species). The work also considers interactions between farmers, gender roles and use of local sources of repellent plant species in an effort to improve the likelihood the new method would be taken up by local farmers.

### 1.2 Background to the study

Sorghum has been the dominant cereal grain in Nigeria in terms of staple consumption and demand in processing industries (Julius, 2007). The north-west part of the country contributes a significant portion of the total country's sorghum production, where more than 70% of the annual production is produced by small-scale farmers (NAERLS & NAFRA, 2009). The production is seasonal and farmers have to store some of their produce to ensure continuous food supply for their family and when needed, to sell grain to pay for other household needs until the next season. Thus, grain storage plays an important role in the livelihood of small-scale farmers. Unfortunately, stored grain losses due to insect pests represent a threat to farmers in realizing this benefit (Udoh *et al*., 2000; Mvumi & Stather, 2003). In Nigeria, these destructive pests often attack cereal and legume grains that serve as staple foods for small-scale farmers (Ega *et al*., 1992; Anonymous, 2003), forcing them to sell their grain early at a low price. For instance, *Sitophilus oryzae* (L) (Coleoptera: Curculionidae), *Sitotroga cerealella* (Olivier) (Lepidoptera: Gelechiidae) and *Callosobruchus maculatus* (F.) (Coleoptera: Chrysomelidae) were identified as major pests of stored maize, sorghum, rice and cowpea in northern Nigeria (Enobakhare &

Wey, 1996; Lale *et al*., 2002). In a survey conducted by Udoh *et al.* (2000), insect pest infestations were reported by most of the small-scale farmers storing grain in northern Nigeria. In a survey conducted by Lale & Yusuf (2000) in northern Nigeria, small-scale farmers were found to have high infestations of *Tribolium castaneum* (Herbst) (Coleoptera: Tenobrionidae) in stored millet, and *T. castaneum* and *Sitophilus* species in stored sorghum and millet (Chimoya & Abdullahi, 2011). The level of effect of the pest infestation depends on the variety of grain, method of storage and the storage structure used. A survey conducted by Lale & Yusuf (2000) in northern Nigeria indicates that the experience of respondents with *T. castaneum* infestations varied with type of storage structure; a large number of respondents reported high infestation rates for underground storage pits, followed in declining order by granaries, polypropylene bags and clay storage pots. However, this may vary with region.

Kebbi state is one of the major sorghum and millet producing area of Nigeria (NAERLS and NAFRA, 2009), where a total of ~ 1.36M ha of productive land is currently used for crop production, of which ~ 420,000 ha are flood plain (Fadama) and the rest is upland, which is only used for seasonal cultivation (COA, 2009). The main crop production area is mainly cultivated by small-holder farmers for the production of sorghum, millet, rice and vegetables such as onion, pepper and tomatoes (KARDA, 2004). Sorghum and millet form a significant part of the food for the people of Kebbi (COA, 2009), where a total output of ~ 220 MT and 210 MT for sorghum and millet respectively were estimated along with others crops such as rice (50 MT) and maize 42 MT in 2007 (NAFRA, 2007). Considering the importance of sorghum as a major source of family food and livelihoods for small-scale farmers in Kebbi state and Nigeria at large, securing improved methods of sorghum storage could further improve food security and the livelihoods of small-scale farmers.

However, storage pests are becoming a threat to the food security and livelihoods of small-scale farmers of Kebbi State. According to Kebbi Agricultural and Rural Development Authority KARDA (2004), the main body with a mandate to oversee and control all agricultural activities in the state, reported that the common storage pests in Kebbi State include *T. castaneum, Rhyzopertha dominica* (Fabricius) (Coleoptera: Bostrichidae), *S. oryzae*, *Lasioderma serricorne* (Fabricius)

(Coleoptera: Anobiidae), *S. cerealella* and C. *maculatus*. Similarly, an unpublished MSc study by the author (Utono, 2007) conducted in Yauri District of Kebbi State found that the major insect pest species were moths (*Plodia interpunctella*, Hubner, (Lepidoptera: Pyralidae) and beetles, such as *T. castaneum and R. dominica*. Farmers' complaints about storage pests are increasing dramatically (KARDA, 2004, Personal communication with extension workers and farmers in Kebbi State). Unfortunately, recent reports do not provide the relative importance of these pests because grain stores were not inspected (KARDA, 2004; COA, 2009). The Kebbi state farmers are aware of the causes of the losses; they speak up about the importance of storage pests to extension leaders (Personal communication with Zonal extension officers of KARDA, Yauri branch), and they respond to the pest problems by either treating their stored grain with insecticides or traditional materials, such as sand or plant materials, to prevent infestation. Although insecticides work well in some cases (Arthur, 1994; Arthur & Campbell, 2008), constraints regarding costs, the environment, human health and insecticide resistance limit their use and efficacy (Yang *et al*., 2005). Control measures that are cost effective, environmentally friendly and safe for humans could be the answer.

In Kebbi state, the specific factors responsible for storage pest problems among small-scale farmers are not well understood. The most likely factors include 1) the types and designs of the materials used to store grain, 2) the methods of storage used by farmers, 3) the types of materials used for pest control and 4) how those materials are used (COA, 2009). The details of significance of these factors in promoting storage pest infestation need to be investigated and addressed to help farmers realise the potential benefits of secure grain storage. Therefore, the first step was to undertake an investigation of how farmers in Kebbi state store and manage their grain, and how this affects the types of insects that attack their grain. The data obtained provided the basis for identifying weaknesses with the existing methods and development of possible novel methods of improvement in order to meet their needs. The main focus was on the consideration of the weaknesses of the locally available plant materials used as grain protectants and identifying potential ways to improve their efficacy. The old methods used by farmers to protect their grain are not necessarily the most efficient or most effective, which is why there is a need to investigate this approach in greater depth. It is reported that a wider acceptance and uptake of a new technology can be achieved if it involves materials and methods farmers are already familiar with and repellent plant species that can be sourced locally (Belmain and Steveson, 2001).

## 1.3 Objectives of the research

The main objective of this research is to investigate the systems of grain storage practiced by small-scale farmers in Kebbi state in order to identify the methods of grain storage and protection used by famers and how these affect the presence of the most important pests that infest their grain. The technologies and practices identified as promising will be improved and tested against insects both in the laboratory and in the field. In essence, the research will be aimed at the group of famers that are most in need of improved methods of grain storage.

### 1.3.1 Specific objectives

* To identify the range of storage structures used by small-scale farmers to store their sorghum and the local plant materials used to protect it from infestation.

* To develop a new bioassay that is more efficient and effective than the standard bioassay in identifying the plants most repellent to *T. castaneum*, the most common pest observed in the survey.

* To develop a novel method of using repellent plants to protect grain stored in bags.

* To understand the roles of gender in the use of local plant materials as grain protectants.

* To analyse differences in the chemical content of locally grown plant that have been sun- or shade-dried, to determine how to maximise their bioactivity against *T. castaneum.*

## 1.4 Research hypotheses

The findings of the farmers' survey were used to state the following null hypotheses:

Ho1: Addition of an extra protective barrier of repellant plant materials in between the layers of a double bag does not make a difference to the level of insect infestation.

Ho2: There is no gender difference in the perception and use of pesticidal/repellent plants as grain protectants.

Ho3: There is no difference in the active ingredients of individual samples of promising pesticidal/repellent plant species.

## 1.5 Expected outcomes and impacts of the research

The research is expected to determine the main insect species attacking the most staple grain of small-scale farmers in Kebbi, and to identify the main weaknesses of the current techniques used to protect grain. The aim is to develop improved methods of protecting stored grain that would work better than the farmers' existing methods. The approach is to involve farmers in the experimental field tests, so they can observe and learn how plant repellents reduce infestations, and consequently increase the chances that farmers will adopt the new system. The new method developed is expected to ensure that at least a proportion of famers' grain is of high quality, hence increasing farmers' chances to gain more income and have access to healthier food. Although it will not be possible to undertake a full field trial to determine how cost effective the new method is compared to other methods, the outcome of the research will provide basic information for decision-making in pest management practices, which should help extension workers in advising farmers on how best to protect their grain from infestations with the most promising treatment identified.

# CHAPTER 2

## Literature review

This review considers the importance of production and storage of cereal grain to the livelihoods of small-scale farmers and how methods of storage influence the types of pest that infest their stored grain. The weakness of the various methods of grain protection and their potential to provide appropriate grain protection for small-scale farmers is considered, particularly in relation to the possibilities of improvement.

### 2.1 Importance of grain production and storage

In recent decades, increased production of cereal grains has played an important role in improving the livelihoods of the poor globally (FAO, 2003). In Nigeria, cereals such as sorghum and millet are produced in large quantity and serve as staple foods for much of the populace (CBN, 1992; Anonymous, 2007), especially in the northern part of the country. As a result, sales of surplus produce have helped to increase their income (Julius, 2007).

The national annual cereal production in the year 2003 was estimated to be ~ 25 million metric tons (MMT; Fig. 2.1), of which the major component was made up of sorghum (~8.0 MMT), maize (~5.3 MMT), rice (~2.5 MMT) and wheat (~60,000 MT; Anonymous, 2003). Since then, grain production has increased to a total of ~27.9 MMT in 2006 (Anonymous, 2007) and ~29.5 MMT in 2008 (Balami *et al*., 2011). The northwestern areas of the country (Sokoto, Kebbi, Zamfara, Katsina, Kano, Kaduna, Niger and Jigawa) contribute a significant proportion of the country's total annual food production, in spite of being relatively arid, and consisting of only a sparse area considered to be 'major crop land' (Fig. 2.2). This region is suitable for growing some of the staple grains. For example, in 2000-2002 and 2008-2009 of the total national production of cereals, 49% of millet, 40% of sorghum, 28% of maize and 22% of rice were produced in these areas, which overall represents ~ 20 - 40% of the three grains produced in greatest abundance nationally (FMA, 2002; NAERLS & NAFRA, 2009). Surprisingly large amounts of rice are produced in spite of being so far from the main rice-growing habitats of the country (Fig. 2.2).

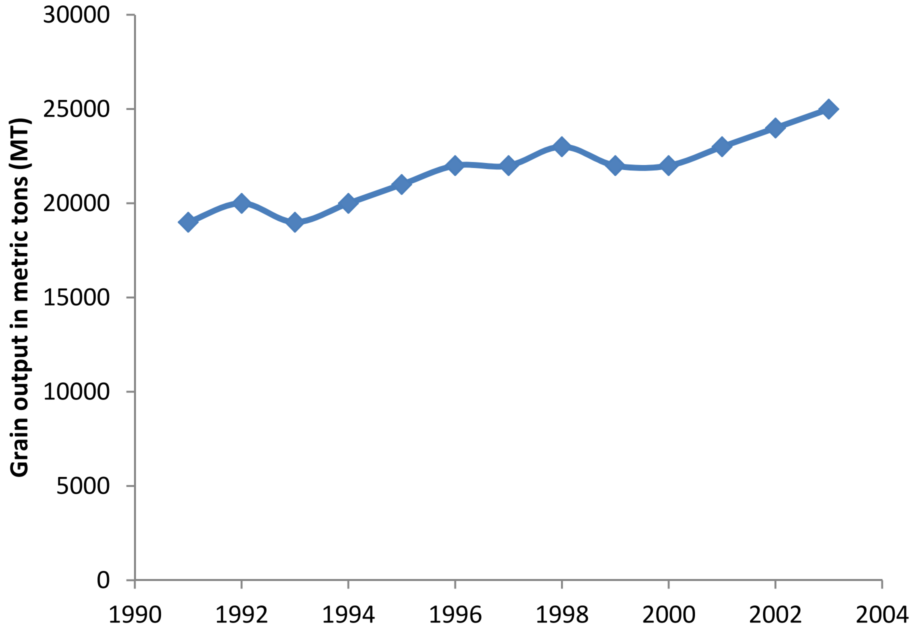



**Fig. 2.1: 1991-2003 Nigerian annual cereal production, in metric tons.** Redrawn from http://www.fao.org/giews/english/sahel/sah036e/sahel036e.htm, accessed on 16/05/2008.

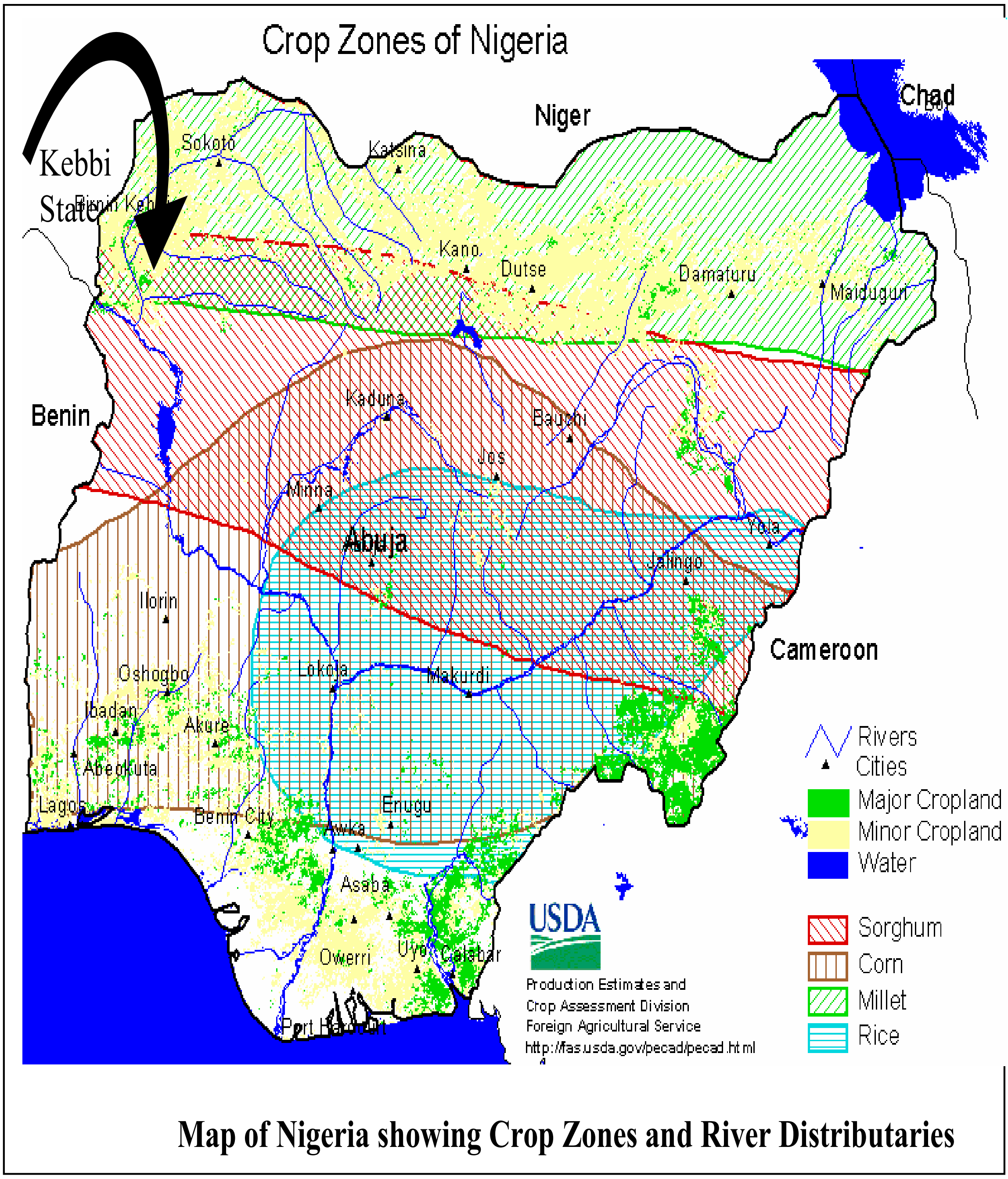



**Fig. 2.2 Map of Nigeria showing different crop zone distributions,** obtained from http://www.fas.usda.gov/pecad2/highlights/2002/03/nigeria/nigeria_rice.htm, accessed on 12/05/2008.

The roles of these grains in human nutrition in Nigeria cannot be overemphasized. They provide sources of calories, protein and oil (Ega *et al.*, 1992). Because they are a source of calories they are stored over long periods of time and processed in a variety of ways for daily nutrition. For instance, a thin porridge called 'Kunu', prepared from sorghum and millet, and a stiff porridge called 'Tuwo', prepared from sorghum, millet, rice and maize, form the daily meal of the people of northern Nigeria

(Ega *et al*. 1992). Hence good storage ensures food availability among small-scale farmers and efforts to ensure continuous availability of good quality cereals through proper production and storage systems can help in maintaining adequate food year round. Apart from being source of adequate food and daily nutrition to small-scale farmers, grain storage provides means of saving surplus to earn more cash when prices are higher (Boxall *et al*., 1997), although, debts and pest problems may force a farmer to sell his stored products early, at a low price.

In Nigeria, over 90% of the annual grain production is carried out by small-scale farmers who used traditional structures for storage (USAID, 2003). In Kebbi state, the short period of rainfall limits the growing season to 6-7 months (May-November) per year (KARDA, 2004; COA, 2009), hence, food security during the rest of the year relies on farmers making every effort to minimize losses during storage.

The aim of good grain storage practice is to maintain a high degree of quality throughout the storage period (Brooker *et al*., 1992), from harvest to disposal, which may take anywhere between a few weeks to several months (Gwinner *et al*., 1990, Adejumo & Raji, 2007) depending on the reasons for storage. The challenge is to identify better ways for farmers to manage all of the factors that affect the quality of the grain they store, particularly subsistence farmers, whose families rely almost totally on the grain for food and cash. Thus, understanding these factors and their effect on stored grain can help in addressing the problems of grain storage losses and improve their food security.

## 2.2 Factors affecting grain storage

The main factors affecting the quality of grain during storage include environmental factors, the design of storage structures and the presence of pests (Christensen & Sauer, 1982; Dejene *et al*., 2004; Arum & Aderinlewo, 2005). These factors can ultimately affect loss of grain weight, loss of nutritional quality, grain discoloration, changes in odour, unwanted germination, infestation of molds and physical damage to kernels (Gwinner *et al* 1990; Brooker *et al*., 1992; FAO, 1994), all of which can have a significant impact on the overall value of the grain.

### 2.2.1 Environmental factors

Adverse environmental factors such as temperature, moisture and relative humidity can affect the condition of stored grain, the size of insect populations in the store and the type of control technology used. A frequent risk in northern Nigeria is fluctuations in ambient temperature and relative humidity. Temperatures in northern Nigeria typically range from 22 - 33 $^{o}$C during the main storage season i.e December-October (Anuforom, 2010), which means that conditions are nearly ideal for storage insects to flourish throughout most of the storage season unless measures are taken to control them. It should also be noted that the presence of relatively large populations of insect pests could increase the moisture content of grain due to their respiration, which can lead to an increase in mold and fungal attacks, as well as a decline in seed longevity (Gwinner *et al*., 1990; McCormack, 2004). Low temperature in the region during the cold season can affect relative humidity, which can increase grain moisture content, making it more suitable for insect development. Some of the control chemicals including botanicals used for grain protection are volatile in nature, thus high temperatures during the storage season can affect the duration of their expected efficacy.

### 2.2.2 Threshed/unthreshed form of grain

The form in which farmers store their grain depends on the type of grain and the type of storage structures available locally. In northern Nigeria farmers store their grain either in a threshed or un-threshed form (Adejumo & Raji, 2007). Threshed grain is stored either as bulk grain mass in a granary or packaged in bags and stored in a granary or store-rooms. Bulk storage leaves grain more vulnerable to pest attack, which is enhanced if the grain also suffered breakage during threshing. The protective husk cover of un-threshed grain, as found in sorghum and millet, is believed to provide some degree of protection from pest attack (Lawrence & Pedersen, 1990; FAO, 1992). However, insect infestations can be found in sorghum and millet stored in both the threshed and un-threshed forms (Lawrence & Pedersen, 1990). Thus, grain should be protected by an additional protective barrier to infestation such as a bag treated with a good pest control agent.

### 2.2.3 Storage structures

The use of traditional storage structures among small-scale farmers have evolved over a long period of time, providing storage in accordance with the culture and environment of the local area (Anonymous, 1978). Nonetheless, the levels of grain losses often depend on the form and material of the storage structures, how the structures are managed and maintained, and the characteristics of the crop to be stored. Losses of ~ 20-65% were reported for traditional storage structures due to ineffective protection from loss-causing factors (Aniche, 2003; Asoegwu & Asoegwu, 2007)

In Nigeria a wide variety of storage structures can be found, including; the mud rhombus, thatch rhombus, polypropylene bag, clay pot, drum, basket, platform, crib, underground pit, local warehouse and bare floor of a house (Giles, 1965; Udoh *et al*., 2000; USAID, 2003; Adejumo & Raji, 2007). The choice of a particular storage structure depends on region, availability and cost of the construction materials. However, in the northern part of the country, polypropylene bags are the most common storage structure used, followed by a granary made of local mud (Udoh *et al*., 2000).

The most common sacks or bags used to store grain are made of cotton, jute, or polypropylene woven bags (Hayma, 1989). The latter is the most suitable for nearly-airtight storage and the one used most by local farmers in northern Nigeria (Udoh *et al*, 2000; Lale & Yusuf, 2000). Storage bags serve as a barrier between grain and pests, and, if the grain is treated with repellent plant material, may protect volatile compounds from evaporating out of treated grain. In spite of their advantages, high losses are incurred nonetheless (Lale & Yusu, 2000). Some of these losses may be preventable, particularly those relating to damage by insect pests, which is the subject of the research project presented here. Impregnating jute bags with repellent plant materials, such as *Chenopodium ambrosioides* or *Lantana camara*, reduces damage to stored legumes by pests such as *Acanthoscelides obtectus* and *Callosobruchus maculatus* (Koona *et al*., 2007). Considering the importance and widespread use of storage bags by small-scale farmers in Nigeria, this research will explore how best to incorporate repellent/deterrent plant material into the use of polypropylene bags to protect grain against insect attack.

### 2.2.4 Modern storage structures

Recent efforts to improve the crop productivity of subsistence farmers have led to the development of new storage containers, such as metal storage bins and concrete storage structures. These are better able to accommodate more grain and provide a better protection against yield losses to storage pests (Anonymous, 1978; Boxall *et al*., 1997). However, the materials used for their construction are too expensive for farmers to adopt, particularly small-scale farmers. Therefore, there is a need to improve the existing traditional storage methods used by farmers.

### 2.2.5 Storage pests

Stored grain is attacked and damaged mainly by pests such as insects, mites, fungi and rodents (Munro, 1966; Hill, 1990; Almasi & Mrdjen, 2004). Several studies have shown that in Nigeria insect pests are a major problem for farmers, notably poorly resourced farmers (Schulten, 1989; Udoh *et al*., 2000 and Chimoya & Abdullahi, 2011).

Generally, the level of loss depends on the initial size of the insect population and their stage of development when storage begins (FAO, 1992), with additional influences, such as the grain moisture content, duration of storage, level of grain nutrients, temperature and relative humidity of the store environment (Dobie *et al*., 1991; Odogola, 1994). The type of damage caused, however, depends on the insect species (Dobie *et al*., 1991; Hill, 1987, 1990; Almasi & Mrden, 2004). Some species feed directly on the grain by boring holes into the grain kernels. These are classified as primary insect pests and the damage they cause paves the way for secondary pest species (Farrell *et al.,* 2002). Weevils and lesser grain borers (*Rhyzopertha dominica*) are good examples of primary pests; the adults bore inside the grain, feeding as they go and then they lay their eggs in the grain. The emerging larvae damage the grain further as they eat their way through grain kernels (Dobie *et al*., 1991; Gwinner *et al*., 1990; Cronholm *et al*., 1998). Secondary insect pests feed either on the surface of kernels that have already been damaged to some extent by primary pests, or kernels that have been broken unintentionally during processing and storage (Hill, 1987, 1990; Cronholm, *et al*., 1998). Good examples of secondary pests include the flour beetle *T. castaneum,* the Indian meal moth *P. interpunctella* and *Ephestia spp*, which are surface feeders and lay their eggs scattered generally amongst grain kernels

(Cronholm *et al*., 1998). Thus, control of primary pests can reduce the effects of secondary pests. Secondary pests, however, are easier to control than primary pests at an early stage of egg-laying because primary pests hide their eggs deep inside kernels. In tropical Africa, annual storage losses of ~30-50% are attributed to storage insect pests (Lale, 2001). In Nigeria, a total loss of ~10-25% was recorded in granary stores over a period of 6 month – 3 years (Aniche, 2003; Adejumu & Raji, 2007). Therefore, further measures are required to devise appropriate control measures to reduce these wasteful losses.

In Nigeria, *Oryzaephilus surinamensis* (L.) (Coleoptera: Silvanidae), *Sitophilus zeamais* (Motsculski) (Coleoptera: Curculionidae), *R. dominica, Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae), *Dinoderus distinctus* (Lesne) (Coleoptera: Bostrichidae), *Dinoderus minutus* (Fabricius) (Coleoptera: Bostrichidae), *Trogoderma granarium* (Everts) (Coleoptera: Dermestidae), *T. castaneum, Cryptolestes ferrugineus, L. serricorne, P. interpunctella* (Hübner) (Lepidoptera: Pyralidae), *S. cerealella* were reported as the most important pest of stored products (FMANR & ODA, 1996). However, the type of pest species and level of infestation depend on the region, type of grain stored and storage methods. For instance, in a survey conducted by Chimoya & Abdullahi (2011) in Adamawa Northern Nigeria among farmers and traders storing sorghum, millet and rice using polypropylene bags, *T. castaneum* and *Sitophilus* species were found to be the most abundant species. Turaki *et al*. (2007) reported *T. castaneum* to thrive better to different cereal flours from northern Nigeria. Infestation of sorghum and millet by *T. castaneum* in Nigeria could be influenced by the presence of mixture of broken grains as a result of the nature of processing method of pounding and beating with stick of unthreshed grain. Breakage predisposes grain to infestation by *T. castaneum* (Tanzubil, 1991, Dobie *et al* 1991). Infestation of sorghum and millet by *T. castaneum* results in a serious loss (Tanzubil, 1991). In Nigeria, even though data on the current amount of losses caused by *T. castaneum are* not available, past studies indicate that beetles account for over 10% losses each for stored sorghum and millet (Schulten, 1989). This could be greater with the recent development of a new grain hybrid and change in the farming and storage systems, which could predispose the grain to more pests and diseases infestations. This provides an indication of the insect as one of the major pest of sorghum and millet in Nigeria.

However, in Kebbi state where sorghum is a staple food, *T. castaneum, R. dominica, L. serricorne, S. oryzae, T. granarium, P. interpunctella, S. cereallela,* are reported to be the most common storage pests (KARDA, 2004). Despite farmers' complaints about these storage pests, the insect pests that cause the most damage and their economic importance are yet to be identified.

***Tribolium castaneum (Herbst)***

This insect is commonly known as the rust flour beetle and belongs to the Order Coleoptera, Family Tenebrionidae (Hill, 1983, Hill, 1987; Dobie *et al*., 1991). Adult *T. castaneum* (Fig. 2.3) are somewhat flat, oblong, reddish-brown in colour and about 3-4mm long (Dobie *et al.,* 1991; Harney, 1993).

Adult females lay ~400-500 eggs, depending on the temperature and the climate, which is one of the factors that contributes to their high population in any colonized environment (Lale *et al*., 2002). Eggs are laid loosely in the foodstuff by the female adult and covered with a sticky secretion, which can make eggs adhere to the side of a container or storage bag and this facilitates transportation from one locality to another (Hill, 1983; Harney, 1993). The eggs laid by *T. castaneum* are small, white and cylindrical (Hill, 1983), mature larvae are yellowish-white, ~ 4-5mm long, slender, with pale brown heads (Munro, 1966). After passing through 7-8 instars, mature larvae develop into pupae, which are yellowish-white, later becoming brown and the dorsum hairy and the tip of the abdomen having two spine-like processes (Dobie *et al.,* 1991). Eggs laid by adult *T. castaneum* hatch in 3-5 days and reach maturity in as few as 30-35 days under optimum condition, i.e 30-35$^{o}$C (Hill, 1990; Harney, 1993), or longer depending on the environmental conditions, such as temperature, relative humidity, light and nutrient availability (Dobie *et al*., 1991; FAO, 1992).

**Fig. 2.3 Dorsal view of adult *Tribolium castaneum***
http://en.wikipedia.org/wiki/File:Tribolium_castaneum.jpg, accessed on 11/04/12.

**Distribution and damage**

*Tribolium castaneum* is thought to have originated in India, but is now found throughout the tropical and sub-tropical parts of the world (Dobie, *et al.,* 1991), with greatest abundance in warm temperate regions and the sub-tropics and limited abundance in less temperate and very hot climates, where temperature can be above 40$^{o}$C (Hill, 1990). Infestations can be found in all types of storage materials, such as granaries, elevators, mills and warehouses throughout the world (Trematerra *et al*., 1996). This pest is reported to prefer grain that is broken either by primary insects or as a result of improper processing and handling (Appert, 1987; Gupta & Singh, 1996; Tanzubil, 1991)**.** Damage caused by adults and larvae may cause both direct and indirect loss, such as poor quality food, seed viability and as a result of insect feeding holes, attract low market value. This pest is found in warm areas of northern Nigeria, attacking all types of processed and damaged whole cereal grains (Lale & Yusuf, 2000; Turaki *et al*., 2007).

***Rhyzopertha dominica* (Fabricius)**

This insect (Fig. 2.4), commonly known as lesser grain borer, belongs to the Order Coleoptera, Family Bostrichidae (Hill, 1983; Hill, 1987; Dobie *et al*., 1991). It is a primary pest of dried stored grain, such as wheat, sorghum and maize, throughout the tropics (Jood *et al*., 1996; Park *et al*., 2008). The adult females lay their eggs in crevices or on the surface of grain, after which the developing larvae bore into the grain (Dobie *et al*., 1991). Both larvae and adults feed on whole, sound grain or milled and cereal flour, causing serious damage to the infested grain, affecting grain weight loss, colour and smell (Park *et al*., 2008; Lale, 2001), which consequently affects the food and market value of the grain. Infestation by this pest facilitates infestation by secondary pests, e.g., *T. castaneum* and fungi (Cronholm *et al*., 1998).

**Fig 2.4 Side and dorsal view of adult *Rhyzopertha dominica*,** http://202.141.78.173/insectpests/Rhyzopertha-dominica.php, accessed on 11/04/12.

### 2.3 Control of storage insect pests

Depending on the resources available and the level of farming and pest control knowledge, farmers often use a range of control strategies, including chemical, biological and cultural methods of insects control (Arthur, 1994). The application of plant-based control materials has been an increasing area of pest management (Isman 2008). However, before an effective storage pest control program can be developed, crucial data must be collected about the storage environments and the status of local pest species.

#### 2.3.1 Chemical control

Insecticides continue to be the most common methods of pests control in developing countries (Udoh *et al*., 2000; Kamanula *et al*., 2011). Their quick action in giving instant insect knockdown can explain their widespread acceptance to users. To control stored crop pests, insecticides are applied directly to the grain or as a fumigant (Arthur, 1994). Fumigation with insecticides can completely control storage pests (Zettler & Arthur, 2000). For instance, methyl bromide used as a fumigant resulted in 100% mortality of adult and immature stages of storage insects, such as, *S. oryzae, R. dominica* and *T. castaneum* (El-Lakwah & Abd-El-Aziz, 2000). Effective control of *T. castaneum* and *S. zeamais* is achieved in traditional granaries using pirimiphos-methyl, with the damage level kept at <8% (Mvumi & Giga, 1994). Giga *et al*. (1991) compared the effectiveness of malathion, pirimiphos-methyl and methacrifos on *S.*

*cerealella, S. zeamais* and *T. castaneum* over an 8 month storage trial and found that untreated maize (control) incurred damage to and losses of 76%, compared to maize treated with malathion (36% losses), pirimiphos-methyl (17%) or methacrifos (10%). Mortality of both 3rd and 4th instar larvae of *P. interpunctella* was successfully achieved using deltamethrin within a few hours of treatment and the percentage of adults emerging was eventually reduced (Locatelli *et al*., 2006). Similarly, the use of sulfuryl fluoride as a fumigant provides protection against all stages of many storage pests, such as *T. castaneum* and *P. interpunctella* (Ducom *et al*., 2002; Drinkall *et al*., 2002; Reichmuth *et al*., 2002). In Nigeria, magnesium phosphide, pirimiphos-methyl and permethrin are the most common insecticides used to control stored crop pests (FMANR & ODA, 1996).

Despite the benefits of insecticides in giving quick action and complete mortality for pest control, some insecticides such as aldrin are toxic to non-target organisms, they persist in the environment and their bioaccumulation in foods has become problematic (Xue *et al.* 2006). Furthermore, broad spectrum insecticides can affect beneficial organisms, lead to outbreaks of secondary pests and resistance in pest population, which leads to increasing costs as a result of the need for frequent re-application of insecticides in an attempt to overcome the pest problems (Metcalf & Luckmann 1994; Yang *et al.,* 2005). Ultimately, the evolution of resistance to insecticides will be the most serious barrier to the successful use of these chemical agents in the future, particularly for storage insect pests such as *T. castaneum*, and *P. interpunctella* (Arthur *et al*., 1988; Arthur *et al*., 1990).

Indiscriminate use of insecticides and lack of knowledge of the appropriate application rate and dose by some farmers contributes to the overuse of insecticides, which results in the development of resistance and detrimental effects to the environment and non-target species (Hill, 1987; Arthur *et al*., 1988, Gwinner *et al*., 1990; Campbell & Campbell, 2001; Yadav & Singh, 1994; Snelder *et al*., 2007). Overuse of insecticides also occurs when farmers apply them on a regular basis, or whenever they see other farmers treating their grain, instead of only where there is an insect outbreak that necessitates treatment. Champ & Campbell-Brown (1970) found that as a result of extensive use of insecticides *T. castaneum* has acquired resistance to dichlorvos, fenitrothion, Tetrachlorvinphos, cyano, diazinon, carbyl, promecarb, dichlorodiphenyltrichloroethane (DDT), lindane, malathion and chorphyrifos (Zettler, 1991; Subramanyam & Hagstrum, 1996; Assie *et al*., 2007). Continuous use of chemical pesticides to control storage pests is likely to result in more insect resistance, health and environmental problems, which is emerging as a particular problem in developing countries. Therefore, there is an urgent need for judicious use of pesticides, which alternatively can be used together with alternative control techniques or changes in the management of preparing and storing grain.

**2.3.2 Physical control method**

Changes in the systems of storage can enhance or suppress pest abundance and the amount of damage caused to grain. A 'hygienic' storage system can serve as an alternative to the application of pesticides. For instance, Gwinner *et al.,* (1990) reported proper cleaning of storage areas during or prior to storage can reduce pest outbreaks at a cost most farmers can afford. Cleaning is necessary to remove all debris, such as rotting grain and insect matter that would otherwise attract more insects and microbial pests, which then become established in the store (Reed *et al*. 1991; Baker & Smith 1990; Hagstrum 2001). Old stock of grain left in stores can hide both eggs and adult insects, which can carry over to the next season. Arthur *et al*. (2006) observed high populations of some insects, such as *Cryptolestes spp* and *Sitophilus spp* in a sample of grain residues collected in a commercial elevator. Hygiene alone is not good enough to give total protection of stored grain from infestation. Good hygiene in combination with protectants improved the efficacy of protectants in storage infestation by *O. surinemensis,* which was better than in stores with good hygiene alone (Herron *et al*., 1996).

In addition to cleaning, combining other cultural practices, such as the use of varieties of grain that are resistant to storage pests and modifications to grain storage structures to control the temperature and humidity, have proved to be successful in insect pest management as an alternative to chemical control. Plants possess some defensive mechanisms against storage pests; Franco *et al*. (2000) found compounds in plant seeds that play a key role in resisting damage by pests and pathogens. Furthermore, Bughio & Wilkins (2002) have identified millet cultivars that are resistant to *T. castaneum* attack; they observed fewer eggs and greater mortality in resistant than in susceptible cultivars. This indicates that identification and cultivation of plants with these characteristics could reduce storage pest problems.

Heating of storage areas to at least 50$^{o}$C for an adequate amount of time are reported to be effective for disinfecting invasive pests, although that depends on the pest species (Roesli *et al*., 2003). The maximum temperature *T. castaneum* can tolerate is ~ 40$^{o}$C (Dobie *et al*., 1991) and a temperature of 50$^{o}$C was found to be lethal to *P. interpunctella* (Locatelli & Biglia, 1995). This is consistent with the findings of Tilley *et al*. (2007) that heat treatment of empty grain stores with 29 kW for 2 hrs before stocking it with grain provides 100% mortality for three insects; *T. castaneum*, *S. oryzae* and *R. dominica*. Similarly, heating grain stores to 50$^{o}$C provides 100% adult mortality of *T. castaneum*, although some degree of tolerance was observed in old instars and pupae (Mahroof *et al*., 2003). Temperature treatment was found to be effective against all developmental stages of *P. interpunctella*, where the pest was successfully controlled at either a cold temperature (2$^{o}$C) or a high temperature of ~65$^{o}$C for 6 hours (Rahemi & Zare, 2002), although, these methods may be too expensive for small-scale farmers to practice.

### 2.3.3 Biological control

The importance of natural predators and parasites in the control of insect pest populations cannot be overemphasized. However, human activities such as use of pesticides to control pest infections have also reduced populations of natural enemies to a level whereby they no longer have a great impact on the pest populations (Hill & Walter, 1982). Considering their importance in the field of pest management, entomologists developed ways of managing natural enemy populations more efficiently in the control of insect pest populations, a phenomenon called biological control (Hajek, 2004). This is defined by Eilenberg *et al*. (2001) as the use of living natural organisms to suppress the populations of specific pest organisms, making it less abundant or less damaging than it would otherwise be (DeBach, 1964). Even though the method is good, it requires knowledge of the biological control agents, how to source and use them (e.g., mass rearing of the biological agents), which may be too expensive for small-scale farmers to practice. Small-scale farmers need methods that are simple, cost effective and, less harmful to their health and environment, and easy to practice.

### 2.3.4 Pesticidal/repellent plants as control agents

A considerable number of African plant species are reported to have the potential for use as grain protectants (Dubey *et al*., 2011). Plant materials, such as leaves, seed, bark or roots, can contain chemical compounds that are toxic or affect the behaviour of pest species, such that damage to grain is reduced. These plants have been known about for many years and used by researchers and farmers in some African countries to protect their grain (Golob *et al*., 2002; Isman, 2006). Many of the African plants have been found to be good sources of insect behaviour-modifying compounds that have qualities that make them repellent, attractive, anti-feedant, oviposition deterrent and/or toxic to insect pests (Hassanali *et al*., 1990; Regnault-Roger, 1997; Bouda *et al*., 2001; Kim *et al*., 2003; Isman, 2006; Chu *et al*., 2010; López & Pascual-Villalobos, 2010). Good examples include Neem tree (*Azadirachta indica),* which produces Azadrachtin (Isman, 2006 & 2008), Retonoid from *Tephrosia vogelii, Methyl salicylate* from *Sacuridaca Longepedunculata*, (Belmain & Stevenson, 2001); Methyl chavicol and Eugenol from Ocimum species (Díaz-Maroto *et al*., 2004; Kasali *et al*., 2005). Some of these compounds are proved to have repellant properties that affect both physiological (growth regulator) and anti-feedant activities of many insect species (Isman 2006).

#### 2.3.4.1 Use of pesticidal/repellent plant products as grain protectants

The most widely known plants used in pest management are pyrethrum, rotenone, Neem, essential oils from various plant species, as well as three plants, ryania, nicotine and sabadilla, which are in limited use (Isman 2006). Since this study is aimed at improving the use of plant materials as grain protectants in Africa, this review will focus only on traditional ways pesticidal/repellent plant materials from Africa are being used by small-scale farmers as grains protectants.

Farmers in African countries often use different plant materials to protect their stored and field crop as alternatives to synthetic pesticides, for the specific reasons that they can be found locally, they do not cost anything and they are easy to prepare and apply (Dubey *et al*., 2011). Depending on the type of plants available and farmers' experience, different plant parts, such as leaves, fruits, seeds, roots, barks and ashes have played an important role in the application of traditional methods of pest control against many notorious African storage and field insect pests (Belmain & Stevenson, 2001; Asawalam *et al*., 2007). The efficacy of these plant materials on particular insects is influenced by the plant species, methods of processing and application of the plant materials, and dosing. Environmental conditions can also affect the chemical composition of individual plants, and hence their efficacy against target insects. Farmers have different ways of processing and using plant materials as grain protectants. For instance, the plants can be used as crude liquid extracts, ground powder, as essential oil extracts (Denloye, 2010), or as dried whole plants layered in-between grain in stores (Belmain & Stevenson, 2001). Use varies with region and locality, however. For instance, in Ghana fresh or dried leaves of *A. indica, Cassia sopera,* root of *S. longepedunculata*, are either used as whole plants or powdered or soaked in water to get the crude extract and mixed with or layer within stored grain (Belmain & Stevenson, 2001). In Kenya, stored product pests are often protected using ground powder or extract from Neem or burned wood ash (Deng *et al*., 2009). In Northern Cameroon, 27 plants species, from the Poaceae and Lamiaceae families are often used either as powder or extracts in farmers' stores as grain protectants (Ngamo *et al*., 2007).

In Nigeria, little attention is given to on-farm use of plant materials as grain protectants, despite the report by Poswal & Akpa (1991) that, due to the cost and erratic supply of synthetic pesticides, small-scale farmers are either adopting alternative methods of leaving grain untreated or using plant materials as grain protectants. This implies that farmers consider plant materials as a cheap source of grain protectant and an alternative to synthetic pesticides. Hence, there is a need to determine how to efficiently and effectively use locally available plant materials. There is evidence from Mann (1998) that in Nigeria plant materials, including *Ocimum species,* are often used by peasant farmers in the northern part of the country to protect their stored cereals and legumes from pest infestation. Salako *et al.* (2008) and IAR (2001) reported that small-scale farmers in Nigeria are either using dried powder or extracts of plant materials, which are mixed with grain to be stored.

Several studies conducted in the laboratory on pesticidal plants grown in Nigeria have established their potential as control agents against several important storage pest species. For example, Asawalam *et al*. (2007) reported that 5% of powder prepared from eight plant species (*Vernonia amygdalina, O. grattissimum, Piper guineeses,* *Chromolaena odorata, Afromomum melegueta, Nicotiana tobacum* and *Capsicum frutescens)* grown in Nigeria proved to be effective against *Sitophilus zeamais.* Conceicao *et al*. (2010) found a high mortality rate of 31% and 86% when *S. zeamais* was treated with 15% and 30% plant extracts from *Mentha pulegium, Lonchocarpus sericeus, Daphne gnidium, Laurus nobilis, Momordica charantia,* and *Ptaroxylon obliquum*. An admixture of 3g of *Dennettia tripetala* (pepper fruit) per 25g sorghum or millet achieved 100% mortality of adult *R. dominica, S. oryzae* and *T. castaneum* (Okonkwo, 2004). A mixture of 25g sound millet grain with 5g and 7g of *O. basilicum* and *Balanites aegyptica* for 24h resulted in significant mortality of *T. castaneum* (Ahmed *et al.,* 2010). In a laboratory experiment, Yusuf *et al*. (1998) reported that powders leaves of *A. indica, Melia azaderach, Zingiber officinale, Eucalyptus camaldulensis, O. basilicum, Capsicum frutescens* and wood ash of *Khaya senegalensis* were effective in the control of maize weevil (*S. zeamais*) in stored maize in Nigeria. Most of this laboratory work may not be feasible in the field because grain needs to be treated with high doses of plants in order to achieve good results, which explains the need for farmers to collect plant materials in bulk to treat their grain. Due to the high evaporation rate of essential oils on one hand and the difficulties in access on the other hand, the use of grain protectants by small-scale famers may be difficult. Attention should be given to the methods of preparation and application that are effective and can be acceptable to farmers. For pesticidal/repellent plants to gain success and acceptance by small-scale farmers, it is important to focus on the development of a best method of application for the most commonly available species that are already known of by farmers and screening and isolation the active compounds from the most pesticidal/repellent plant species (Isman, 2006).

Since plant materials differ in their bioactive compounds (Owusu, 2001, Isman, 2006), mixtures of different combinations could enhance their efficacy as well as reduce the need for large quantities of just one species of plant materials. López & Pascual-Villalobos, (2010) and Ntonifor *et al*. (2010) reported that plant oils and/or powder compounds from two or more types of plant can produce a synergistic control effect. These findings suggest that there is still much to learn about the insecticidal and sub-lethal effects of various plants and plant parts which could help to improve the efficacy of pesticidal plants, to achieve outcomes at least as good as or better than chemical insecticides.

#### 2.3.4.2 Action of pesticidal/repellent plants on storage insects

Different pesticidal/repellent plants exhibit different typess of action on different insect species, depending on the plant species, plant parts, and their bioactive compounds, location where plant can be collected and the method of plant preparation. For instance, crude extract, oil, powder and essential oils obtained from Neem tree parts (leaves, seed & bark) contain some bioactive compounds (notably, *Azadirachtin*) which are confirmed to have broad spectrum insecticidal activities on a range of insect species (Isman, 2006 & 2008). Oil derived from Neem seed (Calneem) can be significantly repellent or toxic to adult *T. castaneum,* depending on dose and other factors, and can delay the emergence of their progeny (Adarkwah *et al*., 2010). Similarly, Ahmad *et al.* (2009) found that the adults, larvae and pupae of *T. castaneum* were affected in various ways by sub-lethal concentrations (1, 0.5 and 0.25%) of Neem oil and Neem leaves on filter paper. In another instance, three stored product pests, S. *oryzae, Cryptolestes ferrugineus* and *T. castaneum* were all killed or repelled by Neem extract (Xie *et al*., 1995).

A number of other plants and their constituent essential oils have been confirmed to show similar effects of repellency, anti-feeding, growth regulation, and even insecticidal properties against a range of stored product pests. Examples of some plants with pesticidal/repellent properties are shown in Table 2.1.

Table 2.1 An overview of pesticidal/repellent plants and their types of action on insects.

| Pesticidal/repellent plant | Insect affected | Type of action | References |
|---|---|---|---|
| *Melia azedaracha, Mentha longifolia, Myrtus communis, Cymbopogon citratus Datura stamonium* (Methanol extracts), | *C. maculatus, T. castaneum, O. surnamensis* | Repellent and lethal effect | Manzoor *et al.* (2011). |
| *Casssio sophera* (Water extract). | *S. oryzae, C. maculatus* | Reducing emergence of adult. | Kestenholz, *et al.* (2007). |
| *Ocimum basilicum, O. graticimum* (Essential oils). | | Fumigant effect | Kéita *et al*. (2000) |
| *C. citratus, Lantana camara, Ocimum basilicum, Tagetes erecta (*Ground powder) | *S. zeamais* | Repellent | Parugrug & Roxas (2008) |
| *Pimpinella anisum, Cuminum cyminum, Eucalyptus camaldulensis, Origanum syiacum* and *Rosmarinus officinalis* (essential oils) | *T. castaneum, E. kuehniella* | Repellent and lethal effect | Tunc et al. (2000). |
| *Cupressus sempervirens, E. saligna* (essential oils) | *S. zeamais, T. castaneum* | Lethal effect | Tapondjou, *et al.* (2005). |
| *O. basilicum* (Essential oils) | *T. castaneum* | Repellent | Deshpande & Tipnis (1977); Mohuddin et al. (1987); Abd El-Aziz & Elsayed (2009); Nenaah & Ibrahim (2011); Mishra *et al.* (2012). |
| | *Acanthoscelides obtectus* | Lethal effect | Regnault-Roger & Hamraoui (1994). |

It is important to note that killing entire pest populations requires the ability to target all life stages, including eggs, larvae and adults so they cannot establish a viable colony in an isolated area of the stored grain. However, repellent materials have the advantage of preventing insects from becoming established in an environment through its effect of repelling insects from a distance (Dethier *et al*., 1960; Pedigo, 1989; Foster & Harris, 1997). Hence, repellents reduce not only damage to stored food, but also the chances of oviposition, egg hatching and larval development in the grain.

A lot is known about the repellent, anti-feeding and pesticidal effects of essential oils of many African plants, such as *A. indica*, *Ocimum* species and *Cymbopogon* species, against storage pest, such as *T. castaneum* and *Sitophilus* species. However, there has been less exploration of the repellent effects of ground dried plant materials on storage pests, particularly in the field. Some plant materials that are readily available to farmers are likely to have repellent effects that could be incorporated into new technologies to protect stored grain. For instance, in a laboratory experiment, odour emanating from bags treated with an extract of *Lantana camara* reduced bean seed damage by bean weevils (Koona *et al*., 2007). The treatment of the outside of bags of grain with repellent compounds may expose pest insects, especially those coming from within the storage area, to a greater repellent effect than the method of mixing grain with protectant plant material, as currently practiced by farmers. This approach to the use of repellent plant materials could be useful to farmers, because it has the advantage of preventing pest invasion at first encounter (Dethier, *et al*., 1960; Olufolaji, 2011), especially for pests such as *R. dominica* and *T. castaneum* that move freely within storage areas (Dobie *et al*., 1991).

**2.4 Response of insects to stimuli in bioassays**

Bioassays have been used as an important tool to study the response of insects to environmental stimuli (Robertson *et al*., 2007). It has been an important technique in determining the efficacy of different plant materials and their compounds against storage crop insect pests (Morgan *et al*., 1998; Lale & Yusuf, 2001; Musa *et al*., 2009; Stefanazzi *et al*., 2011). The response of insects to a test stimulus in a bioassay is important for understanding the way insects locate their host grain and the mechanisms involved. The sensory cues used by insects to locate the host grain depends on factors, such as the type of insect movement (e.g., flight or walking) and behavioural characteristics of the species that may be influenced by the physical features of the bioassay environment (Yinon & Shulov, 1969; Morgan *et al*., 1998), such as whether it is indoors or outdoors, and exposed to air or buried at a depth. Storage pests such as beetles locate and reach their host-food by responding to volatiles released from food, or in some cases through visual cues (Foster & Harris, 1997). The mechanisms by which insects use volatile stimuli to find the source of odours have been highlighted by Kennedy (1977; 1986). Typically, beetles respond to odours by positive chemotaxis, i.e., moving toward the source of odour. The details of the response depend on the insect species and its ability to perceive the odour and follow the trail of odour by increasing its movement toward the source (orthokinesis) or by altering its turning behaviour (klinokinesis) until it detects cues that make it stop at the source (arrestment). Moreover there are other environmental factors that affect the response of insects to their host odour in nature, and hence in a bioassay, which include responses to light (phototaxis), movement that keeps the insect in contact with as many surfaces as possible in addition to the floor, such as walls (thigmotaxis) and movement in response to gravity (geotaxis, i.e., moving up or down) (Kennedy, 1986). This indicates the need to use a bioassay that takes into account the natural responses of insects to any of these stimuli that might affect their responses to test materials. The implication is that a good bioassay should provide an efficient and effective way to study the behaviour of insects and their responses to test materials which may help in identifying the most effective treatment materials to use against the insect species in question.

Small-scale farmers of Kebbi need improved methods of grain protection in order to enjoy the benefits of good grain storage, i.e. grain of good quality for home consumption and to sell in the market to earn more income. However, further information is required to increase our understanding of the small-scale grain storage systems, e.g., the management practices, and how they affect the type and amount of insect pests that attack their grain. This information will help to establish a baseline data set with which it should be possible to identify new interventions that could improve the food security and livelihood of the farmers. To achieve this, farmers' survey and entomological sampling were conducted in the three regions of Kebbi State (Kebbi north, south and central). The data obtained was used to identify; a) which type of storage, and b) which type of grain might benefit most from being treated with botanicals against c) which major pest. The ultimate aim is to develop a plant-based control method that significantly reduces grain loss in the most important staple crop in the area; sorghum.

# CHAPTER 3

## Survey of systems of grain storage and the management of insect pests in stored grain in Kebbi state

### 3.1 Introduction

The aim of the first phase of the project was to determine which grain storage system in Kebbi state had the most grain loss due to insect pests. By 'storage system' is meant type of grain, type of storage structure and species of insect.

In Kebbi state, more than 70% of the grain produced is stored in traditionally-made structures, such as granaries and storerooms, with grain either loose or in polypropylene bags. Grain is stored for home consumption throughout the rest of the year and surplus is sold to obtain income (COA, 2009). Grain storage on farms thus plays an important role in ensuring food security for local populations. However, high post-harvest losses due to insect pests and inadequate storage facilities are the main setback in realising this benefit (KARDA, 2004; COA, 2009). No record of previous surveys have been published for Kebbi state to establish how methods of grain storage affect the grain, to identify the major pest species affecting stored grain, to estimate the extent of relative damage by the main insect pests, or to characterise how farmers respond to grain storage losses. This chapter presents the results of a survey of conducted in three geo-political zones of the Kebbi state (Kebbi North, South and Central, Fig. 3.1). A farmer survey is a valuable tool for gaining an overview of the problems associated with preserving harvested grain, both during pre-storage processing and during the period of storage, as well as giving an insight into the socio-economic situations of the communities investigated. The information gathered has been analysed to give a picture of the main storage problems at the start of the project. This information was also used in guiding the decision as to which system to focus on for my research into a new approach to controlling storage pests that is appropriate and effective for small-scale farmers in Kebbi state and is based on the use of repellent plant materials.

The aim of the survey was to gain a greater understanding of:

* The type of grain that small-scale farmers in Kebbi store in greatest quantity.

*Methods of grain storage used and how these affect the amount of insect pests and relative damage caused.

* How farmers respond to the insect problems and how they view the effectiveness of the methods they use to control the storage pests.

## 3.2 Methodology

### 3.2.1 Study area

Socio-economic surveys and grain sampling surveys were carried out in June and August, 2008. At this time of year farmers are engaged in grain storage activities. The survey was carried out in three regions of Kebbi state: South, Central and North. Four districts were selected in each region, with two villages in each district, for a total of 24 villages, as follows:

**Kebbi South:** Danko-wasagu district (Villages selected: Wasagu, Tudun bichi), Bedi (Beedi, Tungandoro), Birnin-Yauri (Kimo, Makirin) and Shanga (Saminaka, Tungangiwa);

**Kebbi Central:** Bese district (Villages selected: Dogon-Karfe, Bashe), Mungadi (Gunbinkure, Sabonsara), Basaura (Kyande, Kangiwa), Kalgo (Bagarza, Langido);

**Kebbi north:** Gulma district (Villages selected: Bagaye, Lailaba), Alwasa (Sawa, Kaura), Kangiwa (Falde, Sabogari), Bayawa (Tigi, Kwaido). The location of these villages is given in Figure 3.1.

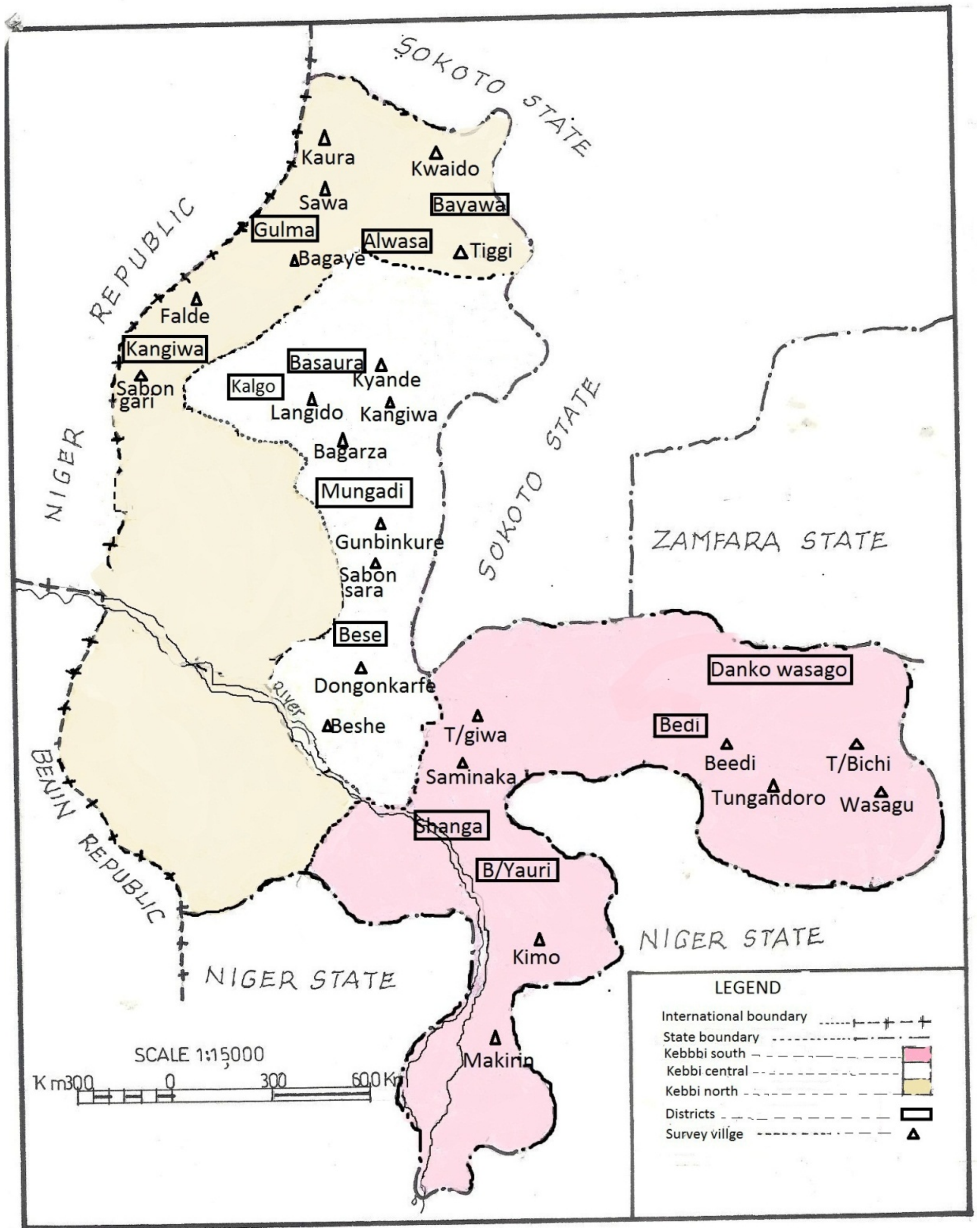



**Fig. 3.1 Map of Kebbi state showing districts and survey villages.**

### 3.2.2 Survey methodology

#### 3.2.2.1 Data collection

A structured questionnaire was pre-tested at NRI by administering it to a pilot group of five PhD students from Kebbi State who were all undertaking research related to agriculture, to check whether the questions were understandable and easy to answer. The final form of the questionnaire (Appendices 1.1) was personally administered by the author to 240 farmers, ten from each of the 24 villages, between July and October, 2008. Hausa, the local language, was used to interview all the respondents in their villages. The questionnaire was designed to collect information about farmers' perceptions of problems associated with stored grain insect pests, the types of insect species damaging their grain, the quantity of grain typically stored, the amount of loss due to stored insect pests, the maximum period of insect pest attack, the type of storage structure used and pest management practiced. In addition, socio-economic information, such as the respondent's sex, family size, farm size, age and educational level were collected. A poster (NRI, 'Insects in a tropical store', Fig. 3.2) containing images of different types of storage insects was presented to the respondents to help in identifying the type of insect pest species in their stores. The data obtained were summarized using simple percentages and, where necessary, subjected to statistically analysis as outlined in Section 3.3.


**Figure 3.2 Poster of storage pests used to help identify insect species found in the grain stores of famers.**

#### 3.2.2.2 Village sampling

A purposive stratified sampling method was used to obtain a representative sample, whereby two villages were chosen from each of four districts in each of the three regions. Village agricultural extension agents in each district provided a list of villages that grew and stored grain. Two villages were selected at random from the list for each district (See map, Fig. 3.1 and section 3.2.1 for the name of the villages**)**.

#### 3.2.2.3 Respondent selection

Wealth ranking (Hodges, 2005) was used to identify sub-samples of farmers to be interviewed; to be as unbiased as reasonably possible, and to have a good representation of different categories of farmers engaged in grain storage in each village, the village head and several key informants in each of the chosen villages were asked to provide a range of indicators to denote the relative 'well-being' of a household, as reflected by grain production and storage practices. The main indicators of well-being were based on the following three categories, and these were used to choose which farmers were to be interviewed:

1) stored grain for a whole season and then sold the surplus at the end of the season,
2) stored grain for more than six months, or
3) stored grain for between one and five months.

Ten households, including both male and female heads of household, were chosen randomly from the list of names given for each village, representing the full range of the above mentioned categories, to make a total of 240 farmers for the study as a whole.

### 3.2.3 Statistical analysis

Data from the questionnaires were summarized in cross-classifying responses, such as levels of infestations against region, or method of storage and storage structures against level of infestations sampled. Contingency-table Chi-square tests of independence were used to test for significant associations between the variables. Furthermore, a two-way ANOVA test was used to test for significant effects of variables on quantitative responses. All tests were run using the R statistical software package (version 2.10.0) R Development Core Team (2012).

## 3.3 Results

### 3.3.1 Socio-economic characteristics of the farmers in the surveyed area

Table 3.1 shows the educational background, age, occupation, family size and farm size of the respondents in the surveyed area. The majority of the respondents (97%) were male household heads. The low number of female respondents could be due to the procedure used for selection of respondents, as only the head of a household was interviewed. Female heads of household are rare in northern Nigeria (Anonymous, 2004). In the villages surveyed, women are mostly confined to the domestic area, where their main responsibilities are cooking and child care. It is the responsibility of a male head of the household to produce grain and manage its storage, for use when needed by women for cooking, with the exception of widows who manage their own grain supplies. However, this does not mean that women do not take part in post-harvest operations; the preparation of grain for storage by threshing and winnowing is undertaken by women. In northern Nigeria, women's participation in agricultural activity is very low, (~16%), and focuses mainly on post-harvest activities (Abdullah, 2008). This is related to religious and cultural norms, prevalent among the Hausa-Fulani Muslim community across the north (Okojie, 1991). In this survey, one of the reasons given by some (male) respondents as to why women are denied access to stored grain is a general belief amongst men that women use grain more quickly than men do.

The majority of the respondents had no formal education (~68%), with relatively few having attended primary or secondary school (Table 3.1). The mean age of the respondents was 42 years. The majority of the respondents (~82%) considered farming as their main occupation, followed by those whose main occupation is trading. A very small percentage considered their paid job (civil servant) to be their main occupation. This indicates that agriculture is the main livelihood in these regions.

The mean family size was relatively large (>13.4), which is reflected in the mean farmland holding size, which must be large enough to support the family with enough food. The majority of the respondents (~ 90%) possessed their own farmland and very few borrowed (~ 8%) or rented land (~ 2%). The majority of the farmers (~ 64%) sourced their seed from their previous harvest, ~20% purchased seed from an open market, ~12% sourced seed from friends and only ~4% sourced seed from the government (KARDA). Some of the demographic data varied between regions, as shown in Table 3.2.

**Table 3.1 Socio-economic background of respondents.** The percentage (%) or mean and standard deviation (St Dev) for some of the socio-economic variables derived from the questionnaire data. Total sample size (N) = 240 farmers.

| Variable | Mean | St Dev |
|---|---|---|
| Age (year) | 42.3 | 11.6 |
| Farm size (ha) | 4.7 | 2.3 |
| Family size | 13.4 | 7.5 |
| Occupation (%): | | |
| Farming | 82.5 | |
| Trading/farming | 12.5 | |
| Civil servant/farming | 5.0 | |
| Education (%): | | |
| Non-formal | 68.3 | |
| Primary | 17.9 | |
| Secondary | 12.9 | |
| Tertiary | 0.8 | |

Figure 3.3 shows that the age distribution of the respondents within the different regions was quite similar, and followed a normal distribution. The majority of the respondents in the north were in the 41-50 and 51-60 age categories, whereas in the south and central regions the majority were in the 41-50 age range. The proportion of respondents in all regions in the 20-30 age range and over 70 years was minimal. The difference in age of all respondents in the different regions and age categories was found not to be significant ($\chi^2$ =7.4, df=10, p=0.6843).

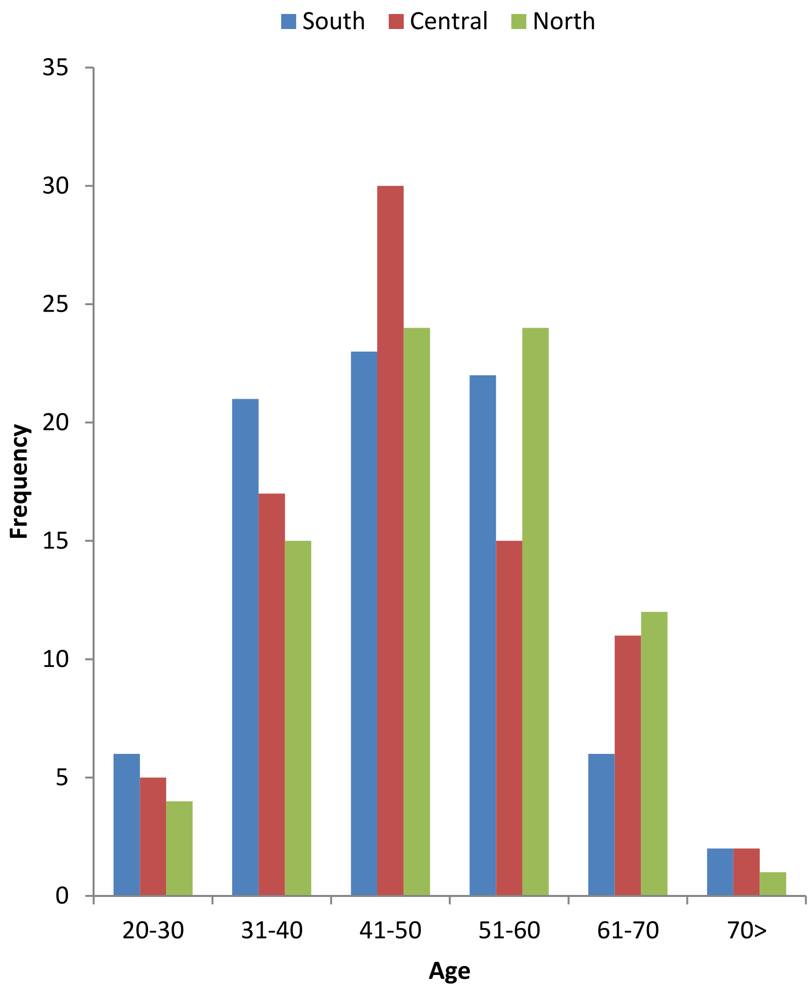



**Fig. 3.3 Frequency distribution of respondents by age in the different regions** (N = 240 farmers, 80 farmers per region). There was no difference in the age of respondents in each age category between the regions ($\chi^2$ =7.4, df=10, p=0.6843).

**Table 3.2 Demographic characteristics within the three regions surveyed.**

| | Central | North | South |
|---|---|---|---|
| Ethnic group | Hausa | Hausa | Hausa/Dakarkari/Kambari |
| Average age of respondent | 42.1 | 44.1 | 40.4 |
| Average farm size (ha) | 6.5 | 3.4 | 7.1 |
| Average family size/household<br>Average of adults<br>Average of children | 12.2<br><br>5.4<br>6.8 | 14.4<br><br>6.4<br>8.0 | 13.6<br><br>6.0<br>7.7 |
| Main pest control measures | Chemical, botanical | Chemical, botanical | Mainly botanical, some chemical |
| Cereal grain grown, in rank order of most to least | Sorghum/millet, maize | Millet, sorghum, maize | Sorghum, maize, millet |
| Time of harvest<br>Sorghum<br>Millet<br>Maize | <br>Nov-Dec<br>Aug-Sept<br>Sept-Oct | <br>Nov-Dec<br>Aug-Sept<br>Sept-Oct | <br>Dec-Jan<br>Aug-Dec<br>Oct-Dec |
| Average quantity of bags stored per household (1bag=60kg) | 34 | 36 | 40 |
| Form of grain in storage | Mainly un-threshed form | Mainly un-threshed form | Mainly threshed form |
| Storage type/ design | Mud granary, bags and store-rooms | Mud granary, bags and store-rooms | Mud granary, bags and store-rooms |
| Main storage pests | *T. castaneum, R. dominica, S. zeamais, C. maculatus, P. interpuntella, L. serricorne* | *T. castaneum, R. dominica, S. zeamais, C. maculatus, P. interpuntella, L. serricorne* | *T. castaneum, R. dominica, S. zeamais, C. maculatus, P. interpuntella, L. serricorne* |

### 3.3.2 Types of grain grown and stored by farmers surveyed

The farmers listed sorghum, millet, maize and rice (in rank order, highest to lowest) as the major cereal grains grown and stored. The time at which farmers harvest and store their grain varies by crop and region. Harvest of sorghum begins in December to January, with most farmers harvesting in December. For millet, farmers harvest earlier, between August and December, with most farmers across the three regions harvesting in September (Table 3.2). The results in Table 3.2 show that harvesting of all grains starts earlier in the north than in the south, where it occurs a few months later. This may be related to the difference in weather conditions, such as rainfall, which begins later and ceases earlier in the north (Adejobi *et al*. 2008; COA, 2009). Grain storage begins a few days to weeks after harvest and drying. In all the regions, some farmers produce more than one kind of grain. Whether farmers stored one or more types of grain depends on their wealth status, reason for storage and the number of storage structures possessed.

Table 3.3 shows that the proportion of farmers that grow and store a given type of grain varies between regions. More farmers in the north grow and store a combination of millet plus sorghum than in the central or south regions. More farmers in the southern region grow and store sorghum or millet alone. Similar proportions of farmers grow and store maize plus millet in the south and north, with the greatest proportion in the central region. A similar pattern was true for maize grown on its own in all the regions; more farmers in the south grow and store maize than in the central or north regions and the range of proportions between regions was least for this type of grain. The chi-square test confirmed that there was a significant difference in the proportion of farmers that grow and store each grain type in the three regions (Table. 3.3; $\chi^2$= 44.7, df = 8, p<0.0001).

**Table 3.3 Relative distribution of the amount of grain stored in each region by grain type.**

| | Percentage of respondents in each category | | | | | |
|---|---|---|---|---|---|---|
| | maize | sorghum | millet | maize/millet | sorghum/millet | Total |
| South | 8.8 | 37.5 | 20.0 | 18.8 | 15.0 | 100 |
| Central | 5.0 | 13.8 | 26.3 | 31.2 | 23.7 | 100 |
| North | 1.3 | 13.8 | 6.2 | 21.3 | 57.5 | 100 |

There was a significant difference in the proportion of farmers that grow and store each grain type in the three regions ($\chi^2$= 44.7, df = 8, p<0.0001).

Concerning the quantity of grain stored by household, the results in Figure 3.4 indicate that, overall, there was a statistically significant difference in the amount of grain grown/stored between types of grain (ANOVA, F=22.62, df= 2, 711, p<0.001). The Tukey HSD test indicates that a significantly greater quantity of sorghum was stored (p<0.001) than any of the other grains, with an average of 67 bags per household (4.04 t) across regions, compared to 31 bags (1.9 t) of millet and 28 bags (1.0 t) of maize, respectively. The mean quantity of sorghum stored was similar across the regions, whereas farmers in the south or central regions store significantly more maize than in the north (p<0.001). The reverse was true for millet, which stored in significantly higher quantities (p<0.01) in the north region than in the central or south regions. The distribution of these two grains show a reverse cline, with largest quantities of maize stored in the south and largest quantities of millet stored in the north, which is reflected in a statistically significant interaction between grain type and region (F=15.41, df= 4, 711, p<0.001). However, there was no significant difference overall in the weight of grain stored between regions (p=0.631)

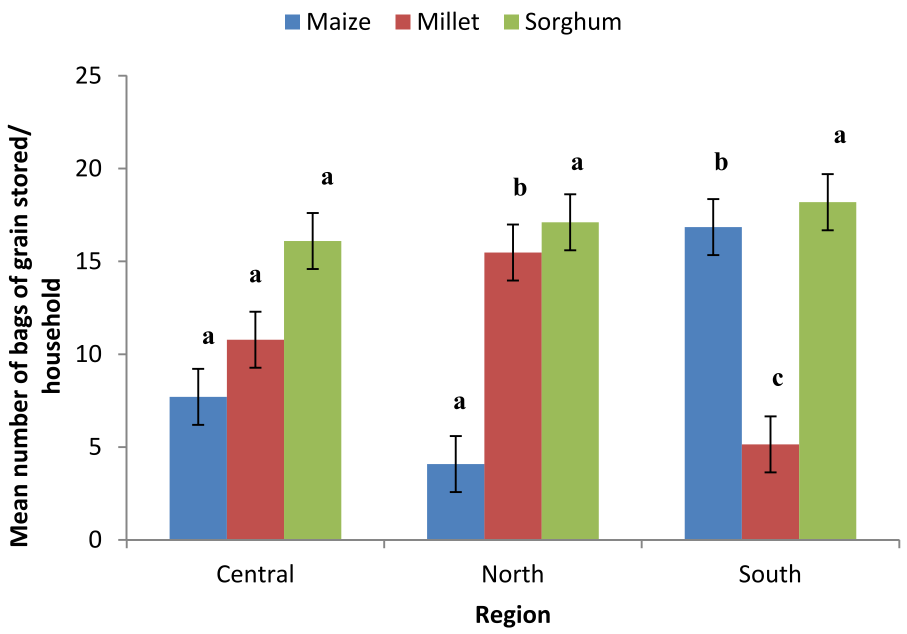



**Fig 3.4 Distribution of the quantity of the main three grains stored per household, by geo-political region, (n=240, 80 per region; each bag =60kg). Error bars are calculated from the ANOVA residuals.** Overall there was a significant effect of quantity of grain store by region (ANOVA, $p<0.001$). Bars with different letters between regions are statistically significant (Tukey HSD, $p<0.05$).

### 3.3.3 Method of processing grain for storage, duration of drying and levels of infestation

***Response of farmers regarding processing of grain for storage:*** Respondents stored their grain in both the threshed (sorghum and maize) and un-threshed (sorghum, millet and maize) forms. Overall, a large proportion of respondents (56%) stored their grain un-threshed, with higher proportions of farmers practising this form of storage in the north and central regions (Table 3.4). The remaining farmers (44%) stored their grain in the threshed form, with a high proportion in the south and similar proportions in the central and north regions. There was significant association between form of storage and regions ($\chi^2$ = 31.391, df = 2, p<0.001). Most farmers (45%) mentioned reduced insect pest damage as their reason for using the unthreshed form for storage, followed by economic reasons (37%), and ease of handling (19%). For those that stored threshed grain, 89% mentioned easy bagging and handling as their reasons, while 11% mentioned reduced insect infestation.

**Table 3.4 Percent of farmers using different methods of preparing grain for storage in different regions.**

| | Threshed | Un-threshed | Total |
|---|---|---|---|
| South | 68.7 | 31.3 | 100 |
| Central | 35.0 | 65.0 | 100 |
| North | 27.5 | 72.5 | 100 |

There was a significant association between form of storage and regions ($\chi^2$ = 31.391, df = 2, p<0.001).

Table 3.5 shows that a greater proportion of the respondents said that un-threshed grain had low levels of infestation, compared to the respondents reporting threshed grain to have low levels of infestation. It is interesting to note that even though unthreshed grain had lower infestation levels, a majority of farmers stored their grain only in the south in threshed form. It would be interesting to know why they do this, because they could reduce losses by simply leaving their grain unthreshed. This is worth future investigation. Similarly, the proportion of respondents who stored threshed grain reported high levels of infestation, which was higher than for those storing un-threshed grain. Only a few of the respondents reported no infestation. The difference in levels of infestation between the forms of storage was statistically significant ($\chi^2$ = 11.1574, df = 3, p<0.01).

**Table 3.5 Comparison of methods of processing grain for storage and level of infestation during storage.**

| | Percentage of respondents in each category<br>Level of infestation* | | | | |
|---|---|---|---|---|---|
| | None | Low | Medium | High | Total |
| Threshed | 10 | 24 | 30 | 36 | 100 |
| Unthreshed | 13 | 53 | 23 | 11 | 100 |

There was a significant difference in the perception of respondents on level of infestation on form of grain storage ($\chi^2$ = 11.1574, df = 3, p<0.01). (*Level of infestation; None= no insects observed in store samples Low= 1-15 observed moving within the store samples, Medium= 16- 40, and High= >40 insects observed within the store samples).

Figure 3.5 shows the duration of grain drying practiced by farmers. In all three regions most respondents dried their grain for ≥ 8 days before storage, with the largest proportion of respondents drying their grain for 8 days, which was a greater proportion of farmers in the north than in the central and south region. The drying period decreased in all regions from 10 days onwards. This may be due to higher moisture content at harvest. The maximum drying time recorded was 18 days, reported by 1% of the respondents from the central region. The difference in duration of drying time between regions was found not to be statistically significant ($\chi^2$ = 5.569, df = 8, p=0.695).

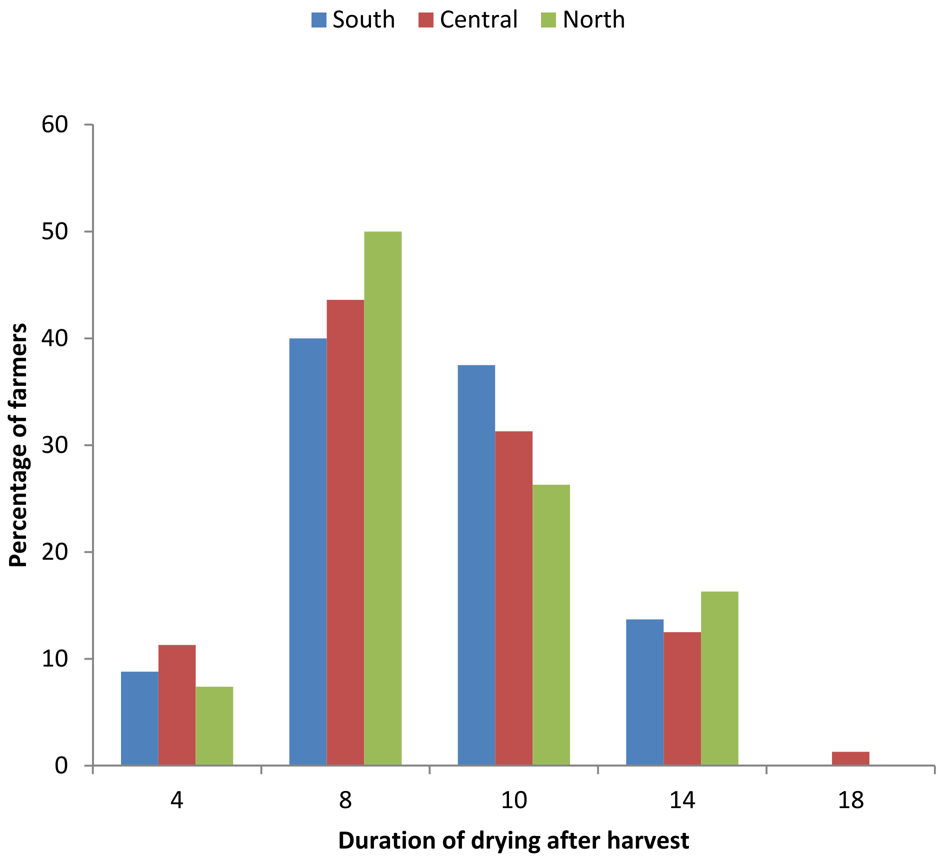



**Fig 3.5 Percentage of farmers in each region that dried their grain for different lengths of time (days), n=240, 80 per region.** The difference in duration of grain drying time had no significant effect between the regions ($\chi^2$ = 5.569, df = 8, p=0.695).

The results in Table 3.6 indicate that levels of insect infestation appear to be highly significantly associated with the duration of drying time before storage. Overall, grain dried for 8 days had the highest number of respondents reporting a low level of infestation (52.3%, Table 3.6). However, the majority of respondents drying their crop for 8 days before storage were concentrated in the north (52%), as reported in Figure 3.4. The farmers who reported the next lowest level of infestation were those who dried crops for 10 days, followed by 14 days. Only a small proportion of the respondents experienced high levels of infestation when their crop was dried for 8 days. Although there were indications of high levels of infestation at 8 and 10 days, the proportion of farmers reporting high levels of infestation was relatively small. High infestation levels were reported by a greater proportion of respondents when their crop was dried for 4 days. There was a significant association of drying time on levels of infestation ($\chi^2$ =31.247, df =12, p<0.001).

**Table 3.6 Relationship between levels of insect infestation and drying time.**

| Percentage of respondents mentioned each drying period | | | | | |
|---|---|---|---|---|---|
| | Drying period (days) | | | | |
| | 4 | 8 | 10 | 14 | 18 |
| Infestation level | | | | | |
| None | 4.5 | 19.7 | 11.8 | 32.4 | 0 |
| Low | 18.2 | 52.3 | 48.7 | 38.2 | 100 |
| Medium | 50.0 | 20.3 | 31.6 | 29.4 | 0 |
| High | 27.3 | 7.5 | 7.9 | 0 | 0 |
| Total | 100 | 100 | 100 | 100 | 100 |

There was a significant association of drying time with level of infestation ($\chi2$ =31.247, df =12, p<0.001).

### 3.3.4 Farmers' perspective on storage practices and storage structures

***Response of farmers on choice of storage structures:*** The granaries used by farmers in the three regions generally measure 7.9 – 35.4 $m^3$ and are made of mud and wood with a thatched roof. Stones are used to raise the floor above the ground for aeration and rat control. Some have side openings (hatches) to provide access to the stored grain, while others are windowless with access to the grain only from the top of the structure. Grain is stored either loose or in bags. The second most frequently used storage structure is a store-room within the main dwelling, which is made of mud with a thatch or zinc roof. Store-rooms are generally used to store grain and other types of food and domestic belongings. Unlike granaries, the floor of a store-room is not raised above the ground. Grain is generally stored either loose or in bags which are loaded into, and emptied out, of the room through a small door. The bags used for storage hold 60 kg of grain, and are made from woven polyethylene strips.

The results in Fig. 3.6 indicate that farmers often use more than one type of structure to store their grain. The users of the different structures are evenly distributed between regions. For instance, <35% of respondents used each of the two structures in each region, except for store-rooms, which have a greater proportion of users (>40) in the south). However, the difference between the proportion of farmers that use store-rooms in the south and other regions was not significant ($\chi^2$ = 4.238, df = 4, p=0.375).

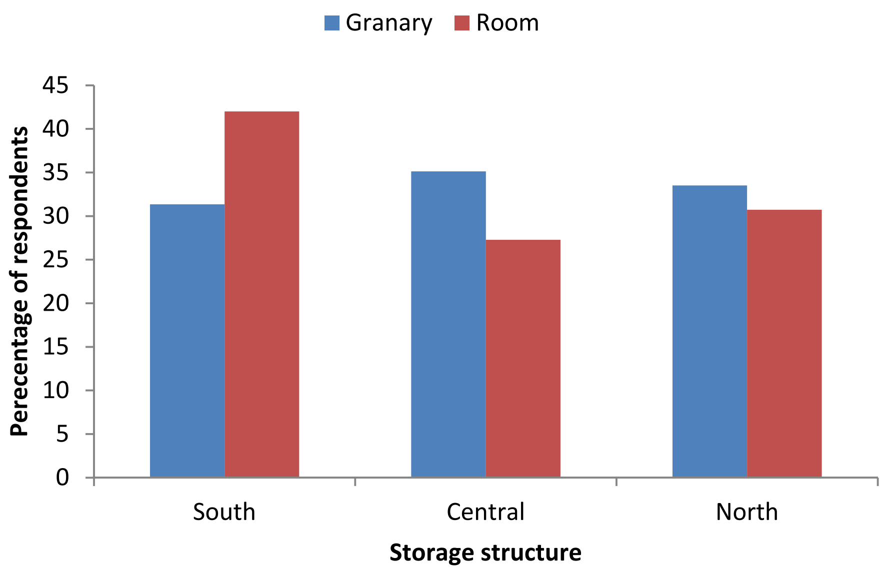



**Fig. 3.6 Distribution of respondents according to location and storage structures used, n=240, 80 per region**. No significant difference in the distribution of farmers that used a particular storage structure between the regions ($\chi^2$ = 4.238, df = 4, p=0.375).

Farmers mentioned a range of reasons for choosing particular storage structures. The majority of farmers (64%) using granaries mentioned reduction in insect damage, followed, in rank order by; better control of how grain is used (22%), because they inherited the granaries (9%), and ease of storage (4%). Of farmers that chose store-rooms for storage, 39% reported ease of storage, followed by 'had no access to a granary' (32%), store-rooms can accommodate more grain (20%) and reduced insect infestation (9%).

***Duration of storage and its effect on levels of infestation:*** The majority of farmers in all regions began to fill in their stores with grain in December- January, and store grain for as long as one year, depending on their circumstances. The majority (67%) gave 'home consumption' as their main reason for storing grain, and 22% gave 'trading' as their main reason. Only 11% combined both consumption and trading as their main reason for storing grain. In all regions, most respondents stored their grain for seven months; 73% in the north, 45% in the central and 38% in the south (Fig 3.7). Some respondents stored grain for up to a year: 30% in the south, 25% in the central and only 8% in the north. In all regions, very few of the respondents stored grain for only 4 months. There was a significant difference in the duration of storage between the regions ($\chi^2$ = 33.569, df = 6, p<0.001). This appears to be due to farmers in the north who tend to store their grain for only 7 months, whereas in the central and south regions there is a tendency for equal proportions of farmers to store their grain for 7, 10 and 12 months.

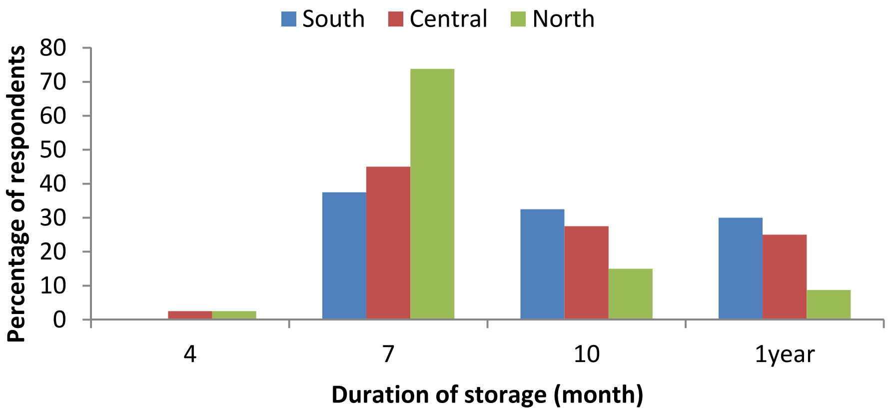



**Fig 3.7 Distribution of respondents according to location and duration of grain storage.** (N=240, 80 farmers per region). There was a significant difference in the duration of storage between the regions ($\chi^2$ = 33.569, df = 6, p<0.001).

Most respondents reported highest levels of infestation occurring in grain stored for 7 months, followed by grain stored for up to 10 months (Fig 3.8). Grain that had been stored for 4 months had the lowest levels of infestations (none + low). The highest proportion of respondents that reported infestations in grain stored for one year had only medium levels of infestation. The data also suggests that if grain is stored well, it can be kept for one year with only medium levels of infestation developing, especially if the region has low level of infestation. According to the chi-square test there was a highly significant association of storage duration on level of infestations ($\chi^2 = 91.74$, df = 9, $p<0.001$).

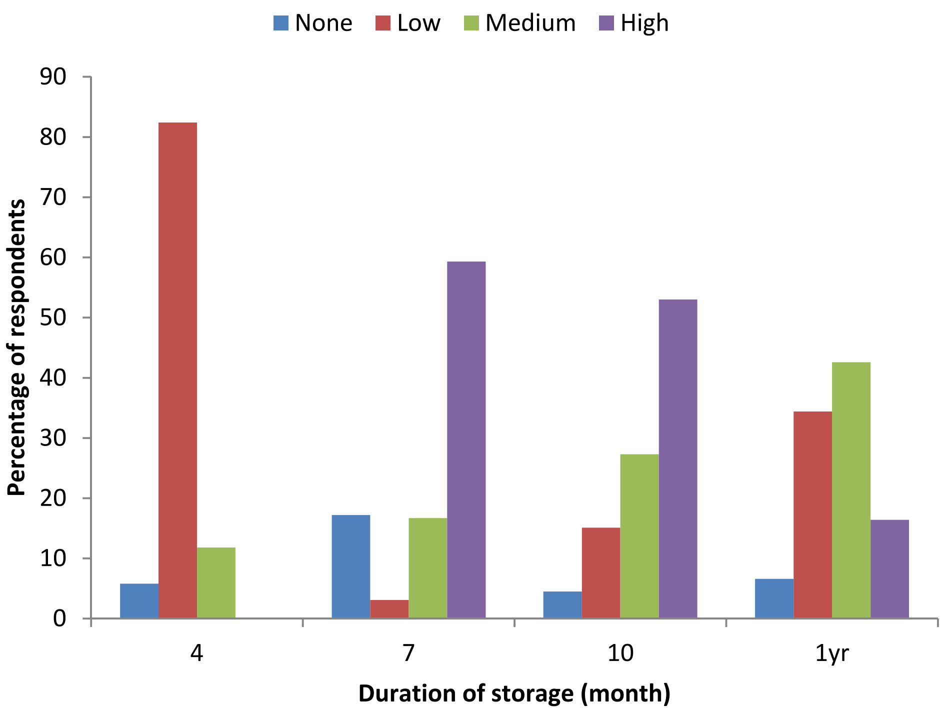



**Fig 3.8 Relationship between levels of insect infestation and storage duration.** (N=240, 80 farmers per region). There was a significant association of duration of storage on level of infestations ($\chi^2 = 91.74$, df = 9, $p<0.001$).

### 3.3.5 Farmers' perspectives on insect pest identification and levels of infestation in stored grain

Farmers were able to identify some of the insects that were found in their stores using the NRI poster (Fig.3.2). However, those with moth infestations did not know that moths produce larvae that cause grain losses. However, 52% of respondents were able to identify *Tribolium castaneum* and *Rhyzopertha dominica* as the pest found in their store using the poster; and 26% identified *Sitophilus zeamais*, 16% identified *Lasioderma serricorne* and 6% indentified *Callosobruchus maculatus* in their grain. Table 3.7 shows that the number of insect species varied by the storage structure used. The greatest percentage of farmers reporting no insect infestations was those using granaries (~49%), while only 13% of farmers using store-rooms had no infestations. A relatively high percentage of farmers using store-rooms (~47%) reported three, four or five insect species in their grain, while only ~20% using granaries reported more than three insect species. Overall, there was a significant difference between the number of insect species reported and types of storage structures used by the farmers ($\chi^2$ = 19.4826, df = 10, p<0.01).

**Table 3.7 Percentage of insect species farmers observed in their grain stores according to the type of storage structures used.**

| Percentage of respondents reporting different number of insects species in their store | | | | | | | |
|---|---|---|---|---|---|---|---|
| | Number of insect species | | | | | | |
| | **0** | **1** | **2** | **3** | **4** | **5** | Total |
| Granary | 48.6 | 31.1 | 0.0 | 13.5 | 5.4 | 1.3 | 100 |
| Store-room | 13.0 | 39.7 | 6.0 | 24.5 | 13.4 | 3.6 | 100 |

There was a significant difference between the number of insect species reported and types of storage structures used by the farmer ($\chi^2$ = 19.4826, df = 10, p<0.01).

The results in Fig 3.9 show that there was a significant difference between the regions in level of damage by insect pests ($\chi^2$ =16.6847, df=6, p<0.01). Overall, a relatively high proportion of respondents (> 40%) reported medium or high levels of damage, especially in the south (~48%). This may link to differences in climate, type of crops, storage structure or control chemicals. The results of this survey (Table 3.9) suggest that respondents from the north tend to use chemical control more than the other regions, likewise respondents in the south stored grain more in the threshed form than the respondents in the other regions (Table 3.4).

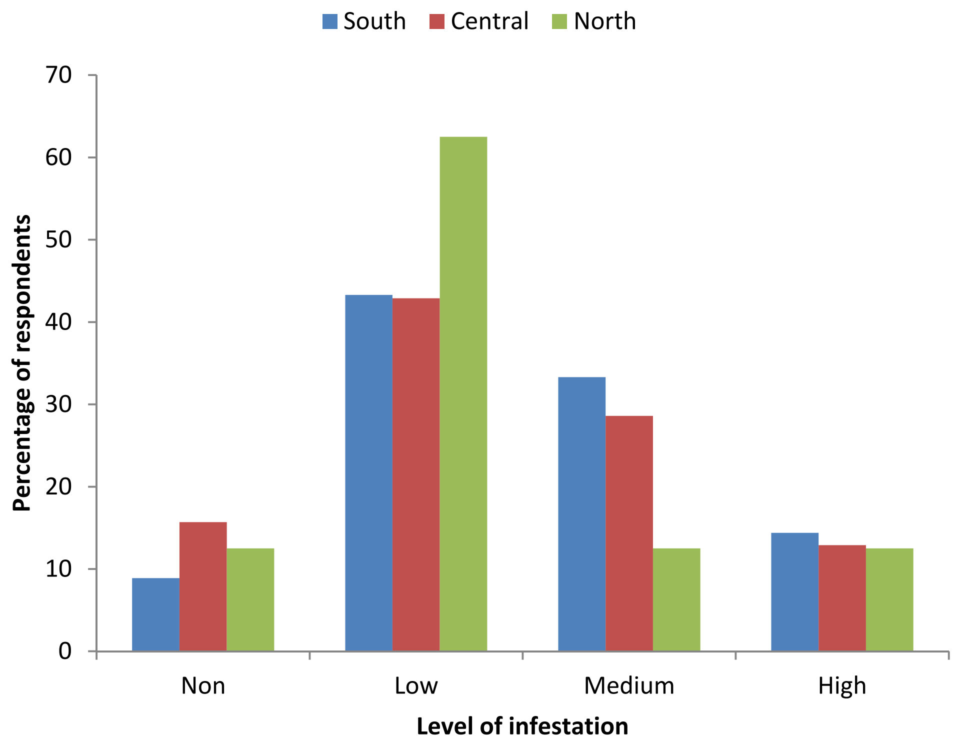



**Fig. 3.9 Levels of insect infestation (damage) in the three regions of the study area.** (N=240, 80 farmers per region). There was a significant difference between the regions in level of damage by insect pests ($\chi^2$ =16.6847, df=6, p<0.01).

### 3.3.6 Farmers' perspective on the effects of seasons during the storage period on pest infestations

Kebbi state experiences three seasons: the rainy season (May to November), the dry season (March to May) and the *Harmattan* (windy) season (December to March), during all of which farmers store their grain. The results in Table 3.8 show that the majority of respondents (60%) reported that they had relatively high numbers of insect species in their grain stores during the rainy season. A high proportion of respondents observed three types of insect species in their grain stores during the rainy season, followed by those that observed two species and four species. However, in the dry season the greatest proportion of farmers reported the presence of no insect species, followed by those reporting one species, and the least proportion reported three species. Only a very few of the respondents reported the presence of insect species during the *Harmattan* season, with the highest proportion of respondents reporting no insect species or five insect species. Overall, the relationship between the proportion of people reporting different numbers of insects species and season was statistically significant ($\chi^2$ = 28.2311, df= 10, p<0.001). High infestation during rainy season may relate with the duration of storage since rainy season occurs 7 months of storage, whereas harmattan is immediately after harvest.

**Table 3.8 Distribution of respondents according to season and number of insect species observed**.

| Percentage of respondents reporting number insect species in each season of storage | | | | |
|---|---|---|---|---|
| | Dry | Rainy | Harmattan | Total |
| No. of insect species | | | | |
| 0 | 43.8 | 12.3 | 43.8 | 100 |
| 1 | 40.4 | 56.4 | 3.2 | 100 |
| 2 | 25.0 | 75.0 | 0.0 | 100 |
| 3 | 20.8 | 77.1 | 2.1 | 100 |
| 4 | 21.7 | 73.9 | 4.3 | 100 |
| 5 | 33.0 | 50.0 | 16.0 | 100 |

There was a significant association between season of storage and number of insects species ($\chi^2$ = 28.2311, df= 10, p<0.001).

### 3.3.7 Farmers' perspectives on pest management practices

In all the surveyed regions, farmers used a variety of pest control methods, including synthetic chemical products (permethrin, cypermethrin+, aluminium phosphide and lindane), botanicals (*Ocimum basilicum*, *Erythropleum guineeses*; wood ash mixed with pepper (*Capsicum frutescens*), *Nuclea diderrichii* dried flowers from tamarind (*Tamarindus indicus*) and *Vernonia amygdalina),* while some used no insect control measures at all.

Table 3.9 shows that there was a significant difference between the regions and the control methods used ($\chi^2$= 19.7713, df=6, p<0.01). The most frequently used methods of insect control in each region were either the application of chemical pesticides (central and north regions) or nothing (south region). Although in every region >20 % of respondents used botanicals on their own or in combination with chemical pesticides. However, overall, ~ 30% of respondents used no control at all. The type of control method used varied with regions; almost half in the north and central regions used only chemicals, while in the south ~ 30% used botanicals. This may be do to availability of botanical in the south and cash to buy chemical in the north.

**Table 3.9 Percentage of all respondents within a region that used a particular form of insect control (n=240, 80 per region)**

| Percentage of farmers using each control method | | | | | |
|---|---|---|---|---|---|
| | | Control method | | | |
| | None | Chemical | Botanical | *Chm/bot | Total |
| South | 37.3 | 20.0 | 30.2 | 12.5 | 100 |
| Central | 28.8 | 45.0 | 17.5 | 8.7 | 100 |
| North | 35.0 | 43.2 | 12.0 | 10.0 | 100 |

There was a significant difference between the regions and the control methods used ($\chi^2$= 19.7713, df=6, p<0.01). *Chm/bot indicates a combination of chemicals and botanicals.

Figure 3.10 shows that among those that used botanicals in all regions, *O. basilicum* (Sweat basil) was the most widely used, with the highest number of respondents in the south, followed by central and north regions, in rank order. Wood ash mixed with pepper was used by 29% of farmers, with the highest number of respondents in the south, followed by central and the north. Only 9% of respondents used *N. diderrichii*, which was only found in the central and north regions. The other botanicals mentioned included *E. guineeses* and tamarind flower, which were both reported by 8% of respondents, and *V. amygdalina*, mentioned by 3% of respondents. However, the difference in types of botanicals used and the region was not statistically significant ($\chi^2 = 5.7289$, df = 10, p=0.837).

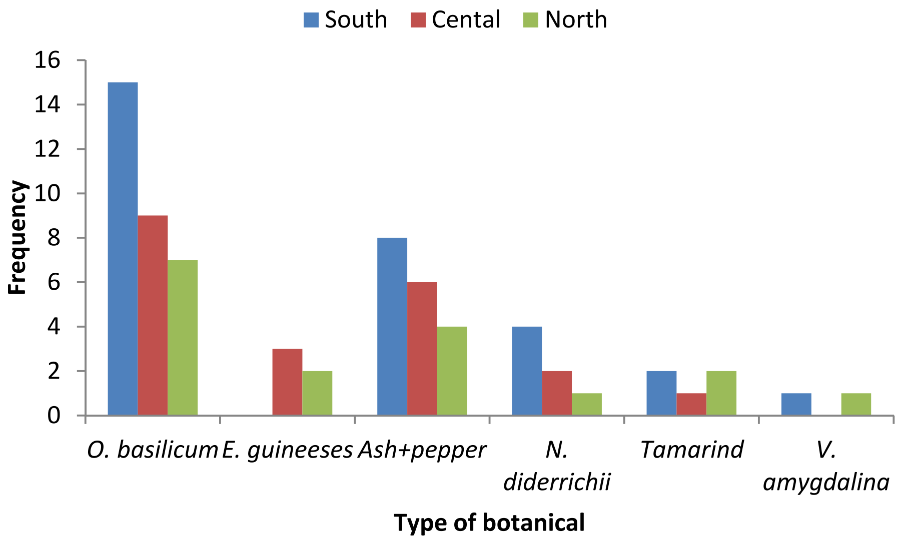



**Fig 3.10 Frequency of respondents applying specific plants for pest control in different regions.** (N=68). There was no statistical significant between types of botanicals used and the regions ($\chi^2 = 5.7289$, df = 10, p=0.837).

When farmers were asked about their views on the effectiveness of botanicals as grain protectants, there was a discrepancy in the way farmers perceived how botanicals work. A majority of respondents that used botanicals (67%) reported that botanicals are an effective method of pest control, as against 33% that reported the method was not effective.

The majority of respondents that used botanicals (75%) sun-dried the plants for 2-3 days, and then applied them to the grain by crushing just the leaves of the plants to powder with a pestle and mortar and then mixing with the grain before loading it into the store. The other 25% of respondents that sundried their botanicals, placed the whole dried plants in layers between and on top of the grain.

The results in Table 3.10 show that the type of insect species recorded in grain and levels of infestation depended on the form of insect control used. For instance, most of the respondents that reported their grain had none to low levels of infestation had used a chemical control method (~78%), followed by those using botanicals (~70%). The majority of respondents reporting medium to high infestations were those that had not used any control measures (~57%), followed by those that had used botanicals (~ 30%). Respondents reporting the lowest percent of high infestation were those that had used a combination of botanical and chemical control (8%). It is interesting to note that respondents using only chemicals or botanicals appear to belong in two groups: those with none or low infestations and those with high infestations, with a dip to only ~ 6% with medium infestations. Overall, there was a significant difference between control method applied and presence of insect infestations ($\chi^2$ = 48.689, df = 9, $p<0.001$). This suggests that the differences in infestations may have been due to differences in preventive and curative control methods used; farmers may be using the control method at the beginning of the storage season as preventive measures, however, some may treat their grain later when the infestation has manifested. However this needs to be investigated further.

**Table 3.10 Distribution of respondents according to infestation level and control method used. (n=240).**

| Percentage of farmers reporting infestation level in each control method | | | | |
|---|---|---|---|---|
| | None | Chemical | Botanical | Chm/bot |
| Infestation level | | | | |
| None | 21.4 | 27.6 | 30.3 | 20.0 |
| Low | 21.4 | 50.0 | 39.4 | 36.0 |
| Medium | 27.1 | 6.3 | 6.1 | 36.0 |
| High | 30.0 | 16.1 | 24.2 | 8.0 |
| Total | 100 | 100 | 100 | 100 |

There was a significant difference between control methods and presence of insect infestations ($\chi^2$= 48.689, df = 9, $p<0.001$).

### 3.3.8 Influence of socio-economic factors on the choice of storage structures and insect pest management methods used by farmers

Table 3.11 shows that granaries are the structure used by most respondents (≥57%) in all age categories, except for the 31-40 yrs group. The most commonly used storage structure in this group was the store-room (>62%). There was a trend for increasing use of grain stores as opposed to store-rooms with age; none of the respondents >70 yrs reported using store-rooms. Most of the respondents that used granaries were not educated or had attended only primary school (>70%), whereas, most of those that used storage-rooms had attended secondary school (>56%) or post-secondary (89%). The difference between the effect of education on choice of storage structure was found to be highly significant ($\chi^2$ = 88.356, df = 3, $p<0.001$). This indicates an association between age and education. The younger generation might be learning about storage with newer technologies from school and the opposite may be for the older generation which had a lower level of education.

**Table 3.11 Influence of age and education on choice of storage structures (n=240).**

| Percentage of respondents in each category | | |
|---|---|---|
| | Storage structure | |
| | Granary | Room |
| Age | | |
| 21-30 | 57.1 | 42.9 |
| 31-40 | 37.7 | 62.3 |
| 41-50 | 78.9 | 21.0 |
| 51-60 | 82.6 | 17.4 |
| 61-70 | 82.8 | 17.2 |
| >70 | 100 | 0 |
| Education | | |
| No formal | 72.7 | 27.3 |
| Primary | 87.5 | 12.5 |
| Secondary | 43.8 | 56.3 |
| Tertiary | 11.1 | 88.9 |

There was a significant difference between the effect of education on choice of storage structure ($\chi^2$ = 88.356, df= 3, $p<0.001$).

Table 3.12 shows that chemical control was most used by young respondents (21 – 40 yrs), whereas botanicals and botanical/chemical combinations tended to be used increasingly with age. Amongst the oldest respondents (61 yrs and older), > 70% used either botanicals or botanical/chemical combinations. Respondents that used chemicals the least (< 10%) were within the age range 51-60 yrs, and were relatively evenly divided between no treatment and some form of botanical treatments. The 41-50 yrs group of respondents reported the highest use of some form of control (>89%) and the highest use of botanicals in some form (~68%). There was a significant difference between the age of respondents and choice of control methods ($\chi^2$=65.38, df=15, p<0.001).

The results of influence of education indicate that un-educated respondents mostly store their grain un-treated or use botanicals. The majority of respondents that used chemicals (>50%) had received primary or secondary education. None of the respondents that had received tertiary education left their grain untreated or used botanicals, which suggest that they are wealthy enough to buy insecticides. There was a significant difference in influence of education on choice of control methods ($\chi^2$= 50.99, df=9, p<0.001).

**Table 3.12 Influence of age and education on choice of control method (n=240).**

| | Percentage of respondents in each category | | | |
|---|---|---|---|---|
| | None | Chemical | Botanical | Bot/chemical |
| **Age** | | | | |
| **21-30** | 35.5 | 42.9 | 14.3 | 7.4 |
| **31-40** | 45.3 | 28.1 | 14.2 | 12.4 |
| **41-50** | 10.8 | 21.6 | 48.6 | 18.9 |
| **51-60** | 46.0 | 7.9 | 32.1 | 33.3 |
| **61-70** | 3.4 | 17.2 | 24.1 | 55.1 |
| **70>** | 0.0 | 28.6 | 0.0 | 71.4 |
| **Education** | | | | |
| **No formal** | 45.2 | 11.2 | 30.2 | 13.4 |
| **Primary** | 37.2 | 51.2 | 11.6 | 0.0 |
| **Secondary** | 25.8 | 51.6 | 12.9 | 9.7 |
| **Tertiary** | 0 | 100 | 0 | 0 |

There was a significant difference between the age ($\chi^2$=65.38, df=15, p<0.001) and education of respondents on the choice of control methods ($\chi^2$= 50.99, df=9, p<0.001).

## 3.4 Discussion

Use and maintenance of good storage systems is key to minimizing losses. Small-scale farmers often use traditional systems for storing their grain after harvest. The grain types and the systems used by farmers vary from place to place, depending on the local environment and cultural practices. In Kebbi state, a survey of farmers was conducted to obtain basic information from the farmers' perspective about their systems of storage, the insect pest species that cause damage and the different management practices used by the farmers. The results confirm that their stored grain is attacked and damaged by a variety of storage insect pests. The level of infestation and amount of damage caused was found to be influenced by many factors, including the grain type, storage system, length of time stored and pest management practices.

***Farmer profile:*** The majority of the respondents (97%) were male household heads, with a mean age of 42 yrs and a mean family size of ~13 persons. The majority (82%) gave their main occupation as farming, and ~90% possessed their own farmland, with ~64% obtaining seed from their previous harvest, although ~20% were wealthy enough to purchase seed from an open market. The majority of respondents (67%) gave 'home consumption' as the main reason for storing grain, and 22% gave 'trading' as the main reason.

***Profile of main stored crop and insect pests:*** Of the variety of staple grains grown and stored in all regions surveyed (sorghum, millet and maize), sorghum was found to be the staple crop grown and stored most abundantly (58.2% total grain grown by weight). Although farmers perceived sorghum as the grain that suffers the greatest proportion of losses to insect pests, mainly *T. castaneum*, other problems included termites, mold and theft. *Tribolium castaneum* is a serious pest commonly found in Nigeria attacking most stored cereals (Chimoya & Abdullahi, 2011). This species thrives in the study area probably because it is well adapted to the weather conditions of the area during the storage season, i.e., temperatures 22-32$^{o}$C (Anuforom, 2010; Dobie *et al*., 1991). Other factors that favour *T. castaneum* include availability of a wide variety of their favoured host food and ability to penetrate into storage material.

The survey results indicate that the proportion of loss caused by storage pests is related to the system of storage practiced. For instance, the length of time grain is dried before storage affects levels of pest infestation during storage. Lower levels of infestation were reported by the majority of respondents who dried their grain for at least 8 days before storage, although this seems to be influenced also by differences in weather conditions between the regions and the moisture content of grain at harvest. Boxall *et al*. (2002) reported that the length of drying time for safe storage depends on the moisture condition of the grain at harvest and weather conditions in a location during the drying period. Farmers drying for only 4 days reported the highest infestations. It could be these farmers harvest their grain when the moisture content is too high for it to dry properly in 4 days. High moisture content supports rapid insect development. The weather conditions in the north are generally more sunny and windy compared to the south, so farmers in the north may have the advantage of quick drying outdoors. Whereas, in the south farmers have to dry their grain for 10 days to ensure low levels of infestation. This indicates that proper grain drying before storage is important for successful and long-lasting storage.

Although a large proportion of respondents overall (56%) stored their grain un-threshed, only 31% did so in the south region, in spite of the evidence that unthreshed grain had lower infestation levels. Further investigation is needed to discover why so many farmers store their grain in the threshed state. The observation that grain stored in an un-threshed form is less susceptible to insect attack is supported by Gwinner *et al*. (1990) and FAO (1992). However, this depends on the insect species and the host grain (Lawrence & Perdesen, 1990). Since threshed grain is more susceptible to pest attack, proper treatment with effective control measures may help. It is interesting to note that a minority of the farmers reported that grain stored in the threshed form should be less vulnerable to pest infestations in spite of the evidence to the contrary.

The survey findings indicate that farmers stored their grain 4 months - 1 year, with the majority storing their grain for 7 months. Levels of insect infestation were reported to be significantly higher in grain stored for 7-10 months compared to that stored for 4 months. Several factors could contribute to this pattern of infestation. First, it takes time for insect populations to grow. Seven months of storage is long enough for an insect population to build-up, particularly with grain that is poorly treated against infestations or not treated at all. Also, the peak in infestations coincides with the rainy season, which reaches its peak in July and August. Gwinner *et al.* (1990) reported that a combination of high temperature, relative humidity and moisture content provides favourable conditions for insect development. The survey also found that most farmers tend to sell their grain stores after about 7 months, possibly to avoid further losses to insect damage. The findings of this survey suggest that farmers need to be supported with improved storage methods so that they can extend the duration of storage without further loss of grain. It could be that the methods of preparing grain for storage, the products used to deter/kill insects and the storage structures themselves cannot protect grain adequately during longer periods of storage. A good combination of products developed from local plant materials to control insects could increase the duration of storage.

Despite the fact that farmers used different storage structures in the different regions, insect infestations were found in all the types of structures used. Grain stored in store-rooms had the highest levels of infestation. This indicates that, despite farmers' additional efforts to protect their grain by putting the majority of it in bags in store-rooms, insect pests still manage to infest their grain. Boxall *et al*. (2002) reported that successful grain storage depends on the type of structure used, how the grain is handled and how well the grain is protected against environmental factors. It was observed that, unlike a granary, the store-rooms used by the respondents were usually a domestic room, with one or more windows, which were often observed to be open to the outdoors. When farmers use domestic rooms to store their grain, it is difficult to prevent insects and other storage pests from coming into the store-room. Bag storage provides a little extra protection against pest attack, and with good management bags can provide long term safe storage (Gwinner *et al*., 1990; Boxall *et al*., 2002). Thus, improvements to the main storage structure used by farmers, and using low cost inputs, i.e., locally-available repellent plant materials, could help improve farmers' food security. Koona *et al*. (2007) confirmed that bags impregnated with plant extracts from *Chenopodium ambrosioides* and *Lantana camara* reduces legume damage by the insect pests *Acanthoscelides obtectus* and *Callosobruchus maculatus*. This method is simple and cost effective, particularly if the materials are locally available and inexpensive. The evidence from the survey in Kebbi suggests that there is an ideal opportunity to research the potential for improving the efficacy of polypropylene bags to store grain by treating them with locally available repellent plant material as grain protectants against the most important pest, *T. castaneum*.

The majority of farmers responded to insect pests mainly by applying chemical pesticides, however, some farmers used no control and a minority used botanicals. Chemical pesticides were used by more farmers in the north than in the other regions and are the most popular method of pest control overall, but especially by young respondents (21-40 yrs). Most farmers rely on pesticides probably because they give quick action as compared to other methods (Gwinner *et al*., 1990). The major worldwide concerns about the application of chemical control, however, are the potential environmental, social and health effects (Yang *et al*., 2005; Xue *et al*., 2006), which necessitates the search for alternative sources. Plant materials such as *O. basilicum*, wood ash mixed with pepper, *E. guineeses*, *N. diderrichii*, tamarind flower and *V. amygdalina* are the plant materials most used by the farmers as grain protectants, particularly in the southern region. However, it was apparent that farmers in the surveyed area did not have a standard method for preparing and applying repellent plant material to their grain stores, which could explain why botanicals did not appear to be very effective in reducing insect infestations. This could also be the reason for disagreements among the farmers as to whether botanicals are effective or not. Belmain *et al*. (2001) reported that the efficacy of plant materials depend on the pest species, the environmental location of the grain stores, the plant species and parts of the plant used and the method of preparation and application used. Hence, an investigation of the optimal method of preparation and application of locally available materials is necessary in order to establish more promising and standard methods.

Generally, the survey focused on the household, including both men and women, however very little information was collected from the female heads of household (only seven), which is not enough to give baseline information about the perception of women regarding storage practices in the survey area. However, of the few female heads of household involved in the initial survey, most were in the north region, where generally untreated grain is stored in granaries, except in the case of two women, who used botanicals. Due to the small amount of information available regarding the perceptions of women, few conclusions can be made, but this will be investigated further in the next phase of the research project. The survey results also show that adults (41-50 yrs) and the uneducated are already using the traditional approach to pest control of storing grain in bags treated with botanicals more than the other age groups, indicating that this group might be most motivated to participate in a proof-of-concept field experiment, to test new methods of pest control.

In summary, the results presented here provide the farmers' accounts of the methods of storage and insect pest management practiced throughout all three geo-political regions of Kebbi state. Sorghum was reported to be the grain that suffers the greatest percentage loss to insect pests, mostly *T. castaneum*. The approach used in the northern region stood out against the others for being significantly more successful at controlling insect pest infestations; the majority of farmers in this area dried their grain for 8 days before storage, used granaries, stored more of their grain in an un-threshed form and used chemical control more than any other control method. Overall, 49% of respondents in the three regions reported low, reasonably acceptable levels of infestations in their grain stores; however, in the north 62.5% reported low levels of infestation, compared to only 43.3% in the south. Therefore, farmers in the south are most in need of new methods to reduce insect damage to their stored grain.

The majority of respondents in the south dried their grain for 10 days before storage, stored their grain in store-rooms, in the threshed form and they used botanical control more than any other control method. However, this survey found that the method of using botanicals was largely ineffective. Possible reasons for this may be that not all farmers prepared and applied the botanicals in an effective way. Therefore, there is a need to establish why farmers had such different perceptions as to how well botanicals work. Possible reasons could be; a lack of proper dosing, timing and method of application and suitability of local storage conditions. There is evidence from the survey that older are likely to be most open to the use of bags and botanical repellent to protect their grain.

# CHAPTER 4

## Assessment of grain loss during storage for small-scale farmers due to insect pests

### 4.1 Introduction

Storage losses due to insect pest infestations have been a problem of major concern among small-holder farmers who use traditional storage structures (Gwinner *et al*., 1990; Mendalis *et al*., 2007). Inspection, sampling and monitoring of grain stores provide baseline information that is useful in identifying and managing problems associated with grain storage, particularly insect pest infestations and are important for helping farmers to verify or correct their own perceptions, which can be biased by beliefs and subjective impressions, as described in Chapter 3. Information generated from standardised techniques can be useful in evaluating the relative importance of loss-causing factors such as temperature, moisture content of grains the presence of insect pest species, grain variety and the effect of storage structures.

Damage to stored grain is easiest to categorise by visual observation of the relative amount of damage to the grain and by measuring the amount of weight loss (Anonymous, 1978). Thus, simple and standardised methods of loss assessment that are rapid and can be easily understood and conducted by farmers are important. Farmers need to be able to identify grain damage in their stores reasonably accurately and estimate the likely economic value associated with each level of grain damage. Understanding this 'damage - economic value' link could help farmers to better understand what quality of grain is required by the market in order to improve their income. The visual damage scale (VDS) method, whereby farmers are asked to assess the status of their grain against an annotated photograph of typical loss categories (described in more detail below) has been found to be simple, rapid, easy to work with and relatively standardized, producing outcomes similar to the more conventional 'count and weigh' weight loss method (Compton & Sherington, 1999). The VDS method has already been established for maize cobs (Compton & Sherington, 1999) and pearl millet (Hodges, 2005), but not sorghum, threshed maize or un-threshed millet. Therefore, for this project, new visual damage scale (VDS) charts were produced for these grains, as described below.

### 4.1.1 Aims

*To obtain baseline data on the insect pest species and associated weight loss they cause in stored grain amongst farmers in the study area.

* To establish a rapid method of assessing the degree of damage to grain and proportional loss for sorghum, millet and threshed maize.

* To compare the precision of the visual damage scale (VDS) assessment method with the conventional weight loss method.

* To help farmers gain an understanding of the process and importance of grain damage assessment through participatory research, so that they can classify degree of grain damage and loss for themselves.

* To identify the target farmer group for the field trial, i.e., the farmers most in need of help with insect control.

## 4.2 Materials and Methods

### 4.2.1 Store sampling

Samples of grain were collected from 150 farmers' stores (50 each for Kebbi central, north and south) of either sorghum, millet or maize from July to October 2008 in a single visit to determine the insect pest species present and to estimate the degree of damage and the proportional weight loss due to insect pests. The store samples were obtained from the farmers that had completed the questionnaire survey presented in Chapter 3. The stores of at least five farmers were chosen for evaluation out of the ten farmers in each village that completed the questionnaire. The sampling was done the same way in all the stores of farmers chosen.

**Fig. 4.1 Local granary storage structures (rhombus) in the survey area**; window type left top and windowless right top.

### 4.2.2 Estimating the proportion of grain loss due to damage caused by insects

***Sampling grain in stores:*** The percentage damage caused by the insect pest species was estimated from samples of grain collected from the farmers' stores, using 50cm, and 1m compartmentalized sample spears (Fig. 4.2 and 4.3). The 50cm spear was used to collect 1kg samples from grain stored in bags, and weight loss was calculated from 100g sub-samples by the 'count and weigh' method. This method involves the removal of damaged grain from undamaged in a 100g sample and then counting and weighing each fraction (see Fig.4.3 below for example). These values are then substituted in the equation below to calculate the percentage weight loss (Adams &Schulten, 1978). The spear was pushed into a bag of grain until at least 2/3 of its length was in the bag. The spear was effectively a long tube, with one side of the tube cut away, so that as the spear is pulled out of the bag, if the opening faces downward the grain in the tube will fall into the collecting container.

$$\% \text{ weight loss} = \frac{(U \times Nu) - (D \times Nd)}{U\,(Nd + Nu)} \times 100$$

Where, U = weight of undamaged

D = weight of damaged

Nu = Number of undamaged

Nd = Number of damaged (Adams &Schulten, 1978)

The 1m sample spear was used to collect grain samples stored loose in granaries and store-rooms. Each sample was taken by inserting the spear into the grain mass straight to the maximum depth, and then rotating the inner tube to ensure that the open compartments filled up with grain, and then rotated back to close the compartments. The spear was withdrawn and the samples were emptied, on a cloth spread on the ground (Fig. 4.3). An assessment of each pile of grain was done by visual analysis (see below). Five samples were taken using the 1m spear from different portions of the stored grain mass. The samples were pooled for final assessment and measuring weight loss.

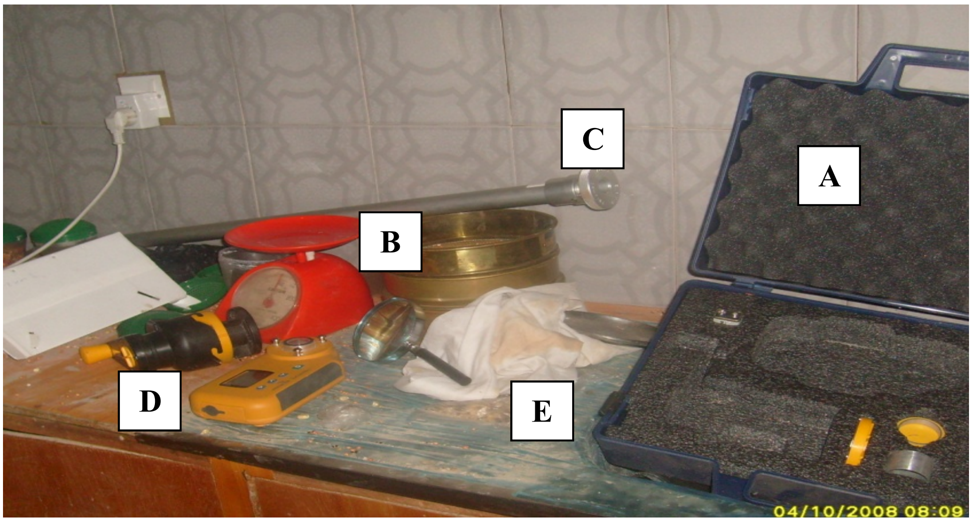



**Fig 4.2 Photograph of the equipment used during grain sampling for weight loss.** A) Case for temperature and humidity probes, B) sieve to retrieve insects from grain samples, C) sampling spear, D) grain meter and E) white cloth on which to lay out grain samples.

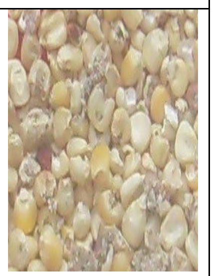
**Fig. 4.3 Details of estimating grain weight loss.** Left: spear samples; Right: volunteers assisting in separating damaged from undamaged grain samples from farmer's stores for weight loss analysis.

***'Count and weigh' method to assess grain damage based on depths:*** To assess grain damage based on depth (layers) in stores, a 1 m compartmentalized spear was used to collect samples of threshed grain (sorghum and maize) from 44 store-rooms. The five compartments of the spear were each assigned a score for Class A-E from top down so that when spear was inserted into grain mass, the compartment at the bottom collected grain from the bottom layer (E), and the compartment at the top collected grain from layer A just under the surface layer. After five spear samples were collected, samples from the corresponding depths were pooled for final assessment and measuring weight loss. A 100g sub-sample was taken from each of the depth samples to calculate percentage weight loss by the 'count and weigh' method.

***Developing a visual damage scale (VDS) for Kebbi region:*** To help farmers learn how to assess losses in their stored grain themselves, a rapid loss assessment method (Compton *et al*., 1998; Compton & Sherington, 1999) was developed. This method involves using a standardised visual damage scale (VDS) poster (e.g., Table 4.1), which was developed for this project following the basic principles of Compton *et al*., (1998) and Compton & Sherington (1999). A scale based on five levels of damage was developed, with the first level being sound grain and each of the other levels increasing in the proportion of damaged grain.

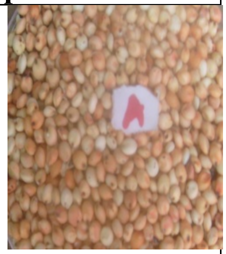
The five levels damage scale was defined in relation to the percentage of grain that showed evidence of insect damage. The range of values that were chosen to define each level was based on levels that farmers typically use to describe the relative use-value of a particular grain. For example, a sample of maize with > 85% damage would not be suitable for home consumption, but would be used as animal feed. Maize with about up to ~70% undamaged grains would be used for home consumption, depending on the financial status of the household (Tables 4.1- 4.4). The percentage categories are not the same for every grain, as discussed in more detail below. These scales are subjective to some degree, but it has been shown that the system is effective because it reflects the basis by which farmers usually assess the potential food or market value of their grain.

**Table 4.1 Damage classes for visual assessment of threshed maize**

| Class | Composition | % weight loss | Remarks on uses by farmers | Sample picture |
|---|---|---|---|---|
| A | No damaged grain. | 0 | Suitable for home consumption, sale to traders or used as seed for next season. |  |
| B | A small percentage of grains with slight damage, about 20% i.e., a few insect holes. | 1.1- 3.3 | Suitable for home consumption, sale to traders, or used as seed for next season. |  |
| C | About 50% of the grains damaged, with insect holes, frass and moth webs present. | 4.5- 7.8 | Damaged grains can be removed and fed to animals. Undamaged grain can be mixed with A or B and consumed or sold. |  |
| D | About 70% of the grains damaged, with insect holes, frass and moth webs present. Change in colour. | 8.2- 15.0 | Depending on the financial status of the household, the undamaged grain can be removed and the rest used for home consumption, not good for selling in the market. |  |
| E | About 85% of the grains damaged, with insect holes, frass and moth webs present. Change in colour. | 17.1- 26.3 | Not suitable for home consumption, rather fed to fowl and other domestic animals. |  |

A clearer picture of this can be found in Appendix 5.1

**Table 4.2 Damage classes for visual assessment of threshed sorghum**

| Class | Composition | % weight loss | Remarks on uses by farmers | Sample picture |
|---|---|---|---|---|
| A | No damaged grain. | 0 | Suitable for home consumption, sale to traders or use as seed for next season. |  |
| B | A small percentage of grains with slight damage about 20%, i.e., a few insect holes. | 1.5-5.1 | Suitable for home consumption, sale to traders or use as seed for next season. |  |
| C | About 40% of the grains damaged, with insect holes, frass and moth webs present. | 6.7-11.4 | Damaged grains can be removed and fed to animals. Undamaged grain can be mixed with A or B and consumed or sold. |  |
| D | About 60% of the grains damaged, with insect holes, frass and moth webs present. | 13.2-22.2 | Depending on the financial status of the household, undamaged grains can be removed and the rest used for home consumption, not good for sale, but with careful grading can be sold at low price. |  |
| E | About 85% of the grains damaged with insect holes, frass and moth webs present. | 24.7. 40.2 | Not suitable for home consumption, rather fed to fowl and other domestic animals. |  |

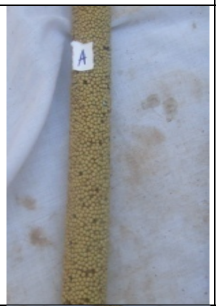
A clear picture of this can be found in the appendix 5.2

**Table 4.3 Damage classes for visual assessment of un-threshed sorghum**

| Class | Description | Standard deviation weight loss (%) | Remarks on uses by farmers | Sample picture |
|---|---|---|---|---|
| A | No damaged grains. | 0 | Thresh and use for home consumption, sale to trader, or as seed for next crop season. |  |
| B | Very few damaged grains. | 1.6-3.5 | Thresh and use for home consumption, sale to trader, or as seed for next crop season. |  |
| C | Less than 50% grains damaged and moth webs present. Few grains are destroyed or missing. | 4.7.-10.1 | Threshed and sale to traders at low price. Can still be used for home consumption or sale to traders after the damaged one are removed. |  |
| D | More than 50% of grains damaged or contaminated with moth webs and other debris. | 13.2- 23.3 | Not good for home consumption, remove moth webbing and tie into bales of about 70-80 sorghum heads and sale to livestock owners. |  |

A clear picture of this can be found in the appendix 5.3

**Table 4.4 Damage classes for visual assessment for un-threshed millet**

| **Class** | **Description** | **Standard deviation weight loss (%)** | **Remarks by the farmers** | **Sample picture** |
|---|---|---|---|---|
| A | No damaged grains. | 0 | Thresh and use for home consumption or sale to traders. Can be used as seed for next crop season. |  |
| B | Very few grains damaged about 30% damaged grain. | 3.4-10.2 | Thresh and use for home consumption or sell to traders. Can be used as seed for next crop season or sold. |  |
| C | 50% grains damaged and moth webs present. Few grains are destroyed or missing. | 11.6- 20 | Thresh and sell to traders at low price. With damaged grains removed, can be used for home consumption or sale. |  |
| D | More than 50% of grains damaged or contaminated, with moth webs and other debris. | 22.4- 28.4 | Not suitable for home consumption, rather fed to livestock. |  |

A clear picture of this can be found in the appendix 5.4

Once the percentage ranges for the five most significant levels of damage for each type of grain had been established, 'standardised' samples were produced representing the mean percentage damaged grains for each level by mixing the appropriate proportions of damaged and undamaged grains. Photographs of the standard samples were used to produce VDS charts for each type of grain representing the typical range of damage experienced in farm stores for each type of grain, i.e., Class A-E (Table 4.1-4.4, and Appendices 5.1-5.4). The mean percent weight loss associated with each damage level of grain was determined by the 'count and weigh' method (Tables 4.5) and added to the VDS charts as a second measure by which to score the class of a sample (Tables 4.1-4.4).

**Table 4.5 Weight loss of maize, sorghum and millet calibrated in the laboratory to include intermediate damage classes by their calculated weight loss.**

| | **Mean percent weight loss expected in 100 g samples** | | | |
|---|---|---|---|---|
| **Class** | **Threshed maize** | **Threshed sorghum** | **Un-threshed sorghum** | **Un-threshed millet** |
| A | 0 | 0 | 0 | 0 |
| AB | 1.2 | 1.6 | 1.6 | 3.3 |
| B | 2.4 | 3.2 | 3.1 | 6.6 |
| BC | 4.0 | 5.2 | 4.2 | 15.4 |
| C | 5.7 | 7.2 | 5.4 | 17.8 |
| CD | 9.4 | 11.7 | 12.3 | 21.1 |
| D | 13.2 | 16.3 | 19.2 | 24.4 |
| DE | 17.3 | 23.6 | | |
| E | 21.5 | 31.9 | | |

Similarly, the same visual damage method was followed for sorghum and millet heads (un-threshed). The visual damage scale was prepared by initially collecting samples from different farm stores and classifying the range of damage into four VDS grades A-D as they occurred in the farmers' stores, based on a sample of ~ 10 sorghum/millet heads for each damage grade (Tables 4.1 & 4.4). However, weight loss for each VDS for these grains was determined by applying a modified version of the 'count and weigh' method of assessing weight loss to each sorghum and millet head (Compton *et al.,* 1998). The calculated mean weight loss for each grade was used as a coefficient for the number of grain heads in that grade A-D and used as a formula (see below) for assessing visual loss in sorghum/millet heads.

Standard samples representing each damage class for each grain were carried in small plastic containers along with the VDS charts to the field to help farmers assess the status of their stored grain. The famers' grain damage and respective weight loss was assessed by collecting samples from farmers' stores and each farmer was asked to compare the sample with standard samples or the VDS chart photo that was most like their stored grain. The corresponding grain damage level on the VDS chart and its weight loss was noted, and if the class score for a farmer's sample fell between two classes on the VDS chart, this was noted in the assessment record, and an intermediate class was declared (Example: AB for intermediate class between Class A and B), as shown in Table 4.5.

The percent weight loss for each farmer's sample in comparison to the weight loss of the corresponding VDS samples was calculated by collecting a 100g sub-sample of the samples drawn from the farmer's stores and analyzed with the 'count and weigh' formula. To assess the weight loss for the samples of sorghum and millet head, the number of grain heads corresponding to each class on the VDS chart was counted and multiplied by the mean percent weight loss for that class in the equation below; these values were added together and divided by the total number of samples of grain heads to give an overall estimate of weight loss.

$$\text{Visual loss for sorghum} = \frac{0 \times NA + 3.1 \times NB + 5.4 \times NC + 19.2 \times ND}{NT}$$

$$\text{Visual loss for millet} = \frac{0 \times NA + 6.6 \times NB + 17.8 \times NC + 24.4 \times ND}{NT}$$

Where:

Values shown refer to the mean percent weight loss for the class designated

NA-ND = number of grain heads in each class A-D

NT = total number of grain heads in each sample – usually 50

### 4.2.3 Insect pest species identification

As many insects as possible were extracted from the grain samples with a Brass Impact Test sieves (U.K.) (Fig. 4.2; mesh sizes: 3.35 mm, 3.0 mm and 2.0 mm). Insect samples were kept in sealed containers and taken to the laboratory at the College of Agriculture, Zuru, Kebbi, Nigeria for species identification using a light microscope (STM- 9T 16-x magnification). The insects were stored at -8$^{o}$C for 1-2 days, and observed under the microscope for species identification, using the method of Dobie *et al*. (1991).

Samples of the pest moth *P. interpunctella* were obtained by hanging sticky Delta traps (20 x 21cm base and 28 x 15cm sides, Agrisense, U.K.), baited with a pheromone lure (rubber septa) containing 0.1 mg of *P. interpunctella* pheromone blend (Z9, E12 – 14:Ac, NRI) in grain storage structures. Each trap was suspended in a central position above stored grain. Pitfall traps baited with a general beetle attractant (Agrisense, U.K.) were set in the stores, for two weeks to catch moths and beetles.

**Fig 4.4 Examples of grain stores with Delta traps (white shapes) and pitfall traps (green shapes).** Top left, room store with a Delta trap suspended in the rafters; top right granary store filled with threshed sorghum and both types of trap; bottom left shows trapped insects that were stuck to the inner surfaces of the Delta traps; and bottom right shows a granary store filled with threshed maize and a pitfall trap.

#### 4.2.4 Statistical analysis

Linear regression analysis was used to determine the correlation between the two methods of assessing weight loss (VDS and Conventional methods). Analysis of variance (ANOVA) tests were performed using statistical software package R-statistic windows, version 2.10.0 (www.r-project.org) to analyse the data for grain weight loss, and chi-square was used for insect sampling data. One way ANOVA was used to test the effect of sampling depth on percent weight loss. A three way ANOVA analysis tested for the following factors that might have a significant effect on weight loss: region (north, central, south), storage type (granary, store-room), grain type (threshed maize, threshed sorghum, unthreshed millet, unthreshed sorghum). The Tukey HSD test was used to compare the means of particular variables. Means are considered to be significantly different when $p \leq 0.05$.

## 4.3. Results

#### 4.3.1 Assessment of grain weight loss according to depths

Weight loss at different depths was estimated only for sorghum and maize stored in the threshed state. Letters A-C were used to represent the compartments of the 1m spear, which was used as a marker of grain depth in sampled stores. Letter A represents the compartment at the end of the spear that samples grain from just below the surface, followed by B down to C, with each compartment 20 cm apart. Grain sampled at different depths was assessed to estimate the percent weight loss overall due to damage by insects during storage. The results of the ANOVA for data in Fig 4.5 show that depth had a significant effect on weight loss ($F= 20.8449$, $df=2, 126$, $p<0.001$). The Turkey HSD test indicates that layer C (deepest layer) had a significantly ($p<0.05$) greater mean (± SE) percent weight loss for sorghum (10.89±0.95) and maize (8.16±1.38) than layer B for sorghum (6.61±0.95) and maize (3.81±0.98) and layer A for sorghum (7.73±1.65) and maize (5.39±1.35). However, these values do not differ between layer A and B for both sorghum ($p=0.931$) and maize ($p=0.728$), respectively.

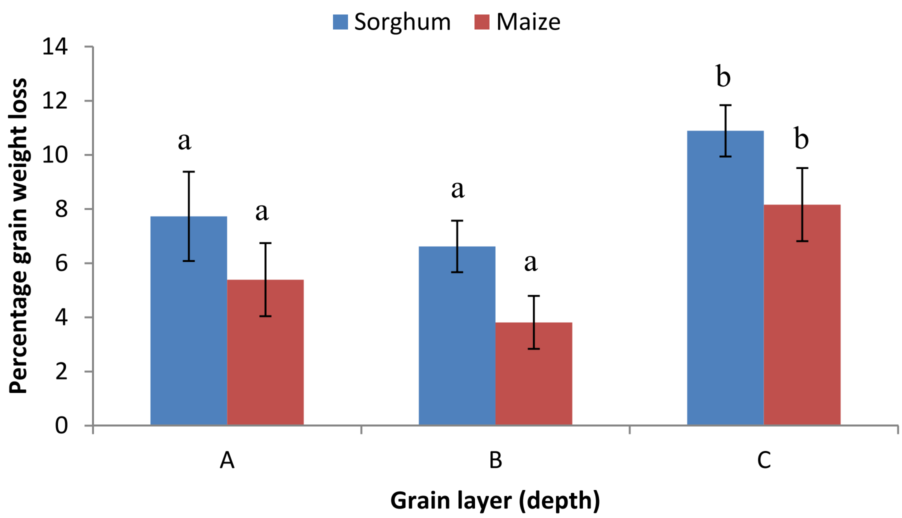



**Fig. 4.5 Comparison of the mean perecent weight loss (±SE) of samples of threshed sorghum and maize at different depths in the grain store-rooms.** (N=44). A = surface, B = midpoint, C = lowest level, each separated by 20 cm. Overall, depth had significant effect on weight loss (ANOVA, $p<0.001$). Bars with different letters are significantly different (Tukey HSD, $p<0.05$).

### 4.3.2 Insect species found in different stored grains

In the grain samples used to assess weight loss, seven insect species were identified in sorghum, maize and millet (threshed and unthreshed data pooled for each grain type, and method of storage pooled for each grain type). Four species were identified as the coleopteran beetles *Tribolium castaneum, Rhyzopertha dominica, Sitophilus zeamais and Lasioderma serricorne*, and the remaining three were lepidopteran moth species (*Plodia interpunctella*, *Sitotroga cerealella* and *Corcyra cephalonica*). In total, 3,707 insects were collected, including *T. castaneum,* which was the most common species (n = 969), with the highest mean number found in sorghum (~11/store), followed in rank order by maize (~9/store) and millet (4/store; Fig 4.6). The species found in the second highest number was *R. dominica* (547), which was found in the greatest abundance in sorghum (~6/store), followed by maize (5/store) and millet (~3/store). A total of 302 *S. zeamais* were recorded, with the highest mean number in maize (~4/store), followed by sorghum (~2/store) and millet (~2). *Lasioderma serricorne* was the least found with similar number (<2) in all the grain. For the Lepidoptera, *C. cephalonica* was found in greater numbers (663) than the other two species; *P. interpunctella* (581) and *S. cerealella* (584), with the highest mean number in sorghum (7/store), followed by maize (5/store) and millet (~4). Amongst all the types of grain, millet had the lowest number of insect species. There was a statistically significant difference in the number of insect species found in the different types of grain ($\chi^2 = 230.62$, df =12, p=0.003).

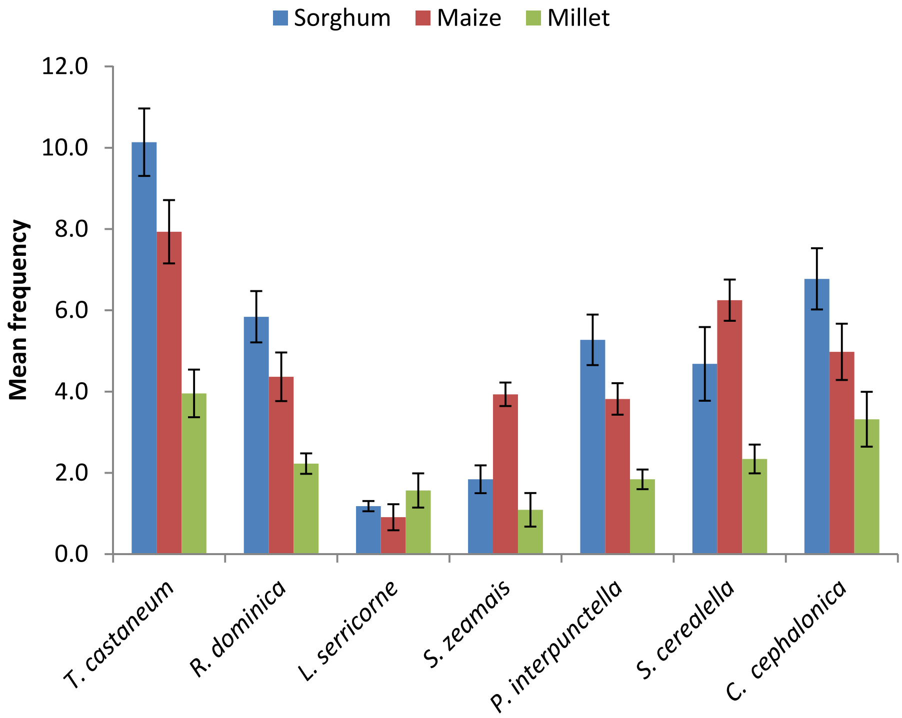



**Fig. 4.6 Mean frequency (±SE) of insect species found in different types of grain stored by farmers in the survey area.** The number of individual insects of each species found in each type of grain differed significantly (3x7 chi-square test; p= 0.003).

### 4.3.3 Comparison of VDS and the conventional method of assessing weight loss

Weight loss measured by the count and weigh method and the VDS method were calculated for maize, sorghum and millet in order to compare their precision. The VDS method was highly correlated with the count and weigh method ($r^2 = 0.756$), i.e., there was a positive linear relationship (t=14.19, df=147, p<0.001, Fig. 4.7), which suggests that data obtained by the quicker VDS method is a reasonable alternative to the more time consuming conventional method.

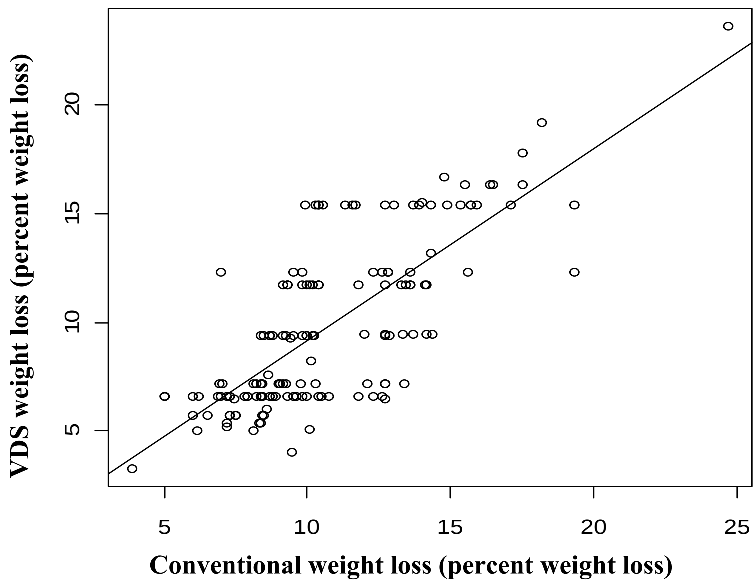



**Fig 4.7 Correlation between the conventional (count and weigh) method and the visual damage scale (VDS) method of assessing percent weight loss**. The straight line indicates a significant linear correlation ($r^2 = 0.756$, N=150, p<0.001)**.**

### 4.3.4 Assessment of grain weight loss

To estimate the percentage weight loss in different types of grain stored in different types of storage structures, grain samples were collected and assessed for weight loss using the conventional ‘count and weigh’ method. The ANOVA analysis showed that the following factors had a significant effect on weight loss: region (F=7.4861, df=2, 126, p<0.001), storage type (F=16.2781, df=1,126, p<0.001) and grain type (F=18.7144, df=3,126, p<0.001). A significant difference in mean weight loss for grain type (p<0.001) was found only between threshed sorghum (13.12±1.01) (mean ± standard error) and all other grains, i.e. threshed maize (10.36±1.01), unthreshed millet (9.52±0.91) and unthreshed sorghum (8.34±1.30), Tukey HSD. There was a significantly greater amount of weight loss (p<0.001) in store-rooms (11.17±0.91) than granaries (8.21±0.81). Irrespective of grain type, a significantly greater amount of weight loss (p<0.01) was found in the south (11.87±1.03) than in the central (9.20±0.92) or north (9.18±0.95) regions, suggesting that the latter two regions experience better grain protection than the south region.

The results in Fig.4.8 show the weight loss observed in the different grain stores (granary and store-room) of famers in Kebbi central. There was no significant effect of protection from weight loss in grain stored between store-rooms and granaries and across all types of grain within the same storage structure (Tukey HSD; p=0.957).

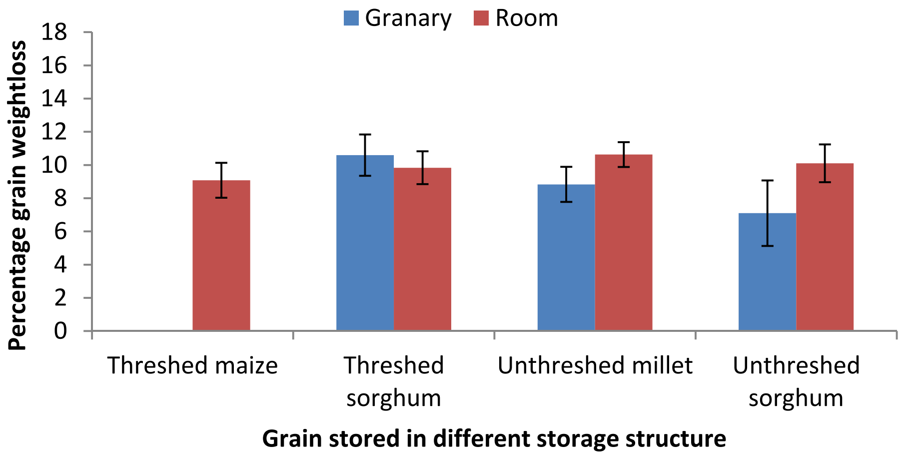



**Fig 4.8 Mean percent weight loss (±SE) observed in different types of grain stored using different storage structures for 8 months in Kebbi central (N= 50 stores).** Standard error bars obtained from the analysis of variance residuals. The ANOVA results indicate no significant difference (p=0.957) in mean weight loss between types of grain and storage structures.

The results in Fig. 4.9 show the weight loss observed in the different grain stores of famers in the Kebbi north. The results indicate that, both granaries and store-rooms provided a similar level of protection with >7% percentage weight loss across all types of grain, although <7% was found in threshed maize stored in granaries and unthreshed sorghum stored in rooms. Threshed sorghum stored in both store-rooms and granaries had the greatest weight loss (>11%). However, there was no significant difference in weight loss between store-rooms and granaries (Tukey HSD test; p=0.209).

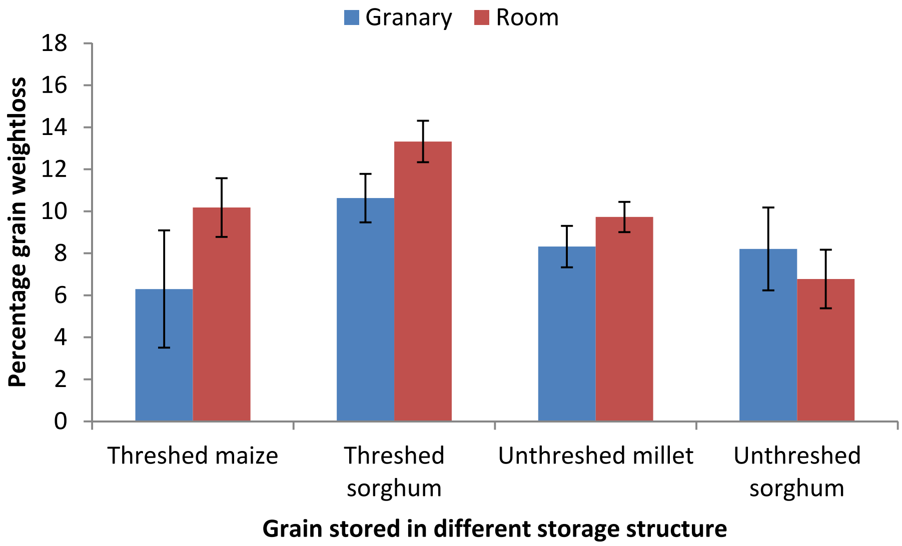



**Fig 4.9 Mean percent weight loss (±SE) observed in different types of grain stored using different storage structures for 8 months in Kebbi north.** (N= 50 stores). Standard error bars obtained from the analysis of variance residuals. ANOVA test indicates no significant difference (p=0.209) in the mean weight loss between the types of grain and types of storage structures.

The results in Fig. 4.10 show the weight loss observed in the different grain stores of famers in Kebbi south. There was a significant effect of mean weight loss between threshed sorghum stored in store-rooms (>17%) and all other grain stored in store-rooms and granaries (<12%), Tukey HSD; $p<0.05$).

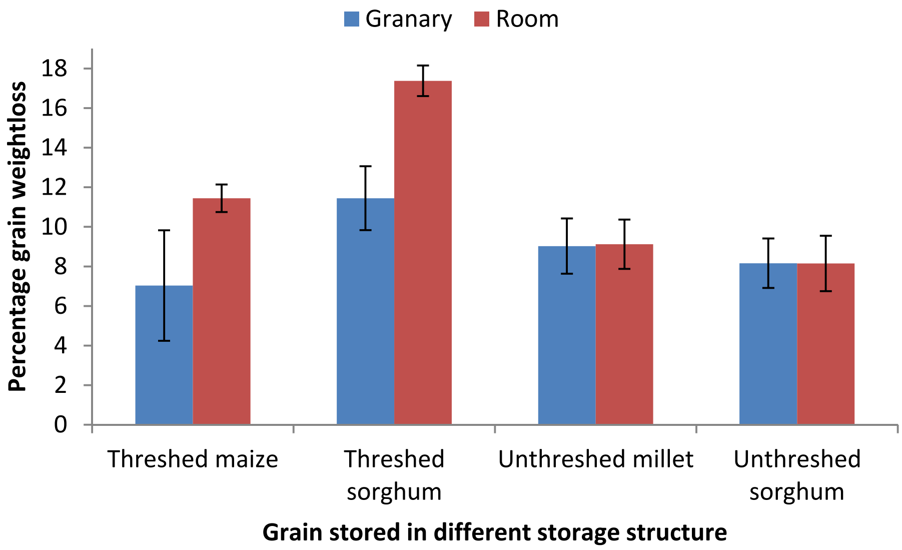



**Fig. 4.10 Mean percent weight loss (±SE) observed in different types of grain stored using different storage structures for 8 months in Kebbi south.** (N=50 stores). Standard error bars obtained from the analysis of variance residuals. ANOVA indicate significant difference ($p<0.05$) in mean weight loss between grain types and types of storage structures. The only significant difference found in the mean weight loss was between threshed sorghum in store-rooms and all other grain in store-rooms and granaries (Tukey test).

## 4.4 Discussion

Knowledge of storage conditions is a key to tackling storage problems. Accurate information about the species of insect causing the worst infestations and infestation levels in different types of farmers' grain stores can help in planning appropriate action at the right time (Loewer *et al*., 1994). The research presented in this chapter was undertaken to gain a better understanding of farmers' grain storage situations. The aim was to develop a 'user-friendly' guide to identify the problems associated with grain storage and to help plan for future improvements. Kebbi state farmers are faced with serious problems associated with grain storage insect pests (KARDA, 2004). However, in the study area the insect pest species that cause the most important problems, the grain that is attacked most seriously and the factors that contribute to these problems have not been well established. The VDS weight loss method was developed to provide a rapid method of on-farm grain loss assessment and identification of insect pests most responsible for the damage. The VDS method had already been developed for un-threshed maize at the laboratory level (Compton & Sherington, 1999), however, the results presented here demonstrate that the VDS method can be used by farmers themselves to obtain accurate assessments of grain loss due to insect infestations in their locality, i.e., for the crops stored most commonly in their area and the insect pests most responsible for their stored crop losses.

The findings of this research indicates that the VDS method is comparable with the conventional method of assessing weight loss and can be used as a rapid method of assessing the degree of damage to grain and proportional loss of sorghum, millet and threshed maize. More importantly the VDS method can help farmers gain an understanding of the process and importance of grain damage so they can classify degrees of grain damage and loss for themselves. Although the method might be open to some degree of error, as it is possible to under-estimate percent damage and weight loss for some insect species, particularly those for which the larvae bore into the grains (Gwinner *et al*., 1990; Cronholm *et al* 1998), and therefore the damage to grains might not manifest during the time of assessments. However, as the name implies, VDS provides a practical and reasonably accurate assessment of grain based on a quick and simple visual observation.

The VDS method presented to farmers included the most common locally stored grains (sorghum, millet and maize), categorised into grades of damage from the 'no damage' to 'most damage', based on ranges of percent weight loss that had been determined previously as standards. The results and information obtained from famers' use of VDS indicates that it would be a useful way to involve farmers in assessing grain infestation and insect pest damage. For instance, during the survey, after a short argument among them, participating farmers were able to agree on the economic value and use of each grade in their locality. They determined that for the stored threshed maize to be marketed, it must have < 50% damaged grains (5.7% weight loss), for sorghum <40% damaged grains (7.2% weigh loss) and for unthreshed millet <50 damaged grains (17.8% weight loss). They also concluded that grain containing even higher percentages of damaged grains could be sold in the market if the damaged grain could be removed and mixed with sound grain. However, when the percentage damaged grains was ~70% for maize, ~60% for sorghum and > 50% for millet farmers felt their only option was to remove damaged grains to be used as feed for the animals and use the undamaged grains for home consumption. The main problem with grain containing high levels of damaged grains is the high content of insect frass and debris. Dobie *et al*. (1990) reported that grain that is heavily infested by insects usually has a strong smell of insect and debris, which discourages potential buyers. The market has a limited tolerance to the quality of grain it can accept, beyond which farmers have no option but to mix some of the high quality grain with the low quality grain and re-grade the combination, or if the grain is highly damaged, use it to feed animals, which is a great economic loss to the household. The information obtained from local farmers about their assessment of the potential use and value of each VDS Class in their locality was added to the VDS chart (Table 4.2 – 4.5), which will provide a useful reference to help all the farmers in the area check the status of their stored grain throughout the storage season and help them make decisions about how and when to improve their grain storage practices.

It is hoped that the VDS can be made available to local farmers throughout the area, since it offers them a quick way of assessing the degree of damage in their grain stores and the associated likely uses and value of their grain in their locality. The VDS chart is easy for farmers to adopt, and they can estimate weight loss in their own stores relatively easily, without much technical experience or specialised equipment, and no calculations are required, which is not the case for the more elaborate and precise methods, such as 'count and weigh' (Harris & Lindblad, 1978).

In the Kebbi study area, information on the most damaging insect species and the type of grain most damaged was not well established until now. Therefore, a part of this study was carried out to obtain baseline data on the insect pest species and associated weight loss they cause in the different storage structures used by farmers. Pest insects are known to move throughout a grain store, due to variation in environmental factors, such as temperature and moisture content of the grain and biological factors, such as the need for insects to continually search out new habitat as their populations expand (Dobie *et al*., 1991; Gwinner *et al*., 1990; Hagstrum, 2000).

Therefore, to gain accurate information about the levels of infestation in a grain store, samples must be collected from a range of depths and across the extent of the store. Data on the position and movement of insects in stores provides information of practical importance for pest management. Accurate information about insect densities and their position are needed to ensure the appropriate control measures are taken.

The finding of the assessment of grain weight loss at different depths indicates that insect infestations and weight loss are greatest in the layer nearest the ground for sorghum and maize. This may relate to responses of insects to environmental factors in the store-room. The layer closest to the floor could provide the most suitable place for insects to oviposit eggs. Jian *et al.* (2006) reported that in many store-rooms downward movement (geotaxis) brings beetles near to the floor, where they can explore a more constant habitat. Hagstrum & Milliken (1989); Dobie *et al*. (1990) and Hagstrum & Flinn (2011) reported that *T. castaneum* and *R. dominica* grow better in warmer and drier regions of a storage space, hence they move to the regions where the temperature is most suitable for them. Given that the survey was undertaken during the peak rainy season (July- August), the bottom layer could be warmer than the upper layers. Moreover, since domestic store-rooms are frequently visited by people living in the dwelling to collect grain and other items, beetles may stay in areas of least disturbance, which would be as deep as they can get in the grain.

The main purpose of the insect survey in the study presented here, however, was to identify the combination of 'most damaging insect species' and the 'type of grain most damaged' for the purposes of the field experiments to develop a new method for controlling stored grain pests. The most commonly found insects, in descending rank order, were *T. castaneum, R. dominica*, *C. cephalonica*, *P. interpunctella*, *S. cerealella* and *L. serricorne.* It is not surprising that sorghum suffered the most weight loss of the crops assessed, as it had the highest number of pest species, and the highest number of insects of each species, mainly *T. castaneum, R. dominica* and *C. cephalonica.* These findings are consistent with the reports of FAO (1992) and Odogola (1994) that the level of damage to stored grain due to insects depends on the type and population size of pests in the grain, as well as the influence of environmental factors that support their development.

The analysis of grain weight loss due to insect damage for the different types of grain stored in the different regions indicated that sorghum stored in the threshed state had more weight loss than sorghum stored in the unthreshed form. The effect of weight loss varied in threshed sorghum kept in store-rooms in the south and all other types of grain stored in both granaries and store-rooms in all regions. This suggests that insects prefer certain types of grain, storage structure and even regions more than other. Granaries are used by many small-holder farmers in many parts of Africa, including Nigeria (Udoh *et al*., 2000; Bett & Nguyo, 2007). However, in the study presented here, it was found that many farmers store their grain in store-rooms associated with the main dwelling, either in bags or just loose in the store-rooms. Similar to the findings reported by Bett & Nguyo (2007), it was found that few famers use living rooms as the storage facility. Considering the nature and the type of room used by farmers for grain storage in the surveyed area, it is not surprising that grain stored loose or in bags in store-rooms had more weight loss than grain stored in granaries. Some granaries that are common in the study area are sealed, with only one access on the roof or a small window on the side. This is contrary to the conditions of store-rooms found in the surveyed area, which have many openings, such as small doors by which to enter the room and sometimes windows. Items other than grain are often stored alongside the grain, which increases the movement of people in an out of the room, and hence, there is a greater chance of grain loss due to insects, moisture and pathogens.

This phase of the study was intended to be a quick survey to determine which type of grain in which region was most in need of improvements to insect pest control. The analysis of damage levels and weight loss has shown that most of the grain observed was damaged above the level accepted for sale in the market. The findings presented in Chapter 3 (Table 3.3) support the findings of this chapter, that sorghum is the major grain stored in the southern region, that it is stored mostly in bags or loose in store-rooms, and therefore the farmers in Kebbi south suffer the most from grain loss, and need the most help to improve their methods of grain storage and protection, especially against *T. castaneum*.

# CHAPTER 5

## Development of a new laboratory-based bioassay to identify new grain protectants

### 5.1 Background

The first survey of small-scale farmers conducted in Kebbi (Chapter 3) indicted that a greater quantity of sorghum was stored than any of the other grains in all three regions investigated (Fig. 3.3). The method of grain storage had a significant effect on the number of pest species attacking grain (Chapter 3.4.5), with the greatest proportion of respondents (54.4%) reporting three or more species of insect attacking grain kept in bags in store-rooms, and *T. castaneum* was the most commonly identified pest. The results presented in Chapters 3 and 4 indicate that farmers that use polypropylene bags to store their grain in store-rooms were more vulnerable to infestation by *T. castaneum* than farmers that use granaries, and hence, their need for supplemental intervention became the focus of the rest of the study.

There are several possible reasons why Kebbi farmers have problems with the efficacy of their botanical grain protectant treatments, such as differences in the efficacy of the species or variety of plants they use, incorrect dosage or poor method of application and suitability of the storage site used. Therefore, the system chosen to identify an improved method for protecting grain from stored crop pests was *T. castaneum* on sorghum stored in bags in store-rooms, using botanical repellents.

It is possible that storing grain in double bags can reduce infestations by acting as a physical barrier to insect movement. However, based on the previous survey it was found that most small-scale farmers cannot afford to store all their grain in double bags, but if farmers could store a proportion of their grain in a way that would provide optimum protection from insect damage, this grain could be sold in the market, thereby justifying the cost of a few extra bags, and perhaps make a profit. A relatively cheap way of improving the protectant qualities of double bags further could be to add repellent plant material to the double bags. The first step in the process of identifying possible improvements to *T. castaneum* control of sorghum stored in bags was to use an appropriate bioassay to test botanical repellent improvements. Therefore, this chapter presents research carried out to test the response of *T. castaneum* to grain in bags treated with repellent plant materials, using laboratory-based bioassays to identify the most repellent plants and the optimal doses.

Typical bioassays test repellency by giving insects a choice of grain that is treated with a candidate repellent and untreated grain, and compare the proportions of insects found in each of the choices after a certain amount of time. During preliminary trials with different types of bioassays, it was noticed that a high proportion of beetles did not ever get to either the treatment or the control grain, but kept moving around the edges of the bioassay chamber. Therefore, it was decided to experiment with a novel bioassay design, based on the known responses of *T. castaneum* to environmental stimuli. This chapter presents research carried out to develop a bioassay that is more efficient (maximum proportion of insects move to either treatment or control grain) and effective (minimal variability in response of insects' to choices) than standard bioassays in identifying plants and the optimal doses that are repellent to *T. castaneum*.

The importance of bioassays in determining the response of beetles to test stimuli and mechanisms involved has been highlighted in Chapter 2, section 2.5. The ability of *T. castaneum* to be stimulated and respond to volatiles from food-host (Seifelnsr *et al*., 1982; Jonfia-Essien *et al.,* 2007) and pheromone (Obeng-Ofori, 1991) have been confirmed, which have been used as an approach in bioassays to study the attractancy or repellency of some plants and their compounds to insects. However, the strength of response, which determines the effectiveness of the bioassay and the test materials, depends on the ability of the bioassay to provide the beetle with choice behaviour as expressed under natural conditions. When testing materials that are attractant or repellent in a bioassay, beetles may respond to the volatile cues carried by a moving air current by positive or negative chemotaxis (Campbell, 2012). However, there are some other environmental stimuli that stimulate other behavioural responses. For example, light can trigger positive or negative phototaxis, i.e., attraction to or away from light, respectively (Reza & Parween, 2006), movement up or down in response to gravity, i.e. geotaxis (Cox & Collins, 2002; Jiang *et al*., 2006) and thigmotaxis (Kennedy, 1986, reviewed in Chapter 2.5). All of these responses can help beetles locate a food source, or avoid contact with toxic chemicals, while keeping them in a protected environment. Ideally, bioassays should recreate the natural environment as much as possible, so that the test itself will give a result that is most similar to what will happen in the natural environment. Otherwise, a poor bioassay can interfere with the natural responses to the volatiles released by host plants and test repellents. It is well known that movement in response to contact is one of the natural behaviours of *T. castaneum* (Surtees, 1963; Yinon & Shulov, 1969; Campbell & Hasgtrum, 2002). This behaviour can affect the efficiency and effectiveness of bioassays to measure appropriately the response of beetles to volatile cue, which highlights the importance of choosing an appropriate bioassay to test the response of the pest to particular stimuli.

A wide range of bioassay methods used to assess the attractancy or repellency of chemical cues to insects are limited in their ability to measure the ‘choice’ behaviour as it would be expressed under natural conditions. For example, in a commonly used bioassay, the ’open arena,’ which tests the attraction or repellency to walking beetles of test compounds placed in the centre of a petri dish (McDonald *et al*., 1970; Duel *et al*., 2011), the beetles frequently spend most of their time walking around the perimeter of the dish, rather than moving out in the open, a behaviour that causes a great proportion of the beetles to never make a ‘choice’, i.e., they do not arrive at the control or treated material by the end of the timed assay (Duel *et al*., 2011; Olsson *et al*.,2006). This is an example of a low efficiency assay; only a limited proportion of the released insects are counted as responding to either of the two choices. Similarly, in the ’food preference chamber’ method, whereby the behaviour of a beetle is recorded as it responds to odours released from four or more chambers, with each chamber containing food that has been either untreated or treated with potential attractant or repellent substances (Loschiavo, 1952; Jang *et al*., 1982; Xie *et al*., 1995; Fields *et al*., 2001; Othira *et al*., 2009), the chambers are close to each other, and a thigmotatic insect may continue to move along the edges of the equipment rather than move across the open area to choose a chamber based on responses to odour alone. The ‘long tube olfactometer’ method, which records the behaviour of a beetle released in the middle of a tube in response to test products placed at either end of the glass tube (Boeke *et al*., 2004b), gives the beetles only a 50:50 chance of choosing one substance over the other, and having made an initial directional choice, they are less likely to move freely through the environment, but are channelled in one direction by the constraints of turning in the tube. This is an example of an assay with reduced efficacy; the outcome can be highly variable, since the choice is 50:50, and an initial random choice becomes the final choice since the insect is forced to make a choice before it has had much exposure to the alternative.

A study by Campbell & Hagstrum (2002) on the behaviour of *T. castaneum* in a bioassay arena investigated in detail this interesting thigmotactic behaviour in beetles. This behaviour can obscure the response of the beetles to the test material and hence may make conclusions as to the strength of the test compound difficult (Arthur *et al*., 2011). Therefore, it would be worthwhile to develop a new bioassay that takes thigmotaxis and the other natural responses to environmental stimuli (phototaxis and geotaxis) into account, yet still be simple and easy to use. In the study presented here, a new modified 'thigmotactic' bioassay was developed to provide a greater understanding of the behaviour of *T. castaneum* presented with candidate repellent plant materials in the presence of stimuli that may mediate the response of the beetle to treat and control grain, which are lacking in standard bioassays.

The most commonly used botanical in Kebbi, *Ocimum* species (Sweet basil, Fig. 3.9) was chosen as a candidate repellent, so that the findings would be of direct relevance to current practice. Another locally grown alternative, *Cymbopogon nardus* (Lemongrass), which is known to have repellent effects on *T. castaneum* (Olivero-Verbel *et al*., 2010) was also chosen as a potentially useful addition to the local practices in Kebbi. The importance of repellency in pest management had been highlighted in Chapter 2 (section 2.4.5.2). Since the effect of repellent plants on insects depends on the range and amount of repellent compounds they consist of (Malik & Mujtaba, 1984; Sarah *et al*., 2006; Isman, 2006; Cork *et al*., 2009), it is important to screen particular plant varieties and cultivars for their efficacy.

Since one of the overall aims was to develop an improved bioassay method, the repellents used in this part of the study were all known to be repellent to *T. castaneum* so that it was certain that the assay should demonstrate repellency. The repellents tested at the laboratory at NRI included: two types of Lemongrass (*Cymbopogon citratus* and C. *nardus*) and two control chemicals known to be highly repellent to *T.* c*astaneum;* citronella essential oils (Wong *et al*., 2005) and methyl salicylate (Thamara *et al*., 2005). The bioassay work was continued in a laboratory in Kebbi, Nigeria, to test local varieties of commonly used repellent plants; Sweet basil (*Ocimum basilicum*), Lemongrass (*C. citratus and C. nardus*), Bitter leaf (*Vanonia amygdalina*), Yellow three (*Nauclea diderrichii*) and a combination of the two most repellent plants; Lemongrass (*C. nardus*) and Sweet basil *(Ocimum basilicum)*, or 'Lem-ocimum', plants that are both grown in the area of the field experiments.

One of the underlying hypotheses of the research presented in this thesis is that the conventional method of mixing repellent botanicals with grain and storing the combination in bags, as practiced by farmers in Kebbi and described by Koona *et al.* (2007) for use against cowpea beetles, does not give optimum protection, probably due to the ability of insects to easily penetrate into the bags in spite of the presence of repellent odours. Therefore, to increase the intensity of exposure of insects to the odours of the repellent plant compared to the attractant odours of the stored grain, and to impede the penetration of insects into the stored grain bag it was decided to test the efficacy of placing the repellent botanicals between the layers of a double bag, with just pure grain in the inner bag. Hence, in this phase of the study an assay was conducted to test the efficacy of a 'treated' double bag (layer of repellent plant material between the two bags, with pure grain inside the inner bag) against a control double bag (only pure grain inside the double bag) to ascertain whether this approach might be more effective than mixing the repellent plant with grain in a single bag.

The main aims of the research reported in this chapter were:

* To design a more effective and practical bioassay method to identify from a range of plant materials, the plants and doses with the most promising repellent effects against *T. castaneum,* and

* To evaluate the effectiveness of inserting a low dose of a repellent plant material between the layers of polypropylene double bags to protect the grain from beetles.

### 5.1.1 Protectant plants and control repellents used to develop new bioassay

The results of a recent survey conducted by the author in Kebbi state (Chapter 3) show that the use of protectant plants varied with region, but overall, 22 – 42% of farmers reported using botanicals either alone or in combination with insecticides (Chapter 3, Table 3.10), and the most common plant materials used by the farmers to protect their grain include *O. basilicum, V. amygdalina, E. guineeses, N. diderrichii* and wood ash mixed with African pepper, although *O. basilicum* was the single most frequently used plant (Chapter 3.4.7, Fig. 3.9 & Fig. 5.1). According to the survey results, however, efforts to protect grain with *O. basilicum* by small-scale farmers in Kebbi did not work consistently well for them (Table 3.11), because the insects still penetrated the treated bags and infested their stored grain. Hence, the systems of storage need to be improved.

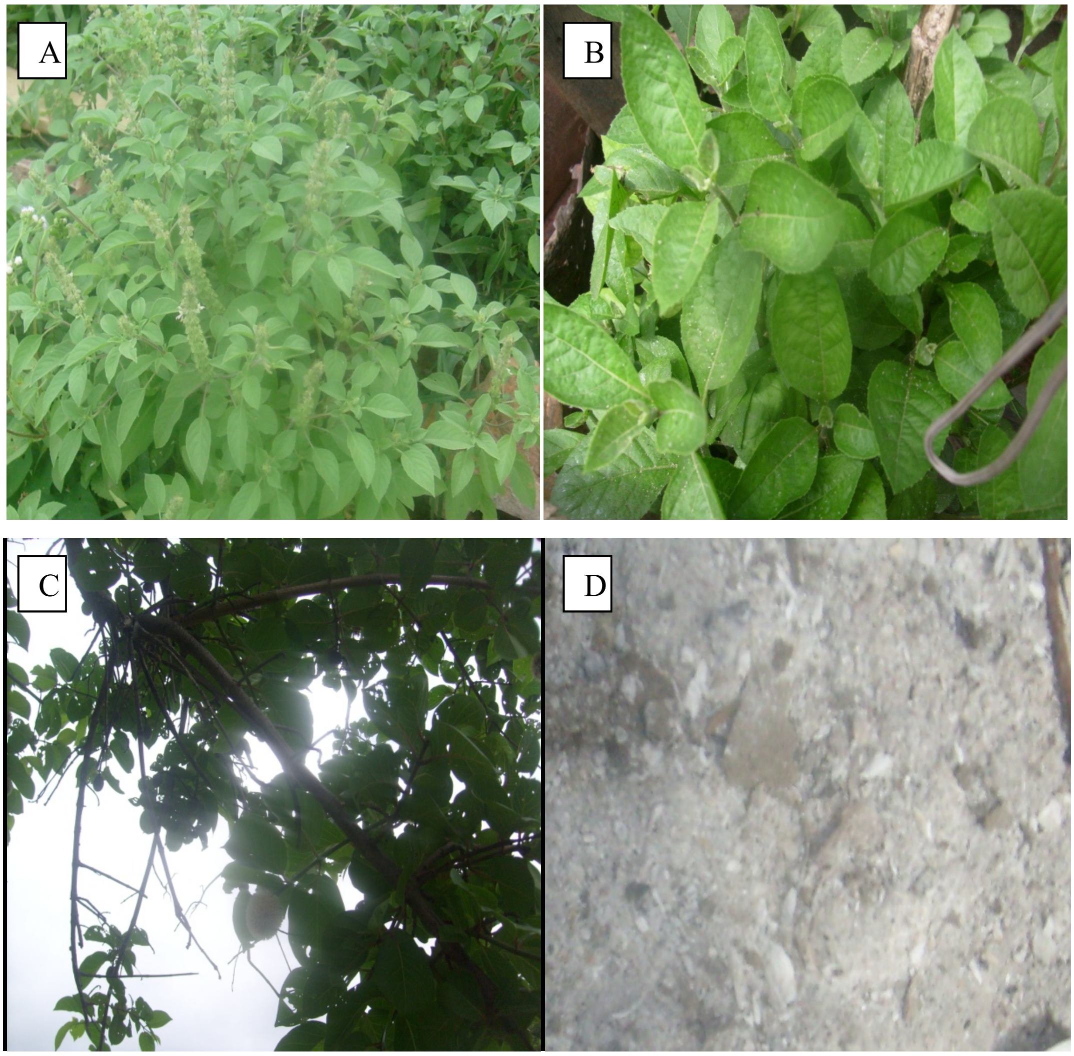



**Fig. 5.1 The most commonly used plant materials for protecting stored grain in Kebbi State,** according to a survey by the author (Chapters 3 & 4). A) *O. basilicum* B) *V. amygdalina* C*) N. diderrichii* D) Wood ash.

The anti-insect activities for some of these plant materials have already been discussed in Section 2.3.5.3 and 2.3.5.4 of Chapter 2. This chapter will focus on *Ocimum* species mainly because it is already a commonly used grain protectant in the study area and there is widespread evidence of its repellent effects against a range of stored grains (Chapter 2).

### 5.1.2 The genus *Ocimum*

The genus *Ocimum* belongs to the family Lamiacea, and is reported to include 60 to 150 species (Simon, *et al.,* 1999; Paton, 1999) which are native to tropical America, Africa and Asia (Darrah, 1980; Paton *et al*., 1999). Members of this genus vary from annual herbs with no rhizome (e.g., *O. basilicum* has only a tap root), to small rhizomes (e.g., *O. bovatum*), to shrubs with quite woody stems (e.g. *O. forkolei*) (Simon *et al*, 1999; Paton *et al*., 1999). Many of the members of this family are known for their aromatic scent (Simon, *et al*., 1999; Paton, 1999).

*Ocimum* species are commonly used for their essential oils and herbs as medicines, food spices, and insect control. *Ocimum basilicum* is used to treat stomach pain and intestinal parasites (Caceres *et al*., 1990). In Rwanda infusions and inhalation of aromatic vapours of *O. suave* are used to cure headache and madness (Janssen *et al*., 1989). In Tanzania, the vapours of *O. suava* are used in treating cough and abdominal pain (Chogo & Crank 1981). In Nigeria *O. basilicum* and *O. gratissimum* leaves are well known for their uses as food additives, as well as folk medicine to manage diseases (James *et al*., 2008). The ground powder of the whole herb is taken by the people of western Nigeria as a treatment for diarrhoea (El-said *et al*., 1969). An ethno-botanical survey in Nigeria by Adjanahoun *et al*. (1991) confirmed that some *Ocimum* species are used for treatment of skin infections, headache, bleeding, typhoid fever, and veterinary uses. Another important aspect of *Ocimum* species are their uses in traditionally pest control. The most commonly used species overall in Kebbi are, *O. basilicum, O. americanum, O. gratissimum and O. suave.*

### 5.1.3 *Ocimum basilicum* (L)

*Ocimum basilicum,* commonly known as Sweet basil, is an annual, aromatic, branched herb that grows 1-2 feet high, with large green leaves, measuring ~ 5 cm in length, with white flower (Arabella & Back, 1980). The stem is glabrous or puberulent with minute hairs concentrated on the two opposing faces of the stem, calyx pilose or pubescent (Paton *et al*., 1999). The plant has been used for medicinal purposes as a digestive stimulant and for treatment of insomnia and constipation. The oils of basil have been used principally in the food and cosmetic industries (Paton *et al*., 1999). Both essential oils and powder of *O. basilicum* have been found to have insecticidal effects on a wide range of insects, which have already been discussed in

Chapter 2. However, the efficacy of *O. basilicum* powder and the effective dose required to repel *T. castaneum* is not known. The study presented here will provide a good basis for establishing effective doses.

### 5.1.4 Lemongrass (*C. nardus*)

Even though it was not mentioned as a plant protectant in the survey, its essential oils are known to have strong repellent and toxic properties against *T. castaneum* (Olivero-Verbel *et al*., 2010) and it was found to be growing in the Kebbi study area during the first research trip to Nigeria (2009-10), although it is mainly used as a traditional medicine there. Since its repellency is already known against *T. castaneum*, it was used as a control plant in the bioassay to compare a plant that is very repellent with one that is likely to be repellent. Due to variability of active compounds in plants the efficacy of some plants to repel insects can be found to be very weak (Isman, 2006). However, the idea of combining two or more plants has shown to be good in improving the efficacy of the combined products compared to using them on their own (Harris, 2002; Agona & Muyinza, 2003). Since Lemongrass and *Ocimum* vary in their chemical compounds they may affect *T. castaneum* in different ways and a combination of the two plants (Lem-ocimum) could have a different effect than the individual plants their own, which could benefit the local practices of the farmers in Kebbi.

### 5.1.3 Controls – citronella & methyl salicylate

Since citronella and methyl salicylate are already reported to have repellent effects on storage pests, particularly *T. castaneum*, they were used as positive control compounds in the bioassays to compare their known repellency with plant materials that have the potential as repellents. Thamara *et al*. (2005) found that treatment of grain with methyl salicylate at a dose of 1.4mg/5ml methanol and treatment of a carton with 0.5g/$m^2$ citronella essential oils dissolved in 95% ethanol (Wong *et al*., 2005) were significantly repellent to storage pests, particularly *T. castaneum*.

## 5.2 Materials and methods

### 5.2.1 Development of a new bioassay

Bioassay methods based on a good understanding of how insects behave under natural environmental conditions can provide valuable information about how they might respond when subjected to test compounds in the field. Furthermore, a bioassay that includes the physical/environmental conditions insects are likely to encounter in the natural environment may overcome problems with many standard bioassays, such as low efficiency and efficacy. In this case an '**efficient**' bioassay is defined as a high proportion of beetles are caught in either treated or untreated grain, with few wandering around in the rest of the assay area, and an '**effective**' bioassay is defined as giving the least variable results and the greatest difference in the proportions of beetles caught in the treated and untreated grain for a given dose. Some bioassays lack the natural conditions that are required by some insects to respond to test stimuli and as a result a high proportion of the insects do not fully respond in the bioassay, which affects the reliability of the outcomes. Hence, extra replicates and experimental materials are required to obtain a significant result. A bioassay that is efficient and effective gives better outcomes by enabling insects to respond to the test stimuli and differentiate clearly between treated and untreated grain. Therefore, a series of experiments was undertaken to compare the efficiency and efficacy of two standard bioassays and a new bioassay in the NRI controlled climate insectary laboratory.

#### 5.2.1.1 Standard bioassays

**Pitfall traps.** 'Pitfall' type trap are based on the geotaxis response of *T. castaneum*; beetles move around the arena, and stop near the edge of a pit; if the contents of the pit are attractive, the beetles express a geotactic response and fall into that opening (Fig.5.2). The pitfall type trap was made up with a petri dish (9cm in diameter, Alpha Laboratories UK). Two eppendorf tubes (1.5ml, Alpha Laboratories UK) with the bottoms cut off were fitted side-by-side on the bottom of the petri dish. The petri dish rested on two centrifuge tubes (15ml, Alpha Laboratories UK), each containing the treated or untreated test materials and the two eppendorf tubes were placed in the centrifuge tubes to allow odour from the tubes to emanate upwards into the petri dish. This bioassay presented *T. castaneum* beetles from the colony at NRI (originally from Malawi and maintained for >5 yrs in environmentally controlled rooms) with a choice between 10g grain (whole wheat, *Triticum aestivum*) treated with 100µl methyl salicylate (10mg/ml) as a test repellent or 10 g of grain treated with 100µl of acetone as the control. A relatively high dose of methyl salicylate was used compared to the effective dose found by Thamara *et al*. (2005) to be sure of a strong repellent effect when comparing different types of bioassay. The treated grain was allowed to dry for 2-3min and then used for the bioassay to test the efficacy of the treatments against *T. castaneum*. Forty, 4 days starved un-sexed, seven-ten day old beetles were introduced at the centre of the dish. Experiments were run for 4 hours and repeated for a total of seven replicates. The positions of the treatment and control grain were randomised for each replicate. At the end of each experiment the number of beetles in the tube with treated grain, in the tube with untreated grain or left in the petri dish, were counted and recorded, and the data was subjected to statistical analysis.

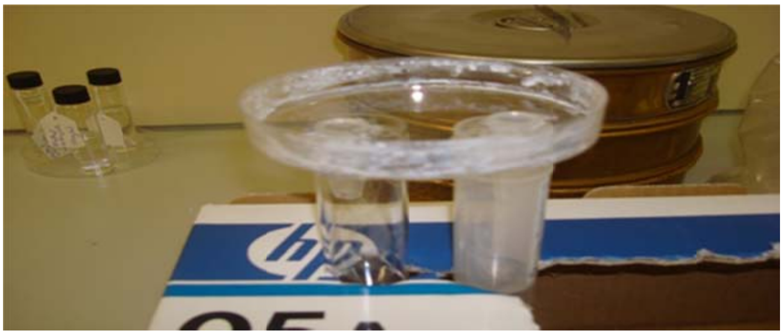


**Fig. 5.2 Pitfall trap type assay** to test response of beetles released in a petri dish (9cm in diameter) to test materials containing grain that was treated or untreated at the bottom of centrifuge tubes (15ml), based on the geotaxis response.

**Open arena**. This bioassay consists of a large open tray arena (58 cm long x 39 cm wide x 8.5cm deep) (Fig 5.3) in which beetles can move around the whole area of the arena and make a choice between grain samples as to which is more attractive to them. The same methods of preparation of repellent materials, beetles, sample size, treatment, number of replicates, and methods of counting and recording of beetles were used as mentioned in the pitfall trap method, except that in this bioassay beetles were presented with a choice between a 20g sample of treated or untreated grain (control) contained in netting bags (8 x 8cm) and placed at opposite ends of the tray. These bags were used because they would not prevent the movement of beetles in or out of the mounds of grain, so that the response to test materials could be evaluated independently of the response to the barrier of the standard grain storage bags.

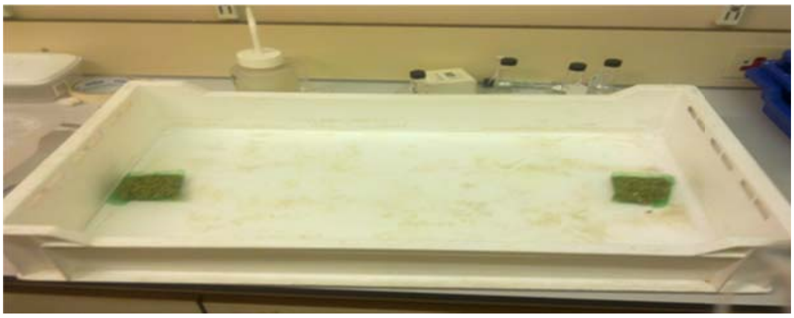


**Fig.5.3 Open tray arena** (58 cm long x 39 cm wide x 8.5cm deep) with green netting bags (8 x 8 cm) containing treated or untreated grain placed at either side of the tray.

During the operation of the bioassays described above, it was noticed that a large proportion of beetles never arrived at either of the grain samples; in the pitfall traps, many beetles walked along the edge of the petri dish and took a long time to approach one of the holes in the floor and there was inconsistency (i.e., high variability in the data) in the mean proportion of beetles that responded to the treated *vs*. untreated bags of grain. Similarly, in the open arena many beetles spent a lot of time walking around the edge of the tray, where the floor meets the walls. The results obtained by these two methods were inconsistent and/or inconclusive, and therefore, a new bioassay was developed.

#### 5.2.1.2 Modified open tray bioassays

The new modified bioassay arena was designed to include features in the environment that affect the behaviour of *T. castaneum* when exposed to grain with and without potential repellent additives. The new assay provided a more natural 'landscape' to the test environment that would enable the insects to explore their surroundings using natural behaviour patterns.

**Open arena with pits**. The first modification was designed to enable natural negative phototactic and geotactic behaviour; the test and control samples of grain were placed in shallow pits in the floor at either end of the arena (Fig 5.4A), whereby 50% of the mound of wheat was below floor level. A control (untreated) bag was placed at one end of the arena, and a treated bag was placed at the other end.

**Open arena with stones and pits (also referred to below as the thigmotactic assay).** The second modification was to place a layer of small stones (10-20mm diameter, Garden Centre pebbles) which were spread throughout the arena (32 cm long x 26cm wide x 6cm deep tray) to enable thigmotatic behaviour. Samples of wheat (20g) were placed in green netting bags (8 x 8cm). This assay provided the beetles with more physical cues they use under natural conditions to search for food without ever having to go out into the open, and provided for thigmotactic, negative phototactic and geotactic behaviour (Fig.5.4 B).


**Fig. 5.4 Modified open tray – 'Thigmotactic bioassay'.** A) open tray arena (32cm long x 26cm wide x 6cm deep) before stones were added, showing 'pits' into which grain samples were placed to allow negative phototaxis and geotaxis, and B) open tray with pits and stones to provide continuous 'edges' for thigmotaxis. Sides of tray treated with silicon to prevent beetle escape.

### 5.2.2 Thigmotactic bioassays of plant materials and positive controls to establish effective repellent doses against *T. castaneum* at NRI

It is important to note that the efficacy of a given treatment to repel insects depends on the active compounds in a given treatment and the concentration required to elicit a threshold of effect, above which can increase responses. Hence, when using plant materials or their active compounds it is important to determine a dose that can work efficiently and effectively on the target insects.

A series of experiments was conducted with dried ground plant materials from the genus *Cymbopogon* (*C. nardus* and *C. citrates* from Malawi, provided and identified by Prof. P. Stevenson, NRI) to compare their repellency with positive controls (methyl salicylate and citronella). Each of the plants was ground to powder using mortar and pestle and mixed with sample of 20g wheat at doses of 0.25, 0.5 and 1% w/w. For the positive controls, three concentrations each of methyl salicylate and citronella were prepared in acetone (1, 2 and 10mg/ml) by serial dilution; 10mg of each compound was added to 1ml acetone to give 10mg/ml acetone. Then, 100µl of the 10mg/ml solution was added to 400µl acetone, which is equivalent to 2mg/ml acetone. Another 100µ1 of the 10mg/ml solution was added to 900µl acetone to obtain 1mg/ml acetone. Samples of grain were treated with 100µl of each concentration (l, 2 and 10mg/ml) of methyl salicylate or citronella. Since pure acetone was used to dilute the other test materials, 100µl acetone was applied to grain as a neutral control. The thigmotactic bioassay was used to test each of the plant materials and positive controls. In each bioassay 40 beetles were introduced to the centre of the tray and allowed a free choice between treated and untreated grain in netting bags placed in the tray pits at either side of the tray. The bioassays were run for 4 hours, after which the beetles in the treated and untreated grain samples were recorded and subjected to statistical analysis and replicated seven times. The assay was repeated seven times.

### 5.2.3 Bioassay to test the efficacy of adding repellent plant materials between layers of double bags

The thigmotactic bioassay was used to test the effect of a new approach to using plant materials to protect grain from beetle infestation that would keep the plant material separate from the grain. The repellent plant material was placed between the layers of the double bag to act as a barrier to prevent beetles attempting to penetrate into the bags. This should be an improvement to the old method used by farmers and described in the literature, e.g. single jute bags impregnated with aqueous extracts of *Lantana camara* to protect grain from legumes beetles (Koona *et al*., 2007).

The experimental bags were made of material similar to that commonly used for grain storage in Kebbi, i.e., woven polypropylene bags. Initially small bags were made from this material by sewing together 10 x 10 cm wide squares cut from larger woven polypropylene bags. The weave of the polypropylene bags was tighter than that used for storage bags in Nigeria, so that the beetles did not penetrate through the material. Therefore, to evaluate how effective the plant materials could be in preventing beetles from penetrating into the bag in treated samples, the polypropylene material was modified to make it easier for beetles to crawl through; either six or 12 strips of the bag (depending on the assay) were removed from the weave. The materials tested (*C. nardus or C. citratus* and the two positive controls; methyl salicylate and citronella) were applied between the layers of the double bags as stated below.

A 200g sample of whole wheat grains was loaded into one of the small polypropylene bag and tied up with string. A paste of a low dose (0.5%w/w) of dry powdered plant material (*C. nardus* or *C. citratus)* or 2mg/ml of citronella or methyl salicylate (positive controls) and acetone (neutral control) was spread on the second bag. This was allowed to dry for ten hours before the grain bag was inserted into the second bag (treated bags). The thigmotactic bioassay (Fig. 5.4B) was used to evaluate the effect of these treatments (double bag treated with test materials) in protecting beetles (n=40 for each test) that were allowed a free choice between the treated or control bags (Fig. 5.5). This assay was run for 8 hours, after which the number of beetles in the treated or un-treated bags were counted and recorded. The experiment was repeated seven times. The data obtained were subjected to statistics using ANOVA and the mean differences were separated using Tukey HSD test for multiple comparisons.

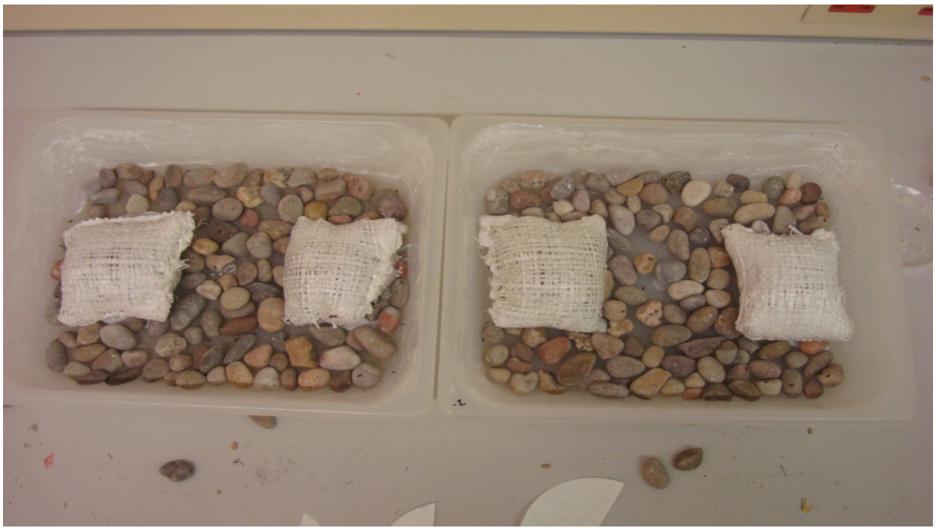


**Fig. 5.5 Comparison of the effect of double bags treated with test materials in preventing beetle penetration into grain using the Thigmotactic open try arena bio-assay**. Each tray (32cm long x 26cm wide x 6cm deep) contained double woven polypropylene bags (10 X 10 cm) either treated or untreated and placed at either end of the tray at a distance of 20cm between them. Each bag had strips of plastic removed from the weave of each side to allow beetles easy access to the bags to test the efficacy of the test materials in preventing beetle penetration into the bags.

### 5.2.4 Grain and plant materials used for the bioassay in Nigeria

The thigmotactic bioassay was validated in the laboratory of the College of Agriculture Zuru's laboratory (Kebbi, Nigeria) to determine if the efficacy of results found in laboratory tests at NRI would be found to be similar for local populations of *T. castaneum*, using the range of locally grown plant materials Sweet basil (*O. Basilicum) V. amygdalina, N. diderrichii* and Lemon grass (*Cymbopogon nardus)* that are used by or available to the farmers locally to protect their grain. These species were chosen because there is evidence in the literature (Chapter 2) that these plant types are repellent to a range of stored crop pests. However, information on the efficacy of these plant materials on *T. castaneum* infesting sorghum is limited, and no published information was found on the efficacy of dried powder of these plants on *T. castaneum*. If Sweet basil and Lemongrass should prove to be repellent in the bioassays, the proposal is to test the efficacy of combining the two species to test for the possibility of an increased effect due to synergy.

Whole wheat grain had been used as the test grain for the preliminary tests at NRI because it is the grain used to maintain the *T. castaneum* colony there. Whole sorghum grain (*Sorghum bicolor*) was used for bioassays to validate the NRI results in the field in Kebbi State, Nigeria, because sorghum was chosen as the target grain for this project, and it is known to be a major host of *T. castaneum* in Nigeria (Lale & Yusuf, 2000; Turaki *et al*., 2007) and in the first survey of small-scale farmers in Kebbi sorghum was reported to be the grain that suffered the greatest proportionate loss to insect pests during storage, mostly by *T. castaneum* (Chapter 3). The same methods of plants preparations, application, beetle counting and recording was followed as mentioned in section 5.2.2 above, except that in this case efficacy of a combination of low dose (0.5% w/w) of the two plants Lemongrass (*C. nardus)* and *Ocimum* (*O. basilicum*), called Lem-ocimum, shown to be most the most repellent treatment for *T. castaneum* in the NRI assays, was tested. The data were subjected to statistical analysis as described for the bioassays above.

### 5.2.5 Plant collection and preparation

All the plant materials were harvested fresh in Nigeria during August to September 2011, except for Lemongrass (*C. nardus* and *C. citratus*) used in the preliminary experiments at NRI, which were from Malawi. The plants collected in Nigeria were air dried in shade and kept in clean plastic bags. The Lemongrass that was used at NRI had already been shade dried in Malawi before being brought to the UK. On the day of an experimental run, whole dry plant material was ground to a powder manually with a laboratory pestle and mortar, and weighed to the required amount.

### 5.2.6 Insect culture

A culture of *T. castaneum* maintained at Ahmadu Bello University, Zaria insectary was used for the bioassays conducted at the College of Agriculture, Zuru laboratory. The Ahmadu Bello culture was reared on a mixture of wheat flour and yeast in the ratio 50:5 by weight, as done at NRI. After thoroughly mixing the two plants in a 2.5L glass jar, 100 unsexed adults of *T. castaneum* were introduced into the culture jar. The upper neck inside the surface of the jar was coated with Fluon and the jar was sealed with filter paper gummed with paraffin wax to prevent the beetles from escape. After 25 days, the newly emerged adult beetles and the parent stock that were still alive were removed. Five days later, newly emerged adults were collected, and every three days thereafter for use in the experiments. The newly emerged adults were collected using forceps and transferred to a new jar with no food and starved for four days before they were used in a bioassay test. The cultures were maintained and the bioassays were conducted in a controlled environment room at 26±3$^{o}$C, 67±5% RH, with a light regime of 16:8 light: dark.

### 5.2.7 Statistical analysis

Where relevant, proportion-responding data was analysed using a Generalised Linear Model (GLM) with binomial errors and a logit link function. Analyses of deviance were performed, and multiple comparisons (Hothorn, *et al.,* 2008) were made using the Tukey HSD method. In other cases, analysis of variance was used, as indicated below. The tests were run using the 'R' statistic software package (R Development Core Team, 2012).

## 5.3 Results

### 5.3.1 Effects of modifications to standard bioassay on the response of beetles to test samples

The results in Fig 5.6 compare the efficiency of the different bioassays; i.e., the proportion of the total number of beetles used for the assay that made 'no choice' and were collected in the arena instead of in either the treatment or control grain bags. The results show that generally the proportion of beetles making 'no choice' decreased as the bioassay method increased in complexity of the environment, from the unmodified assay (left side of plot) to the modified assays (right of plot), even though in the first two assays (pitfall and open arena) the results are almost the same. The data were analysed using a GLM with binomial errors and logit link. The resulting analysis of deviance indicates a significant effect of type of bioassay on the proportion of beetles which made no choice ($\chi^2$=82.42, df=3, p<0.001). A significantly (p<0.001) greater proportion of beetles made no choice in the open arena (0.45±0.029) (mean ± standard error) and pitfall type trap (0.37±0.033) than in the open arena with pits (0.26±0.027) or the thigmotactic 'open arena with pits and stones' bioassay (0.12±0.0197; Tukey HSD comparisons). This suggests that most modifications to the standard bioassays improved the efficiency of their use of the total number of beetles tested.

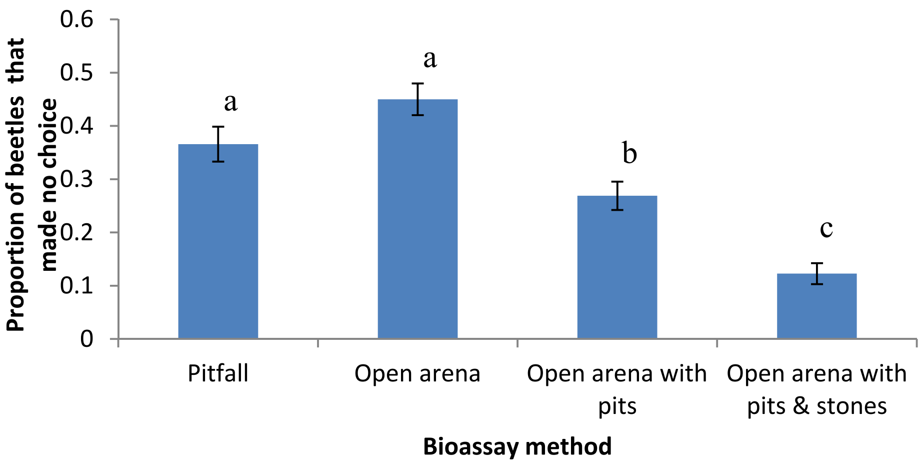



**Fig.5.6 Comparison of the efficiency of four types of repellent bioassays,** i.e the mean proportion (± SE) of beetles that were not collected in either the treated (100µl of 10mg/ml methyl salicylate) or untreated grain (control) samples, out of the total number of beetles (n=40) in each run of the bioassay. Each method was repeated seven times, and lasted 4 hr per assay. Overall, the type of bioassay had a significant effect on the proportion of beetles that made no choice (Analysis of deviance, GLM, p<0.001). Bars with different letters are significantly different (Tukey HSD, p<0.001). Standard error bars were calculated from analysis of deviance residual.

Figure 5.7 compares the efficacy of the types of bioassay tested, i.e., the proportion caught in untreated grain of those beetles that were caught in either treated or untreated grain. The proportion caught in untreated grain can be taken as a measure of the repellency of the treated grain. These results show that there was a gradual increase in the proportion of beetles repelled by the treated grain with increasing complexity of the assay environment, from the pitfall assay (on the left of plot) to open arena with pits and stones (on the right of plot). The analysis of deviance with chi-square test indicates a significant effect of 'type of bioassay' on the proportion of beetles repelled by treated grain ($\chi^2$=14.169, df=3, p<0.01). However, Tukey comparisons indicate that a significantly (p<0.01) greater proportion of beetles were found in untreated grain in the thigmotactic 'open arena with pits and stones' bioassay (0.81±0.024) than in the open arena assay (0.69±0.356) or in the pitfall trap assay (0.65±0.041; Tukey comparisons). There was no significant difference (p=0.485) in the mean proportion of beetles found in untreated grain between pitfall and open arena or open arena with pits, or between open arena and open arena with pits, but the thigmotactic bioassay was significantly different than the pitfall and open arena assays.

The results shown in Figs 5.6 & 5.7 considered together suggest that the reduction in the proportion of 'no choice' beetles as a result of bioassay modifications (Fig.5.6) has also increased the ability of beetles to differentiate more clearly between the treated and untreated grain (Fig. 5.7).  This effect is most clearly shown for the thigmotactic bioassays.

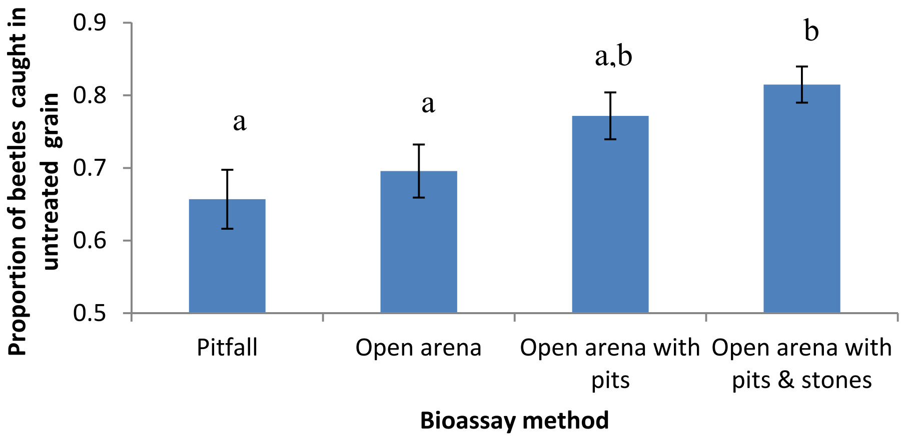



**Fig. 5.7 Comparison of the efficacy of four types of repellency bioassays**, i.e., the mean proportions (±SE) of beetles caught in the untreated grain out of the total number of beetles that were caught in either the treated or untreated grain. Standard error bars were estimated by analysis of deviance program, n=7 replicates, and lasted 4 hr per assay. The number of beetles in each replicate varied; i.e. the number caught in grain bags (treated + untreated) out of the 40 beetles released in each run of the experiment. Overall, 'type of bioassay' had a significant effect on the proportion of beetles caught into the untreated grain (Analysis of deviance, GLM, $p<0.001$). Bars with different letters are significantly different (Tukey HSD, $p< 0.001$).

### 5.3.2 Effect of duration of beetle exposure to different concentrations of methyl salicylate

An experiment was run with the positive control methyl salicylate to determine if the response of beetles to the test compounds could be affected when exposed to various concentrations of methyl salicylate (10mg/ml, 2mg/ml, 1mg/ml) against the control (acetone alone) for different amounts of time (2hrs, 10hrs). The results (Fig.5.8) show that significantly more beetles were repelled when exposed for a longer time than a shorter time (Analysis of deviance, $\chi^2$=21.07, df=1, p<0.01). The response of beetles to different concentrations of the compounds also varied significantly; 10mg was more repellent than 2mg or 1mg ($\chi^2$=26.54, df=2, p<0.001). There was no significant interaction effect between period of exposure and concentration ($\chi^2$=1.154, df=1, p=0.283), as is clear in Fig. 5.8.

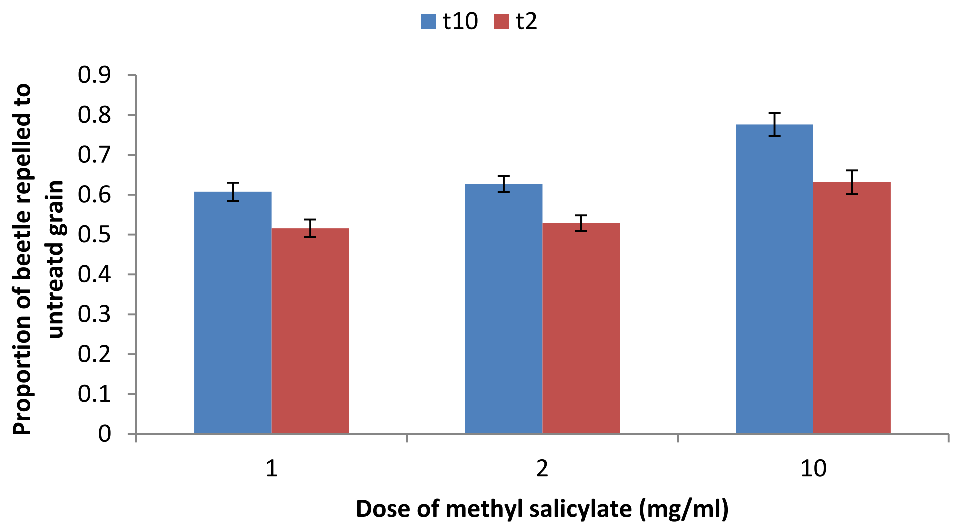



**Fig. 5.8 Comparison of the mean proportion (± SE) of beetles found in the un-treated grain pit when subjected to a choice bioassay** between untreated (acetone only) and treated (methyl salicylate diluted in acetone) wheat samples at different concentrations and durations of exposure to the assay. t2 = two hour exposure, t10 = ten hour exposure. Standard error bars estimated by analysis of deviance program, n=40 beetles per replicate, seven replicates at each dose and treatment duration.

### 5.3.3 Bioassays with various plant materials and positive controls to establish effective repellent doses against *T. castaneum* at NRI

A series of experiments was conducted to determine the efficacy of various plant materials compared with positive controls (methyl salicylate and citronella), and to establish effective repellency doses against *T. castaneum*. The results in Fig. 5.9 indicate that citronella demonstrated a progressive dose effect on the beetles, which increased in repellency as the concentration of citronella increased from 1 to 10mg/ml. Thus, the repellency was dose dependent. This increase in percentage of beetles repelled to the untreated grain as the concentration of citronella increased was found to be statistically significant by Analysis of deviance, GLM ($\chi^2$=44.87, df=1, $p<0.001$). This suggests citronella could be a useful alternative as a repellent against *T. castaneum*.

**Citronella**

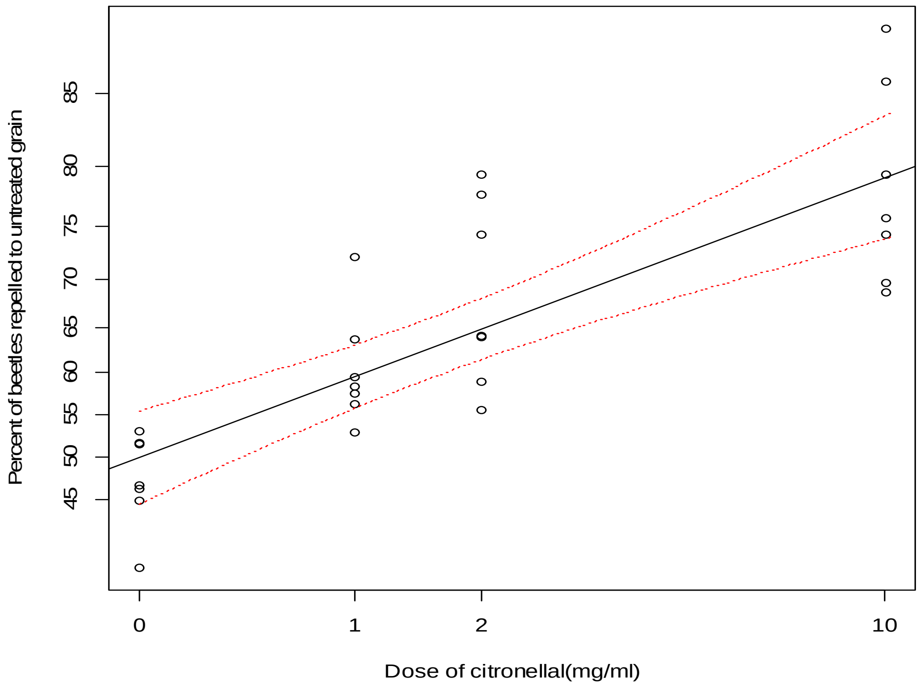



**Fig. 5.9 Relationship between increase in concentration of citronella and percentage beetles repelled**. The logit line in black and red lines (95% confidence interval on the logit) were obtained from GLM analyses with binomial and logit function, n=40 beetles per run, repeated seven times for 4-hr long run at each dose. The increase in dose of citronella had a significant effect ($p<0.001$) on the percentage beetles repelled (Analysis of deviance, GLM).

The results in Fig. 5.10 show methyl salicylate had a positive dose-response effect on *T. Castaneum,* i.e., the greater the concentration of methyl salicylate from 1 to 10mg/ml acetone on 20g of grain, the greater the proportion of beetles found in the untreated grain. The Analysis of deviance (GLM) test indicates that the difference in the increase in percentage of beetles found in the untreated grain as the concentration of methyl salicylate increased was statistically significant ($\chi^2$=57.901, df=1, p<0.001).

**Methyl salicylate**

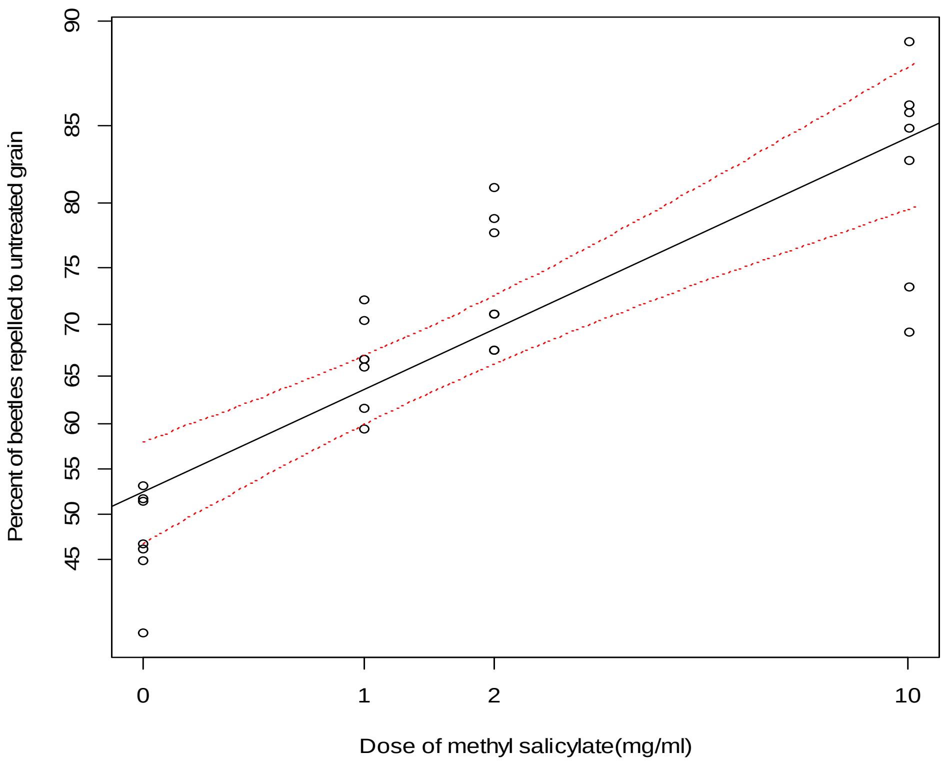



**Fig. 5.10 Relationship between increase in concentration of methyl salicylate and percentage beetles repelled**. The logit line in black and redlines (95% confidence interval on the logit) were obtained from GLM analyses with binomial and logit function. n=40 beetles per run, repeated seven times for 4-hr long run at each dose. The increase in dose of citronella had a significant effect (p<0.001) on the percentage beetles repelled (Analysis of deviance, GLM).

The results in Fig. 5.11 indicate that the greater the concentration of *C. nardus* from 0, 0.25, 0.5 to 1% w/w of 20g of grain, the greater the percentage of beetles repelled to the untreated grain. The Analysis of deviance test indicates that the difference in the increase in percentage of beetles repelled to untreated grain as the concentration of the *C. nardus* increase was statistically significant ($\chi^2$=227.02, df=1, p<0.001).

***Cymbopogon nardus***

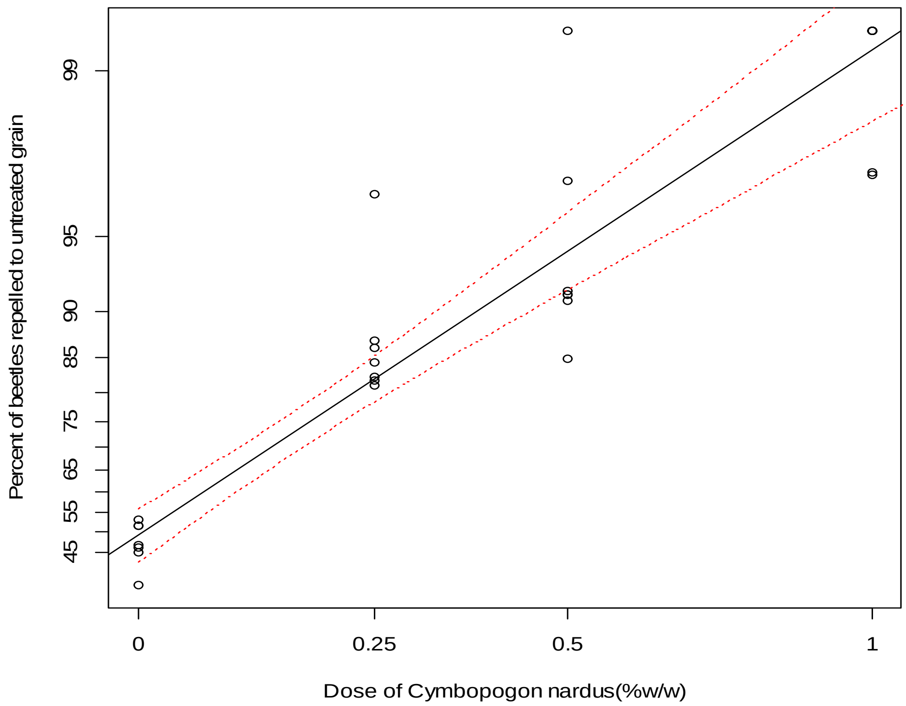



**Fig. 5.11 Relationship between increase in concentration of *C. nardus* and percentage of beetles repelled**. The logit line in black and red lines (95% confidence interval on the logit) were obtained from GLM analyses with binomial and logit function. n=40 beetles per run, repeated seven times for 4-hr long run at each dose. The increase in dose of citronella had a significant effect (p<0.001) on the percentage beetles repelled (Analysis of deviance, GLM).

The same trend was observed with *C. citratus* (Fig. 5.12); the percentage of beetles repelled to the untreated grain increased as the concentration of *C. citratus* increased from 0, 0.25, 0.5 to 1% w/w of 20g of grain. An Analysis of deviance test indicates that the difference in the increase in the percentage of beetles repelled to the untreated grain as the concentration of *C. citratus* increased was significant ($\chi^2$=207.41, df=1, p<0.001). Overall, all the plant materials have a significant effect on *T. castaneum,* with *C. nardus* giving the most effective repellent effect over the range of doses tested. These results suggested that all of these plant materials were suitable for testing in a wider field experiment.

***Cymbopogon citratus***

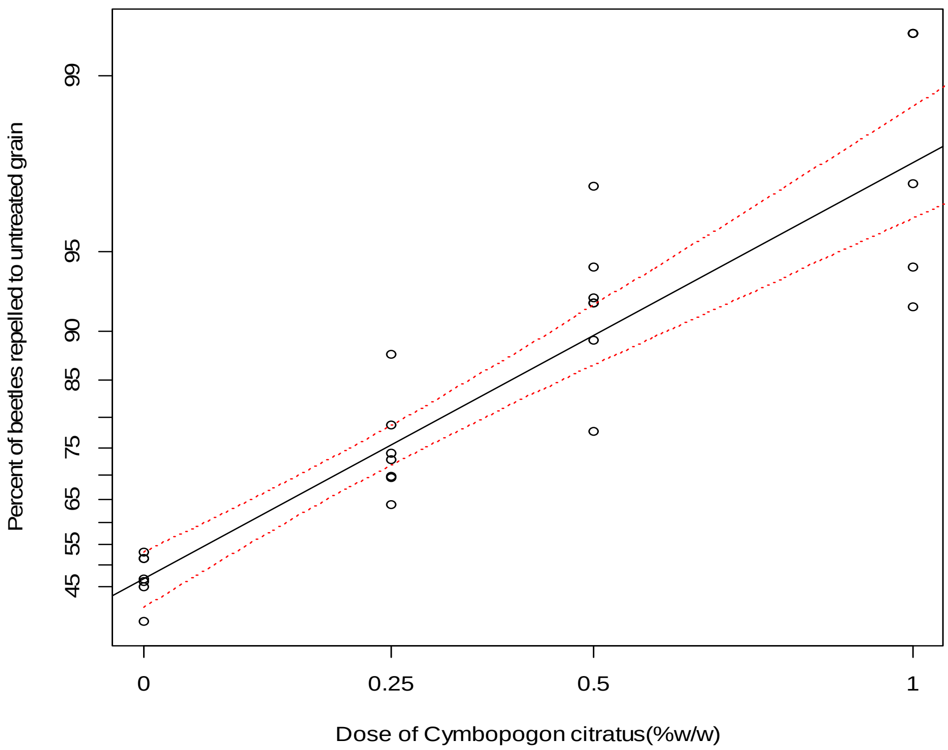



**Fig. 5.12 Relationship between increase in concentration of *C. citratus* and percentage of beetles repelled**. The logit line in black and red lines (95% confidence interval on the logit) were obtained from GLM analyses with binomial and logit function. n=40 beetles per run, repeated seven times for 4-hr long run at each dose. The increase in dose of citronella had a significant effect (p<0.001) on the percentage beetles repelled (Analysis of deviance, GLM).

### 5.3.4 Bioassay to test for repellence efficacy of different plant materials used by farmers in Nigeria as grain protectant against *T. castaneum*

In the experiment conducted at NRI, the efficacy of two plant species (*C. nardus & C. citratus*) and two chemical compounds based on different doses had been established. The experiment conducted in Nigeria was to use the same procedures to confirm if the same or similar efficacy could be achieved with some of the Nigerian plant materials used by farmers. The results in Fig. 5.13 show that in all the four plant materials tested, Lemongrass (*C. nardus*) (lemong) tended to produce the highest repellency, with only a small proportion of beetles (0.06±0.0074) found in the treated grain, followed by *O. basilicum* (ocbas) with 0.15±0.022 proportion of beetles in the treated grain. However, a combination of the two plants (lem+oc) produced the greatest repellent effects on *T. castaneum* (0.02±0.0074), which was greater than the individual plants on their own. *Nauclea diderrichii* (nauldid) and *V. amygdalina* (vemam) had the least repellent effect, with 0.27±0.027 and 0.28±0.028 proportion of beetles in the treated grain, respectively. The analysis of deviance showed that 'type of plant' had a significant effect on the proportion of beetles caught in the treated grain ($\chi^2$=304.130, df=5, p<0.01). However, a pairwise multiple comparisons with the Bonferroni correction indicates that the difference between the means of each treatment were significant between the means of all the treatments except between vernam and nauldid (p=0.205). This suggests that ocbas, lemong and more importantly a combination of both (lem+oc) show promising potential for use in the future for improving small-scale farmers' methods of grain protection.

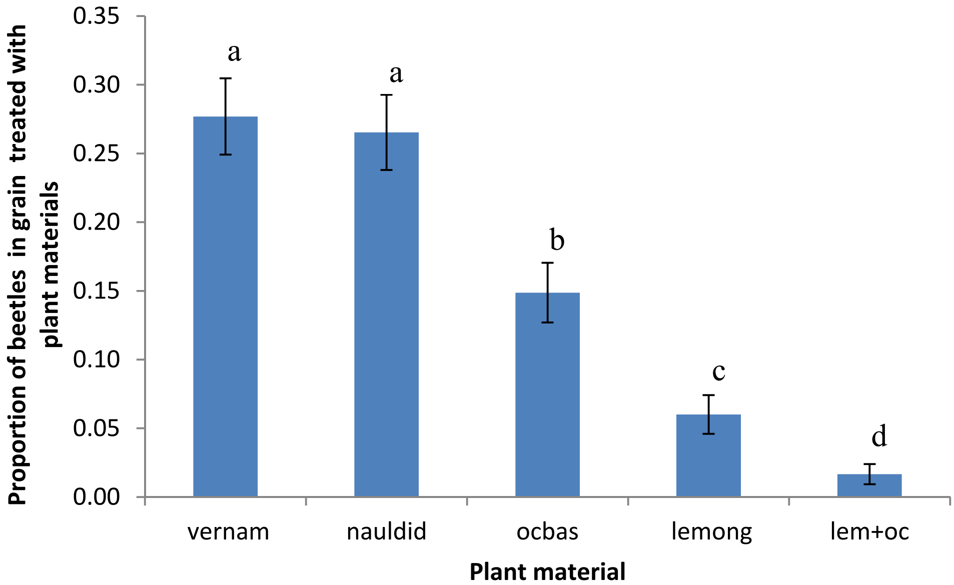



**Fig. 5.13 Comparison of the mean (±SE) proportion of *T. castaneum* in grain treated with different plant materials**. 1% w/w was used for *V. amygdalina* (vemam); *N. diderrichii* (*nauldid*); *O. basilicum* (ocbas), Lemongrass (*C. nardus*, lemong) and a low dose 0.5% w/w combination of *O. basilicum and* Lemongrass (lem+oc). N= 40 beetles per replicate, replicated eight times for 4-hr. Overall, 'type of plant' had a significant effect on the proportion of beetles caught in the treated grain (Chi-square, $p<0.01$). Bars with different letters are significantly different (Bonferroni correction, $p<0.001$**).**

The results in Fig 5.14 further indicate that an increase in the concentration of the plant materials from 0, 0.25, 0.5 to 1% resulted in an increase in the proportion of beetles that went to the untreated grain samples and decreased in the treated grain. This shows that the repellent effect of the plants on *T. castaneum* is dose dependent. This difference in the reduction of the proportion of beetles in the treated grain with increasing dose of plant materials was statistically significant ($\chi^2$=245.142, df=1, p<0.001). An interaction between the dose and type of plant materials was also significant ($\chi^2$=39, df=4, p<0.05), as can be seen by the greater effect of lem+oc with dose than for other types of plant.

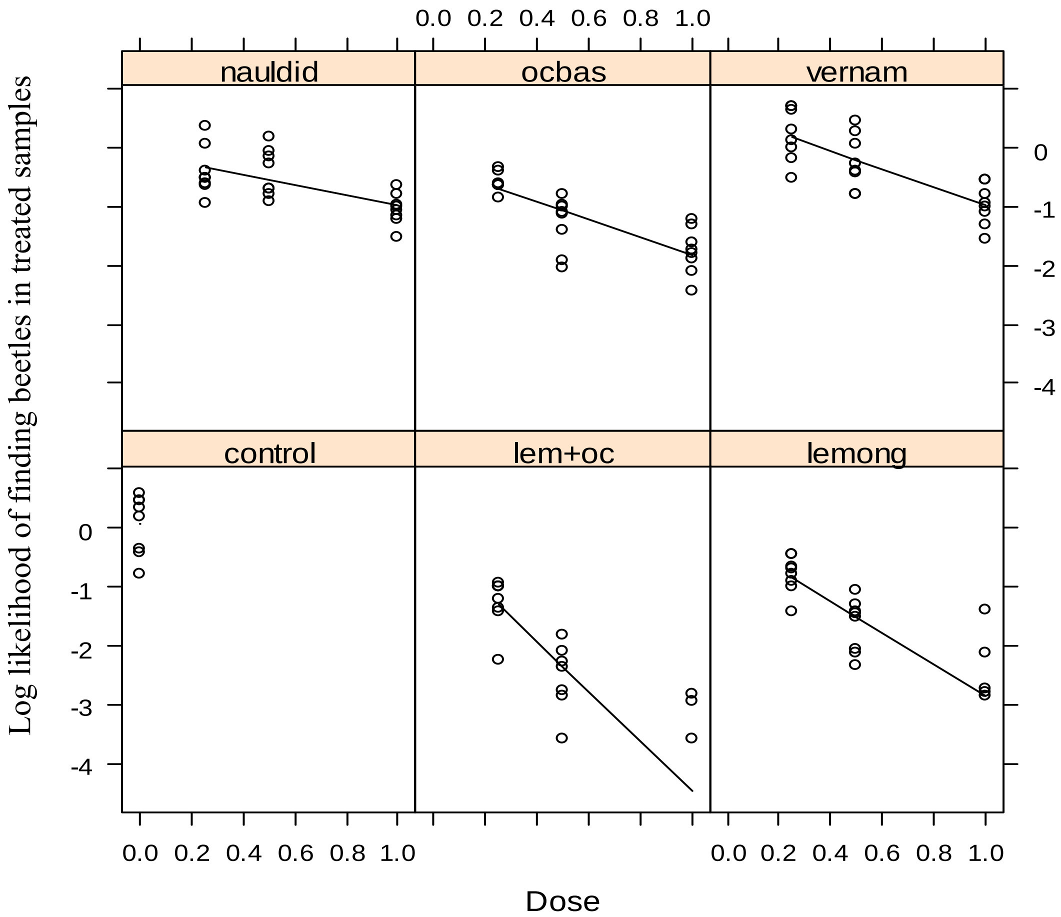



**Fig. 5.14 Relationship between increasing concentration of various plant materials (nauldid, ocbas, vemam, lemong and a combination of plants lem+oc) and the proportion of *T. castaneum* found in treated grain over 4-hr long experiment**. Concentration: 0, 0.25, 0.5 and 1% w/w of 20g sorghum grain, n = 40 beetles per run, repeated eight times for four hours for each plant material and dose experiment (p<0.001).

### 5.3.5 Efficacy of treated double bags to protect grain from insects

The results of an experiment to determine if adding a low dose of plant material in between layers of a double bag containing grain could reduce the chances of beetles penetrating into the bag are presented in Fig. 5.15. To test the efficacy of this method, it was necessary to first test whether or not beetles can be prevented from entering bags. Hence, a few strips (six strips) from the woven bag material were removed from both sides of the double bags to test how easily beetles can penetrate into the bags. On average, only 20 % of beetles could penetrate the bags. However when more strips (12 strips) were removed on both sides, beetle penetration increased to ~ 40%, which suggests this is the best type of double bag for testing the repellent effects of plant materials.

The results of the experiments carried out with double bag with 12 strips removed and then treated with test materials indicates that the test materials had a significant effect on the percentage of beetles penetrating into the treatment bags (ANOVA; $F=20.927$, $df=4$, $p<0.001$). Tukey HSD tests indicate that a significantly ($p<0.001$) lower percentage of beetles penetrated into bags treated with C. *nardus* (4.50±2.14) compared to methyl salicylate (19.18±2.14), Citronellal (24.58±2.14) or the control (32.86±2.14) bags. Similarly, there were significant differences ($p<0.01$) between the mean percentage of beetles found in bags treated with *C. citratus,* Citronellal and the control bag, but not between bags treated with *C. citratus* and *C. nardus* ($p=0.151$). The mean percent of beetles found in bags treated with methyl salicylate was only significantly different than the control bag and *C. nardus* ($p<0.05$). Citronella treated bags had the least effect on *T. castaneum* and this was not different from the control ($p=0.093$). This suggests that the movement beetles into treated bags was affected more by plant materials than the positive controls, with a tendency for *C. nardus* to be the most effective.

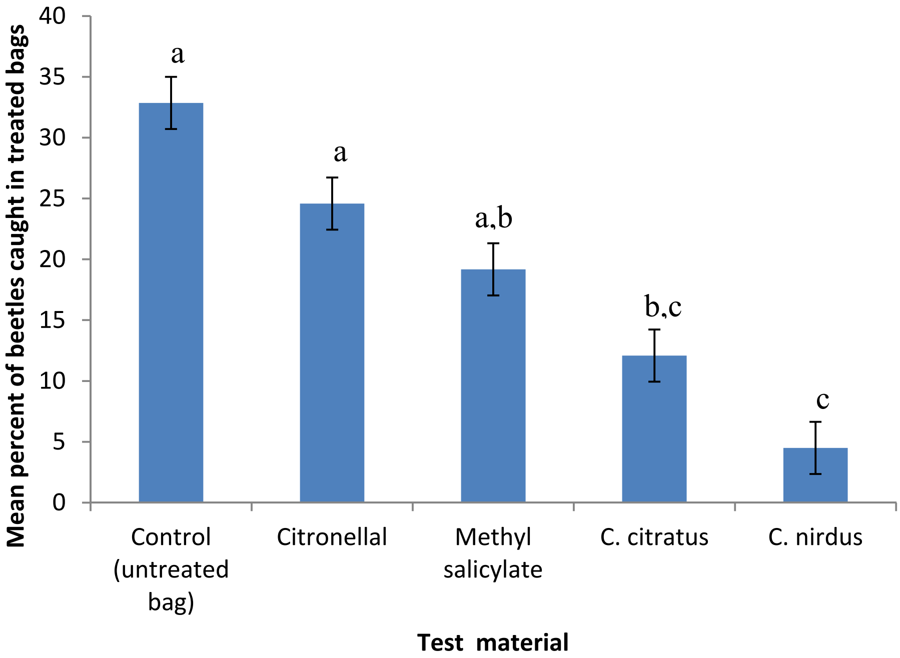



**Fig. 5.15 Comparison of the mean percentage (±SE) of beetles found in 200g samples of sorghum in double bags treated with 2mg/acetone citronellal or methyl salicylate (positive controls), or 0.5% w/w of dried *C. nardus* or *C. citratus* and untreated control bags**. Standard error bars were calculated from ANOVA residuals, n=40 beetles per replicate, replicated seven times over 8-hr for each run. Overall, the test materials had significant effect on the proportion of beetles caught in the treated bags (ANOVA, $p<0.001$). Bars with different letters are significantly different (Tukey HSD, $p<0.001$).

## 5.4 Discussion

### 5.4.1 The new 'Thigmotactic' bioassay

The ability of a particular bioassay to measure efficiently and effectively what it is expected to measure, i.e., the response of insects to a test stimulus, depends on the kind of orientation cues available and their ability to influence the test insects, which facilitates a positive response. Therefore, the purpose of this study was to determine if modifications to standard bioassays could help with a new improved bioassay that is better in efficiency and effectiveness in outcomes i.e., enabling insects to respond to the test stimuli and differentiate clearly between treated and untreated grain.

This was confirmed by the results of a laboratory experiment conducted with *T. castaneum* exposed to four types of bioassay arenas with different physical environmental structures to determine the effect of having some physical cues in giving a better insect response to the test stimuli. In their study of the response of *T.*

*castaneum* to an arena with a patchy environment, consisting of a smooth floor arena with patches of grain, Campbell & Hagstrum (2002) observed that a greater number of *T. castaneum* were found inactive in corners and edges of the arena than in the patches of grain, a behaviour which Kennedy (1986) termed thigmotaxis. If there are no objects in the arena, insects sometimes stop moving around, or become limited to following the edges of the arena without ever moving across open spaces toward the target treatments. This behaviour can obscure the response of insects to the test material and consequently limit the efficiency of a bioassay. It might be as a result of this limitation that even in the presence of an airflow containing attractive odours that Olsson *et al*. (2006), Duehl *et al*. (2011) and Campbell (2012) observed a high number of *T. castaneum* not-responding to pheromone or food attractants in their bioassays.

In the present study four types of bioassays were tested that differed in the type of stimuli and physical features that were present, which presented different orientation cues and resulted in different responses in the beetles: 1) the pitfall assay relied on positive geotaxis to bring beetles to the source of odour, 2) the open arena assay, with no physical cues, relied on random searching to bring beetles nearer the source, but the entire arena was flat, so many beetles stayed near the walls of the arena and did not search the whole arena, 3) the open arena assay with pits, made use of the positive geotaxis and negative phototaxis responses to get beetles to move down into the grain bags, and 4) the open arena with pits and stones (Thimotactic assay), which included features that stimulated positive geotaxis, negative phototaxis and thigmotaxis responses. The bioassay results indicate that there were more beetles not responding to either treatment or control grain in the pitfall and open arena assays, suggesting that few beetles responded to the treated and control materials in these assays. However, the addition of pits to provide some physical cues, such as negative phototaxis and positive geotaxis, increased the efficiency of the assay; a greater proportion of beetles got to the grain bags. The efficiency of the assay increased even more when stones were added to provide thigmotaxis, which increased the movement of beetles throughout the whole area of the arena. The results of this important study suggest that the significant increase in the proportion of beetles that moved to the grain bags as a result of modifications to the bioassay increased the numbers of beetles contributing to the choice score, and hence, increased the efficiency of the bioassay.

The reduction in the proportion of beetles making no choice and the increase in the proportion caught in bags of grain (treated and untreated) in the new bioassay could be as a result of: a) since the beetles are thigmotatic, the addition of stones all over the floor of the arena was thought to give the beetles a more continuous touch stimulus, which enhanced the amount of time they moved around the whole area of the arena, thereby reducing the chances of the beetles to gather around the corners of the tray and increased the chances to detect the odour cue; a trend observed also by Campbell & Hagstrum, (2002). In the study presented here, in the open arena and pitfall arena a greater proportion of beetles were observed to walk continuously around the edge of the tray or Petri dish, hide behind the netting bags used to contain the test grain or gather in the corners of the tray in the open arena assays, b) the observed behaviour of the beetles in an open arena assay to hide under the netting bags could validate what Romero *et al.* (2010) said that the beetles have a preference for shelter, hence putting the bottom half of the netting bags of grain into the sunken pits allowed the beetles to shelter and remain in the pits even if the beetles had not been attracted or repelled by odours emitted from the bag of grain.

The new thigmotactic method allowed study of the behaviour of beetles in response to some of the features encountered in their natural environments. This study suggests that improvements to the standard bioassay to include some of the features that are more like their natural environment can help to gain a clearer understanding of insect responses to the test stimuli and make it possible for the beetles to differentiate more clearly between treated and untreated grain.

### 5.4.2 The effect of repellent plants and chemical compounds on *T. castaneum*

Repellency is an important component in pest management that can affect the behaviour of some insects (Dethier, *et al*., 1960; Yinon & Shulov, 1969; Isman, 2006), although the effect and intensity depends on the source of repellent used, their active compounds, and the type of insect. The purpose of the study in this section was to use the new bioassay to identify plant materials with repellent effects on *T. castaneum* and establish an effective dose that would be used to develop a simple and effective method in the field to reduce infestations of *T. castaneum*. The dose experiment was first established at NRI with ground dry plant materials of two species of Lemongrass (*C. citratus* and *C. nardus)* and two positive controls methyl salicylate and Citronellal, by testing three doses of each (section 5.2.2).

The results of the experiment conducted using the new bioassay method with two plant species and positive controls, indicates that the new method is effective enough to prove that the test materials possessed compounds that are repellent to adult *T. castaneum*. Moreover, the linear response relationship exhibited by the beetles across different concentrations (doses) of the test materials indicates that the repellency of the materials against the beetles was dose dependent. Although all the materials tested were repellent to *T. castaneum, Cymbopogon* species were shown to be more repellent at all doses than the two compounds already known to be repellent to *T castaneum.*

The repellent effect of the two *Cymbopogon* species on *T. castaneum* was not a surprise since evidence of repellency of *Cymbopogon* species essential oils on beetles, particularly *T. castaneum,* was confirmed by Zhang *et al*. (2011). In particular, essential oils from *C. citratus* were confirmed to be repellent against *T. castaneum* by several research groups (Olivero-Verbel *et al*., 2010; Stefanazzi *et al*., 2011; Manzoor *et al*., 2011). Citronella, which is an important component of *C. nardus*, was found to be repellent against *T. castaneum* (Wong *et al*., 2005). Although all the studies reported above conducted with *Cymbopogon* species were done with essential oils, the present study was conducted with dried powder of the *C. citratus* and *C. nardus*. The evidence that powdered plant materials can be effective insect control materials have been shown by Okonkwo (2004), Asawalam *et al*. (2007), Musa *et al*. (2009) and Ahmed, *et al*. (2010), and is one of the techniques used by small-scale farmers to protect their grain from pest infestation (Belmain & Stevenson, 2001; Deng *et al*., 2009). The NRI method with the two *Cymbopogon* species suggests that the two species significantly repel *T. castaneum* even at a dose as low as 0.5% w/w, with increasing efficacy with increasing concentration of the plant materials. This was established to provide the basis for conducting a similar research study with the aim of using plant materials that farmers already use to protect their stored grains in Kebbi, but by using plant materials in a more effective way.

The laboratory experiment conducted with four plants (*O. basilicum, N. diderrichii, V. amygdalina* and *C. nardus*) in Nigeria proved that the method practiced by farmers to protect their stored grain with ground dry plant materials was effective against *T. castaneum*. However, the differences in efficacy experienced by the farmers, as reported in the survey (Chapter 3) could be related to the specific plant materials and methods of application used, as observed in field experiment reported here; each of the plants tested varied in their effect on *T. castaneum*.

*Cymbopogon nardus and O. basilicum* grown from Nigeria demonstrated more repellency than *V. amygdalina* and *N. diderrichii.* The repellency of *V. amygdalina* and *N. diderrichii* was only found at a high dose (1% w/w) when compared with the control and it even appeared to be attractive to the beetles at the lowest dose tested (0.25% w/w). Surprisingly, no literature was available to provide evidence of repellency of these two plants on *T. castaneum* except that the major compound in *V. amygdalina,* 1.8-cineole, was reported to provide moderate repellency against *T. castaneum* (Obeng-Ofori & Rechmuth, 2009). However, it could be that the Nigerian grown *V. amygdalina* is low in concentration of the major compound, which may be the reason for its ineffectiveness at low doses, and a low dose may not be enough to provide a threshold of response. However, *V. amygdalina* was reported to be repellent to the beetle *Callosobruchus maculatus* (Musa *et al*., 2009) and *Sitophilus zeamais* (Asawalam & Hassanali, 2006). More research is required to make conclusion on the repellent efficacy of these plants to *T. castaneum*.

As expected, the high repellency exhibited by the dried ground leaves of *O. basilicum* combined with *C. nardus* is related to their respective chemical compositions. Mikhaiel (2011) and Mishra *et al.* (2012) reported that essential oils from both *O. basilicum* and *C. nardus* are known to possess compounds that repel *T. castaneum.* Even though the two plants demonstrated repellency to *T. castaneum,* their degree of repellency varied, with *C. nardus* having a greater repellency than *O. basilicum*, which suggests that either the two plants possess different concentrations of the same compounds or different compounds that effect *T. castaneum* differently. The response exhibited by *T. castaneum* to the plant materials suggests that repellency was dose dependant and repels beetles more effectively at a dose of 1% w/w, although a lower dose of 0.5% w/w was equally good. However, the low response of the beetles to the lowest dose (0.25w/w) indicates that the beetles can tolerate low doses, which could develop resistant over succession.

Since the two most repellent plants were found to vary in their efficacy it would be interesting to test their efficacy by combining them at a low dose (0.5% w/w), to test for synergism. This was supported in Harris's (2002) report that combinations of two or more control materials in a synergism can improve the efficacy of the combined products. Synergistic effects are important in pest management because they entails the use of low doses and combinations of materials which can lead to products with a multiple mode of action (Harris, 2002), hence, reduced chances for pest resistance to treatments developing. This development of synergistic combinations has been reported by Lòpez *et al*. (2008); they found that a greater mortality of *R. dominica* and *S. oryzae* was achieved when a binary combination of estragole, methyl eugenol, eugenol and linalool compounds were used than when used individually. Extract of chilli pepper mixed with extract of garlic (*Allium sativum*), onion (*Allium cepa)* and *C. citratus* leaf extract were found to be very effective against some leaf eating insect pest of crops (Stoll, 1988). The two-fold combination of *Nicotiana tobacum* powder with *Tagetes minuta*, *Tephrosia volgelli* and *Azadrachta indica* was found to be more effective in reducing plant damage and mortality of the bean bruchid *Acanthoscelides obtectus* than the use of *N. tobacum* alone (Agona & Muyinza, 2003).

Evidence from research reported here indicated that a combination of *O. basilicum* and *C. nardus* at low doses gives a greater level of efficacy than the individual plant on their own, hence synergism is important and indicates that the two plants can be used together to develop a simple and effective method to control this pest in the field. Thus, in real sense this could reduce the amount of plant needed to be collected for grain protection; this is one of the factors that limit the successes of plants as control materials (Isman, 2006). However, the greater challenge is how farmers can best treat their grain with this combination so they can have better protection for their stored grain. The method of mixing grain with plant material to be stored in a polypropylene bags practiced by small-scale farmers of Kebbi was not always effective (Chapter 3) and needs improvement.

### 5.4.3 Effect of adding plant material in between layers of polypropylene double bags on beetle penetration

The farmers' method of mixing grain with powdered plant material or plant extract is thought to not provide efficient protection from pest attacks. These methods also leave residues which may increase labour time for processing. Some plant materials used in grain protection are reported to be toxic (Isman, 2006) and the methods used by farmers to remove residue from grain before consumption may not ensure a total removal of residues (Belmain *et al.*, 2001). It is our hope to develop a new technology using the materials similar to what farmers use to protect their grain, but with a better efficacy. It is hoped that the new method will take into account all the discrepancies with the farmers' existing methods so that more confidence in and acceptance of plant materials for pest control by the farmers can be gained. The main idea is to use the double bagging method, but adding repellent plant materials between the two bags and keep the grain in the inner bag without having contact with repellent plant materials.

The research in this chapter demonstrated that when plant materials were spread in between the layers of double bags, beetles could detect the presence of the test plant compounds in the treated bags and avoided it, hence the proportion of beetles that entered the treated bags was reduced. However, this study elucidates that dried plant materials were more effective in preventing beetles penetration into the treated double bags than the positive control (methyl salicylate & citronellal) used. The reason for low efficacies of both methyl salicylate and citronella compared to plant materials could be that as they are highly volatile, with high evaporation pressure (Sarah *et al*., 2006) and the experiment was run for over eight hours that the positive controls evaporated before the end of the observation period. Since the volatile compounds in plant materials are in a complex form with other compounds, the release might be more slowly and hence explain the longer and greater effect of the plant materials compared to the positive controls. Since there is evidence from the laboratory work that some of the plants tested are good at repelling beetles, it may be possible to develop an effective botanical repellent with these plants against *T. castaneum* using polypropylene bags as the grain protectant in the field.

The type of study reported here has not previously been done. The previous work was on treating grain to be stored in bags by either admixing grain with plant materials or treating bags with plant extract or oils, which resulted in low efficacy (Othira *et al*., 2009). The method proposed here could be an improvement to the previous methods used by farmers to store their grain in polypropylene bags. This double-bag treated method is economical and only a small amount of plant material is required by farmers to protect their grain from infestation. It has the advantage of saving farmers time on winnowing residues of plant materials before being able to use the grain. However, the effect of the technology on long term storage is yet to be evaluated. This may require testing in the field along with farmers' current methods, using local polypropylene bags used by farmers to validate the efficacy found in the laboratory. The two repellent compounds may be dropped for the field experiment due to the fact that they are highly volatile and may not be cost effective to be used by farmers, especially in the hot climate of Kebbi.

# CHAPTER 6

## Field study of the repellent activity of Lem-ocimum-treated double bags against three species of storage pests of sorghum in northern Nigeria

### 6.1 Background

This work describes field experiments carried out in collaboration with small-scale farmers in Kebbi, northern Nigeria. The aim of this study was to test the efficacy of using double bags impregnated with Lem-ocimum, a combination of dried ground plant materials (*Ocimum basilicum and Cymbopogon nardus*), to repel stored crop pests by a method developed in the laboratory at NRI (Chapter 5). The importance of plants from the genus *Ocimum* and *Cymbopogon* as grain protectants is described in Chapters 2 and 5. Regnault-Roger & Hamraoui (1994), Parugrug & Roxas (2008) and Mishra *et al*. (2012) have confirmed their effectiveness against a range of stored crop pests. Furthermore, the efficacy of *C. nardus* and *C. citratus* were confirmed to be effective in reducing storage infestations of certain beetle species (Boeke *et al*., 2004a; Parugrug & Roxas, 2008; Manzoor *et al*., 2011). Surprisingly, no published field research on the efficacy of *O. basilicum* against *Tribolium castaneum* could be found, and most of the literature reviewed in Chapter 2 was related to laboratory-based studies based on essential oil extracts of repellent plants, not whole plants materials. Even though the use of repellent plant materials is one of the main ways farmers in Africa treat their stored grain (Belmain & Stevenson 2001; Golob *et al*., 2002; Deng *et al*., 2009), few publications on the efficacy of ground *O. basilicum* against *T. castaneum* could be found. There is evidence that the efficacy of repellent plant materials can by synergistically enhanced by combining two or more types of plant (Agona & Muyinza, 2003; Operaeke *et al*., 2005; Talukder & Khanana, 2011). The efficacy of a combination of the two plant materials chosen for the research presented in this thesis (*O. basilicum and C. nardus)* has not been previously field-tested.

The findings of surveys described in Chapter 4 indicted that *T. castaneum* is the major beetle pest of sorghum stored by small-scale farmers, followed by *R. dominica*

(Fig. 4.10). The existing traditional method of grain protection is to mix repellent plants directly with the grain and store the mixture either in bags in store-rooms or in granaries. Even though repellent plants were considered to be a cheap and affordable means of pest control by the farmers, most of them commented in the survey that they do not get satisfactory results. Hence, they either change to other control methods or leave their grain untreated. Since small-scale farmers in Kebbi state who keep their grain in store-rooms lose more grain due to insect pests than those that store their grain in typical granaries (Chapter 4, Fig. 4.8) it was decided that this study would focus on improving control of *T. castaneum* in store-rooms.

The laboratory experiments described in Chapter 5 demonstrated that Lem-ocimum treated double bags were highly effective in repelling *T. castaneum;* and therefore, the next step was to test Lem-ocimum treated double bags in famers' grain stores. The aims of the fieldwork presented in this chapter were to develop a model system that illustrates how locally available repellent plant materials could deter target pest species when applied to bags of stored grain held in grain stores typical of a local area. These studies were conducted with the collaboration of 82 farmers who had been consulted during the first two sets of interviews (Chapters 3 & 4). By involving local farmers, and responding to their perceptions, it is hoped that the new method will be more readily acceptable within the local community than if it was presented as a fixed package of recommendations. Therefore, the following chapter presents the findings of a follow-up interview after the field trial, with participating famers, comparing their views on the new approach to the other methods practiced in the area.

The field trials were designed to take into account the following perspectives of the participating farmers; most farmers cannot afford many extra bags, and they may need encouragement before they will spend much time and effort in collecting and drying botanicals and preparing the plant-treated double bags. Also, farmers can earn cash for high quality grain that is well-protected from insect damage. It was decided that a field trial requiring the cooperation and help of so many farmers should be designed to test a method that would be most certain of providing farmers with bags of grain that were well-protected from insect damage, even if only a relatively small proportion of their bags were treated. The benefits of this approach are: at the end of the trial farmers might be encouraged to use botanicals more in the future if they had been shown a method that worked well, the method can be scaled up or down, depending on the number of double bags each farmer could afford each year, and the field trial would be able to obtain cost-effective preliminary data in a single field season, within the time and financial constraints of a PhD-level project.

Therefore, the field trials were designed to answer the following questions:
*How much extra protection from insect infestation and loss of grain is achieved by using a double bag compared to a single bag?
* How much extra protection is provided if double bags of grain are treated with Lem-ocimum, compared to untreated double bags?
*Is there a correlation between the overall number of Lem-ocimum treated double bags in a store and the level of protection? i.e., is there a mass effect of larger numbers of treated double bags providing more protection than expected?
*Does the number of untreated single bags in a farmer's store-room affect the efficacy of Lem-ocimum treated double bags?

## 6.2 Materials and Methods

### 6.2.1 Site of experiments

Field experiments were conducted from September 2011 to March 2012 in three villages (Tondi, Maga and Wasagu) of Kebbi south. This area was chosen because the results of the surveys reported in Chapter 3 indicated that sorghum was the main grain grown and the greatest proportion of farmers already used repellent plant materials as grain protectants (Chapter 3 Fig. 3.9). Moreover, farmers in this area lost more sorghum grain due to insect damage than farmers in the other regions of Kebbi. Thus, farmers in this area would have a greater understanding of the problems and would benefit the most from participation in the field experiments.

### 6.2.2 Selection of farmers and their stores for the trial

A survey was conducted to identify the stores with the best conditions for these experiments, based on the following characteristics:

- Sorghum stored in polypropylene bags in store-rooms
- At least 15 x 60 kg bags of sorghum per store-room
- All sorghum stored in the threshed form

- *Tribolium castaneum* infestations already present in store-rooms
- Levels of infestation similar across the whole group of farmers

A 1kg sample of sorghum was collected from at least three different bags in each store-room, using a 50cm spear (Fig 4.2 & 4.3). The samples were sieved to count the number of live adult *T. castaneum* present. Low infestations (1-25 *T. castaneum* per 1 kg sample of grain) were found in 28 store-rooms, moderate infestations (26-50 *T. castaneum*) were found in 162 store-rooms and high infestations (≥50 *T. castaneum*) were found in 23 store-rooms. The store-rooms with moderate infestations were chosen for the field experiments, because the greatest number of store-rooms fell in this category.

### 6.2.3 Interactive meetings with famers to determine how they would be involved in the experiments

A meeting was held in each village, with an agricultural extension worker from the local government, the village head and the group of 82 farmers who owned the 162 store-rooms that had been chosen for the experiments to agree on how the farmers would participate. After a formal introduction, the author explained to the farmers that he had a new method of pest control that he would like to test in their stores. The new method of protecting stored sorghum with Lem-ocimum treated double bags was explained and the aims of the field work were described, i.e., to test how the new method could be applied to improve control of stored grain infestations. The farmers were encouraged to present their perspectives and ask questions. Most of the farmers agreed to participate in the experiments, and agreed in advance to do nothing to control insect pests in their store-rooms during the experiments.

### 6.2.4 Preparation of experimental storage bags

Farmers normally stored their grain in polypropylene bags large enough to hold 60kg of grain. Considering that the trial would require 420 treated bags, it was not practical or affordable to use the same size bags for experimental purposes. Therefore, it was decided that 5kg bags of sorghum would be the optimal size for experimental bags. Polypropylene bags that are normally used by farmers to store their grain were purchased from farmers and traders, and cut and sewn to a size small enough to contain 5 kg samples of sorghum grain.

### 6.2.5 Plant material and grain treatment

Fresh bags of healthy sorghum were purchased from the King of farmers' store (Yauri village) and fumigated with phosphine for 4 days prior to the start of the experiments to kill any live insects in them. Fresh leaves of *O. basilicum* and *C. nardus* (Fig. 6.1 – 6.3) were collected from various farmers and other locations in Tondi, which were shade-dried for 3-4 days, packed in polypropylene bags and stored in a relatively cool, dark place. On the first day of the experiments, the leaves were ground to a powder with a mortar and pestle used by local farmers. A 50:50 (by weight) combination of ground *O. basilicum* and *C. nardus* (Lem-ocimum) was used to produce 1% w/w of 5kg sorghum grain. This plant powder was mixed with 10g of starch per 100ml of water to make a paste. The starch was used to ensure the plant paste would adhere to the bags. The plant paste was spread all over the outside of half the 5kg bag (Fig. 6.4) and kept to dry in a room for 24 hours. The treated bags were loaded with grain and then inserted into a second bag of the same size and sewn shut with string. The untreated double and single bags used as controls were constructed in the same way, but they were not treated with any plant materials.

**Fig. 6.1 *Ocimum species* growing wild, probably *O. africanum*, Kebbi state, Nigeria.** See Chapter 8 section 8.3.1-8.3.2 for plant identification.

**Fig. 6.2 *Ocimum basilicum* cultivated in back-yard garden, Kebbi State, Nigeria.**

**Fig. 6.3 *Cymbopogon nardus* cultivated in Kebbi state, Nigeria.**

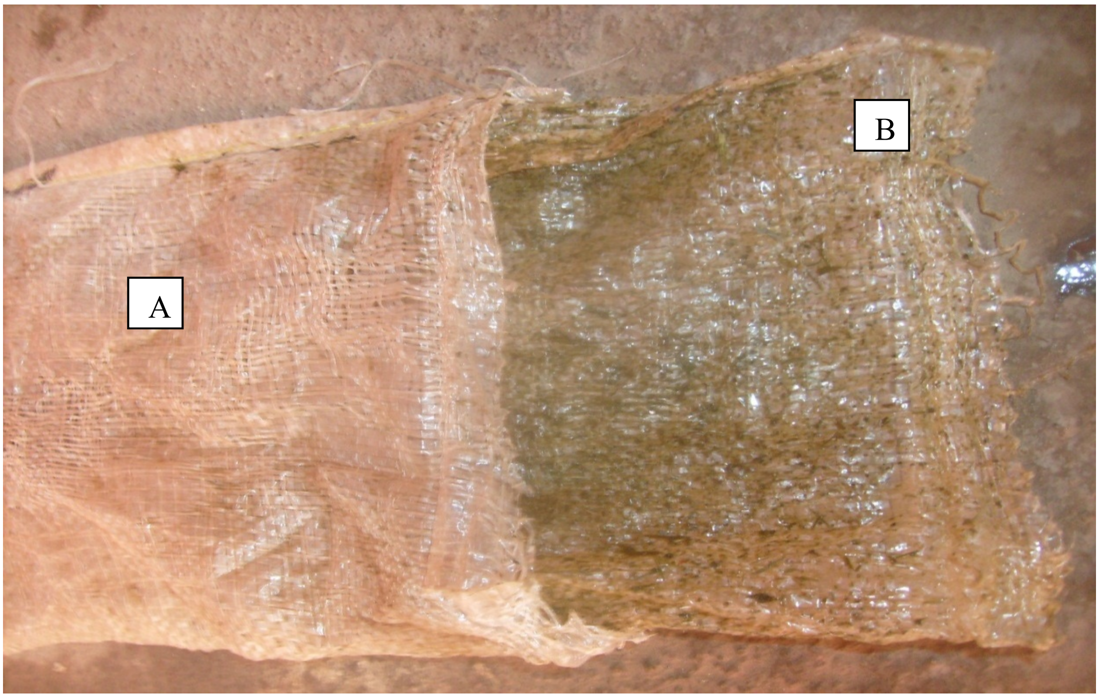



**Fig. 6.4 A new double-bag method for preparing repellent bags**. A layer of repellent paste, consisting of a 50:50 (by weight) combination of the dried powder of two repellent plants (*O. basilicum* and *C. nardus*), is placed between the two bags.
A) Outer, untreated bag and B) treated inner bag that contains the grain.

### 6.2.6 Experimental set-up

The main aim of the field experiments was to determine if *T. castaneum* infestations of sorghum can be controlled by storing the grain in Lem-ocimum treated double bags in store-rooms typical of those used by farmers in the Kebbi area. Moreover, the field experiments were designed to determine if the repellent effect of the treated double bags depends on parameters such as: 1) the absolute number of treated bags in the store-room and 2) the number of untreated bags in store-rooms with moderate levels of pre-existing *T. castaneum* infestation. Therefore, the following two experiments were conducted; 1) Relative efficacy of single bags, double bags and Lem-ocimum treated double bags in repelling *T. castaneum* infestations, and 2) Effect of the number of Lem-ocimum treated bags in a store-room on the level of protection from insect pests in the treated bags.

Of the 162 store-rooms with moderate infestations of *T. castaneum* that were selected for this study, 30 store-rooms were used for Experiment 1 and 120 store-rooms were used for Experiment 2. These store-rooms were owned by 42 participating farmers, with 1-4 store-rooms per farmer.

**Experiment 1: How much extra protection from insect damage do double bags provide compared to single bags? Does lem-ocimum treatment significantly increase the protection of grain stored in double bags?** A subsample of 15 store-rooms from the 162 store-rooms identified with moderate infestations in each of two villages (Tondi and Maga), i.e., 30 store-rooms in all, were used for this experiment. In each store-room, three 5kg bags of sorghum were prepared as follow; one untreated single bag, one untreated double bag and one Lem-ocimum treated double bag were placed on top of the farmers stored grain (a variable number of untreated 60kg single bags) with at least 10cm between each of the test bags (Fig. 6.5). The same distribution of bags was repeated for all 30 store-rooms in the two villages. Single bags were not treated with plant materials, because the overall aim of the project is to find a method of adding repellent plant materials without mixing it with the grain.

**Fig. 6.5 Example of a grain store used for Experiment 1**. One each of a 5kg untreated single bag, an untreated double and a Lem-ocimum treated double bag were placed on top of 60kg untreated farmers single-bags of sorghum grain.

**Experiment 2: Does the number of Lem-ocimum treated double bags in storerooms affect the level of beetle infestations in the treated bags?** To test this, a variable number of 5kg Lem-ocimum treated double bags of threshed sorghum grain were placed in 120 store-rooms. The store-rooms were divided into three groups of

40 store-rooms, and assigned one of three levels of Lem-ocimum treated double bags per room: small (2-3 treated double bags), medium (5-8 treated double bags) and high (9-18 treated double bags). The decision as to how many 5-kg treated double bags were to be added to each store-room was based on a small, medium or high percentage (10%, 33% or 50%, respectively) of the total number of 60kg untreated single bags of grain already kept in each store-room. These percentages are notional, since treated bags held only 5kg of grain and untreated bags held 60 kg of grain. However, this system provided a practical and consistent way of assessing how to prepare each grain store, and resulted in a range of densities of treated bags positioned on top of piles of untreated bags of stored grain. For each level (small, medium and high), the actual number of treated bags added to a store-room depended on the number of single bags of grain the farmer had placed previously in that particular store-room. For example, if 26 bags were found in farmer's store-room, then 10% of 26 bags (rounded to the nearest whole bag), i.e., 3 treated bags were added to that store-room, and placed evenly over the top of the farmer's bags (Table 6.1 and 6.2). The store-rooms treated with the different levels of treated bags (Fig. 6.6- 6.8) were labeled and maintained over a five month observation period.

**Table 6.1 Number of untreated 60kg single bags in each group of small, medium and high level of Lem-ocimum treated double bags.**

| | **Number of treated double bags : single untreated bags of sorghum in store-rooms** | | | |
|---|---|---|---|---|
| | Minimum | Maximum | Mean number of untreated bags | n |
| Level of treated bags | | | | |
| Small (10%) | **2**:15 | **3**:34 | 23.3 | 40 |
| Medium (33%) | **5**:16 | **8**:23 | 21.5 | 40 |
| High (50%) | **9**:17 | **18**:35 | 22.5 | 40 |
| Total | | | | 120 |

Numbers of treated double bags in bold beside numbers of single bags (not in bold).

**Table 6.2 Weight (Kg) of treated double bags and untreated single bags in each group of small, medium and high level of Lem-ocimum treated double bags.**

| | **Weight (kg) of treated double bags : single untreated bags of sorghum in store-rooms** | | | |
|---|---|---|---|---|
| | Minimum | Maximum | Mean weight of untreated bags | n |
| Level of treated bags | | | | |
| Small (10%) | **10**:900 | **15**:2040 | 1398 | 40 |
| Medium (33%) | **25**:960 | **40**:1380 | 1290 | 40 |
| High (50%) | **45**:1020 | **90**:2100 | 1350 | 40 |
| Total | | | | 120 |

Weight of treated double bags in bold beside weight of single bags (not in bold).

Table 6.2 shows the overall weight of treated bags placed in store-rooms beside the overall weight of untreated farmers' bags in store-rooms for different levels of treated bags. This table highlights that although the percentages of treated to untreated bags varied from 10% to 50%, the ratios of weight of treated bags of grain to weight of untreated bags of grain was very much smaller, and that only 1.1% ('small' level) to 4.4% ('high' level) of grain in a storeroom was actually protected by the Lem-ocimum treatment. However, this approach was the most practical way of undertaking a first field test of the efficacy of the new treated double bag method.


**Fig. 6.6 Example of a low level (10%) of 5kg Lem-ocimum treated bags placed on a pile of 30 x 60kg untreated bags in a store-room used for Experiment 2**. In this case, 3 treated bags were placed evenly on top of 30 untreated bags of grain stored by the farmer.


**Fig. 6.7 Example of a medium level (33%) of 5 kg lem-ocimum treated bags placed on a pile of 60 kg untreated bags in a store-room used for Experiment 2**. In this case, 5 treated bags were placed randomly on top of 15 untreated bags of grain stored by the farmer.

**Fig. 6.8 Example of a high level (50%) of 5 kg lem-ocimum treated bags placed on a pile of 60 kg untreated bags in a store-room used for Experiment 2.** In this case, 10 treated bags were placed evenly on top of 20 untreated bags of grain stored by the farmer

### 6.2.7 Grain sampling to assess insect numbers and amount of grain damage

The treated double bags were sampled every 4 weeks for a 5 month period to assess 1) the type and numbers of the three most important beetle species that attack threshed sorghum stored in bags (*T castaneum, R. dominica* and *L. serricorne*) to compare the protectant effect of the new double bag method on a range of important pests, and 2) the amount of grain weight loss. An initial sample of 100g was collected from each of the 5kg treated bags in each store at the beginning of the experiment to determine the baseline weight loss before placing the bags of grain in their respective store-rooms. Thereafter, samples of grain (100g) were collected from the 5kg treated bags in each store, every 4 weeks, to measure the infestation levels of the three target insect species and grain weight loss. For example, if there were four treated bags in a store, each bag was speared three times; the number of live and dead insects of each species was counted, and the average of the three counts was rounded to the nearest whole number and recorded. The numbers of live adults were counted separately from the number of dead insects to find out whether the beetles that gained access to the bags might be reproducing within the treated bags or move through without laying eggs or died. The sample was taken from a different corner of the bag each time. Weight loss was determined using the 'count and weigh' method of estimating weight loss (Adams & Schulten, 1978) described in Chapter 4.3. The data collected were recorded each month and subjected to statistical analysis.

### 6.2.8 A follow-up survey to evaluate the perception of participating farmers on the efficacy and acceptability of the new technology

The views of participating farmers on the efficacy of the new double bag method and their readiness to accept the new method was assessed by a short survey (Figures 6.9-6.11). At the end of the trial, 42 farmers participated in a follow-up questionnaire to find out whether their views had changed since they participated in the experiment. They were asked 1) whether they thought the new method worked, 2) if they thought it protected the grain better than what they had done before, 3) if the new method did reduce grain loss in their experience, did it reduce it enough to encourage them to use the treated double bags in the future, and 4) if the new method was too much work compared to the amount of grain lost?

**Fig. 6.9 Farmers looking at the treated grain after the experiment, comparing different levels of treatments with their untreated grain and giving their views.**

**Fig. 6.10 King of Farmers from Zuru, comparing different treated grain with their stored grain after the 5 month experiment.**

**Fig. 6.11 Farmers looking at the grain after the experiment, comparing different treatments with their untreated grain and giving their views.**

### 6.2.9 Statistical analysis

The data were analyzed using the R statistical software package (version 2.10.0) R Development Core Team (2012). The slopes of the increase in numbers of insects of the three target species (*T. castaneum, R. dominica* and *L. serricorne)* infesting the treated double bags in each store-room over the 5 months of the experiment were calculated, taking into account the use of repeated measures in the study design (i.e., the same treated bags in the same store-rooms were sampled repeatedly over the 5 month experiment). A one-way ANOVA was used to test for significant effects of treatments in Experiment 1 (untreated single bag, untreated double bags and treated double bags) and in Experiment 2 three levels of treated bags (small, medium and high) in store-rooms on beetles infestation and weight loss. The differences between means of specific treatments were analysed for statistical significance using a Tukey HSD test. A linear regression model was used to predict the effect of number of famers untreated single bags on the rate of increase in number of beetles in treated bags in the same store-rooms with levels of treated bags, and multiple regression was used to predict the effect of number of farmers' bags, months of storage and levels of treated bags in store-rooms on the rate of increase in infestation and weight loss in treated bags, using equations shown below. The significance of the interaction was tested using ANCOVA. Data from the questionnaires were summarised in a cross-classifying response; correlating the responses of participating farmers on the efficacy, ease of preparation, implementation and cost effectiveness of the treated double bags that were tested in their store-rooms.

A linear regression model: $\boldsymbol{R = a + bT}$

Where $R$ is the rate of increase of beetles, $a$ & $b$ are constants and T is the number of treated bags.

A multiple regression model: $\boldsymbol{Response = a + bF + cM + dT}$

Where *Response* is either rate of increase of beetles or weight loss; $a$, $b$, $c$ and d are constants, $F$ is the number of farmers bags, $M$ is the number of months and $T$ is the number of untreated bags.

## 6.3 Results

### 6.3.1 Experiment 1: Effect of adding 1) double bagging and 2) double bagging plus Lem-ocimum on the level of insect infestations in bags of stored grain

The main aim of this field experiment was to determine how much better protected sorghum was when stored in a double bag than in a single bag under typical storage conditions, and how much additional protection was provided if the double bag was treated with Lem-ocimum. The three most numerous insect species found infesting stored sorghum in this experiment were *T. castaneum* > *R. dominica* > *L serricorne*.

***Tribolium castaneum***

The results in Fig. 6.12a show the trend in mean number of *T. castaneum* per sample (100g) per month obtained from untreated single bags, untreated double bags and Lem-ocimum treated double bags. The result shows a continuous monthly increase in number of beetles from the first month to the fifth month in all the treated bags. The increase was rapid in untreated single bags followed by untreated double bags and treated double bags. The results in Fig. 6.12b show that the differences in the rate of monthly increase in number of beetles between the three treatment bags was significant (ANOVA; $F=101.5$, df =2 and 87, $p<0.001$), and the difference between the means of each of the three treatments was found to be statistically significant (Tukey HSD test; $p<0.001$). Hence, these results suggest that addition of a second bag on a single bag deters *T. castaneum* from infesting grain and, more importantly, the addition of Lem-ocimum significantly enhances the deterrent properties of double bags against *T. castaneum*.

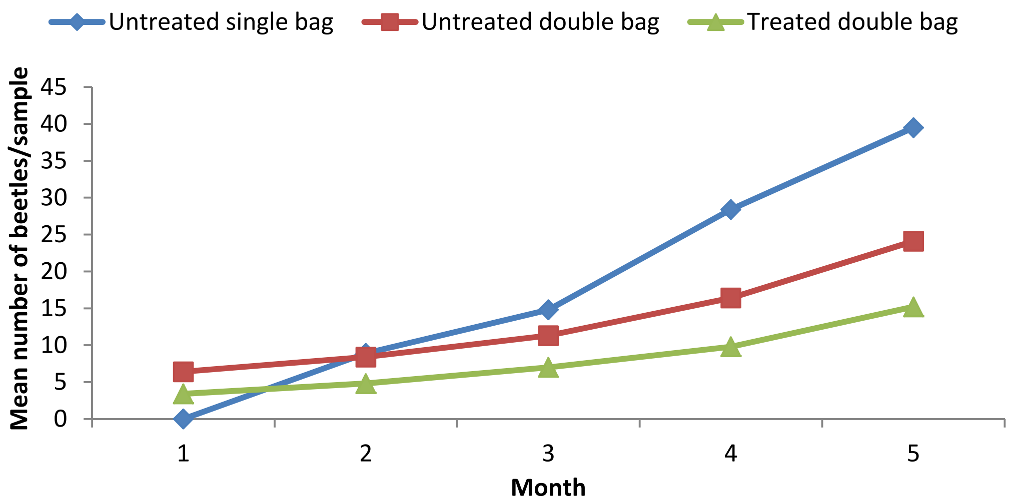



**Fig 6.12a Mean number of *T. castaneum*/sample/month obtained from untreated single bags, untreated double bags and treated double bags (n=30 store-rooms/treatment/month).**

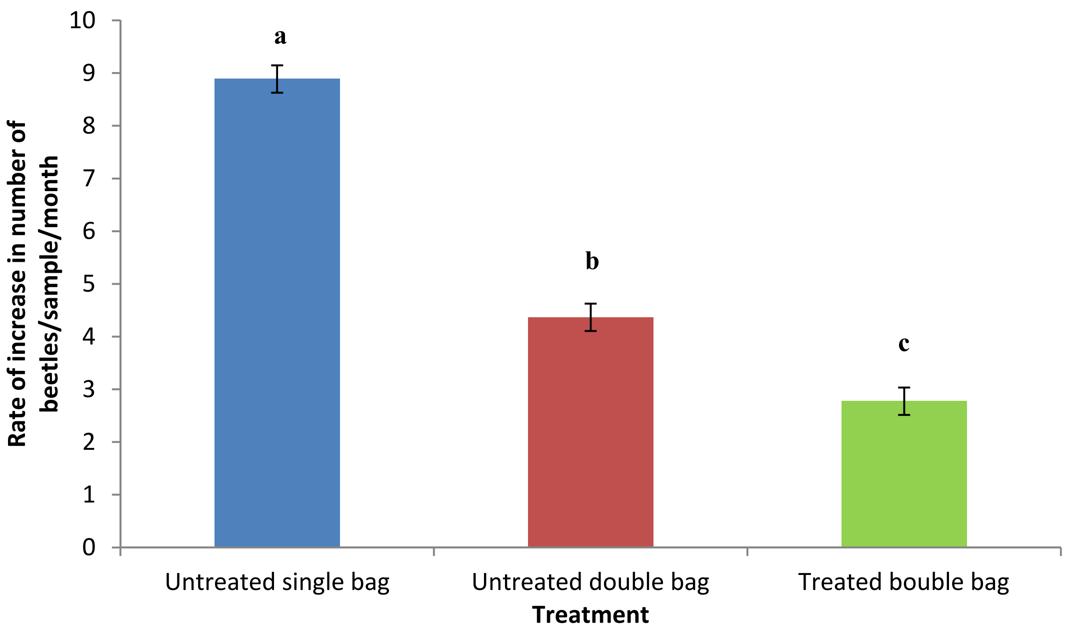



**Fig. 6.12b Mean (±SE) rate of monthly increase in total number of *T. castaneum* found in 100g samples of grain per store-room for each treatment.** A slope was obtained by measuring the rate of increase in number of beetles from the beginning of the experiment to the fifth month of the experiment for each store and analyzed using one way ANOVA. Standard error bars were calculated from the residuals of the ANOVA. n=30 store-rooms/treatment which were each sampled monthly for five months. Overall, the treatment bags had a statistically significant effect on the monthly rate of increase in the number of beetles (ANOVA, $p<0.001$). Bars with different letters are significantly different (Tukey HSD, $p< 0.001$).

Figure 6.12c indicates that there was a monthly increase in number of live *T. castaneum* per store in all the treatments, i.e., a build-up in the population. The differences in the rate of monthly increase in the number of live *T. castaneum* between the treatments was statistically significant (ANOVA; $F=105.9$, $df=2$ and $87$, $p<0.001$). The difference between the means of each of the three treatments was also found to be statistically significant (Tukey HSD test; $p<0.001$). This indicates that the double bag method with added plant materials had a combined repellent effect that can deter live adult *T. castaneum*. The reverse is the case with untreated single bags, which suggests they are more suitable for the live adult *T. castaneum* to penetrate through and multiply in than the other treatments.

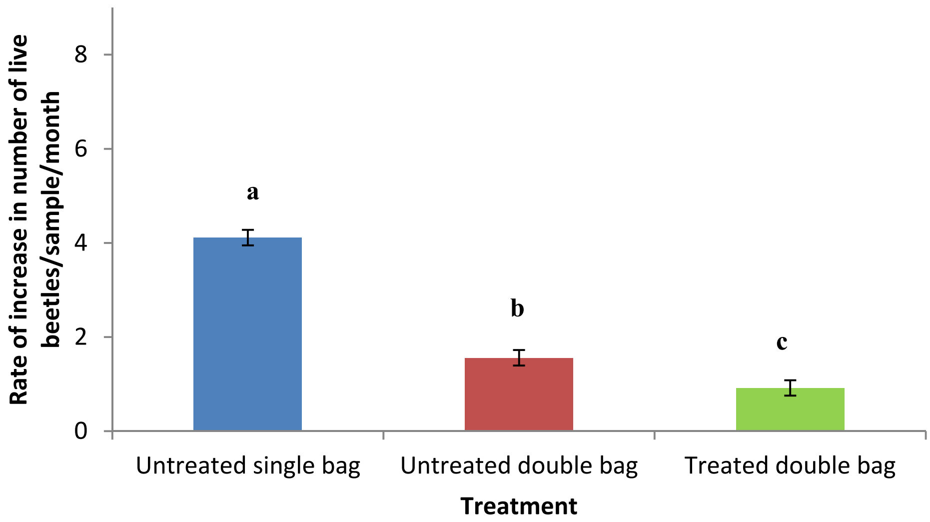



**Fig. 6.12c Mean (±SE) rate of monthly increase in number of live adult *T. castaneum* per sample, per store for each treatment (see text in Fig. 6.12b for details).** Overall, the treatment bags had a statistically significant effect on the monthly rate of increase in the number of live beetles (ANOVA, $p<0.001$, see text). Bars with different letters are significantly different (Tukey HSD, $p< 0.001$, see text**)**

The result in Fig.6.12d indicates that there was also a statistically significant increase in the number of dead adult *T. castaneum* per store for all the treatments (ANOVA; $F=63.27$, df $=2$ and 87, $p<0.001$). However, the difference in the monthly rate of increase in number of dead *T. castaneum* between the means of each of the three treatments was only found to be significant between untreated single and treated double bags, and untreated single and untreated double bags ($p<0.001$), but not between untreated double and treated double bags ($p=0.124$; Tukey HSD), suggesting that the addition of plant material to double bags did not have a significant effect on the mortality rate of *T. castaneum*. However, the differences found between single bags and the two double bag treatments could be due to a larger number of beetles moving around in untreated single bags than the other treatments.

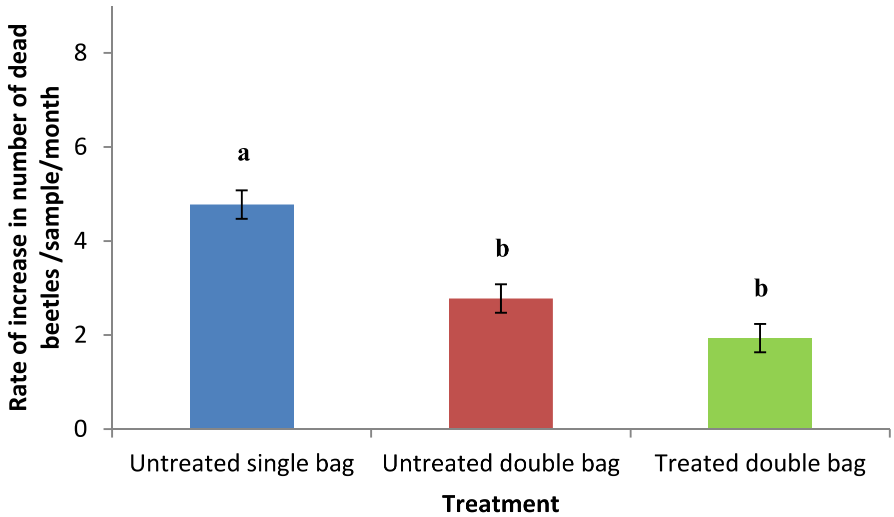



**Fig. 6.12d Mean (±SE) rate of monthly increase in number of dead adult *T. castaneum* found in 100g samples of grain per store for each treatment (see Fig. 6.12b for details).** Overall, the treatment bags had a statistically significant effect on the monthly rate of increase in the number of dead beetles (ANOVA, $p<0.001$, see text). Bars with different letters are significantly different (Tukey HSD, $p< 0.001$, see text**).**

***Rhyzopertha dominica***

*Rhyzopertha dominica* is another pest found in the stored sorghum experiment. The results in Fig. 6.13a show the trend in the mean number of *R. dominica* per sample per month obtained from each of the bag treatments. The results show a continuous monthly increase in mean number of *R. dominica* from the first month to the fifth month of storage in all the treatments. The increase was rapid in untreated single bags followed by untreated double bags and treated double bags. The results in Fig. 6.13b indicate that, the difference in the rate of monthly increase in number of *R. dominica* between the treatments was found to be statistically significant (ANOVA; F=10.37, df=2 and 87, $p<0.001$). The difference between the means of each of the three treatments was only found to be significant between untreated single and treated double, and untreated single and untreated double ($p<0.001$), but not between untreated double and treated double ($p>0.341$), Tukey HSD. This is suggesting that the repellent property of Lem-ocimum had little effect on *R. dominica*.

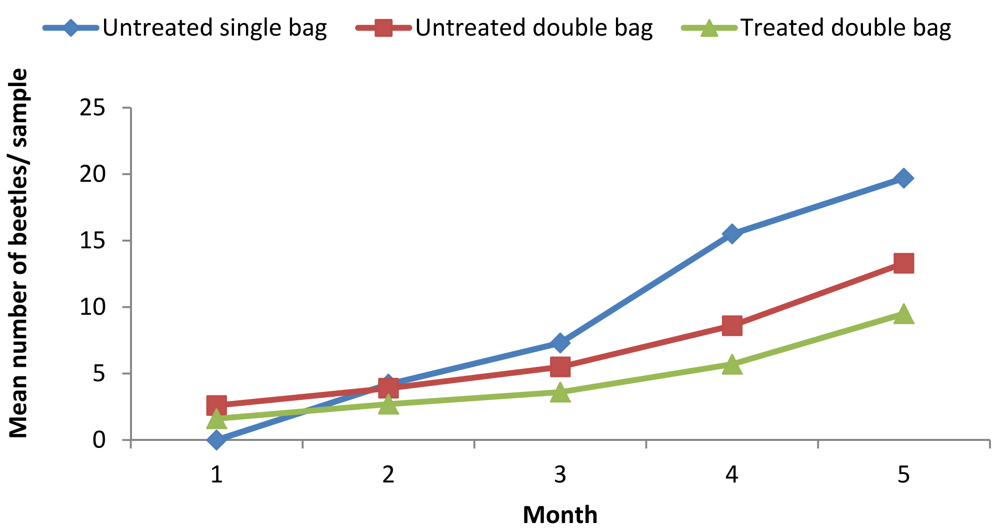



**Fig.6.13a Mean number of *R. dominica*/sample/month obtained from untreated single bags, untreated double bags and treated double bags (n=30 store-rooms/treatments month.**

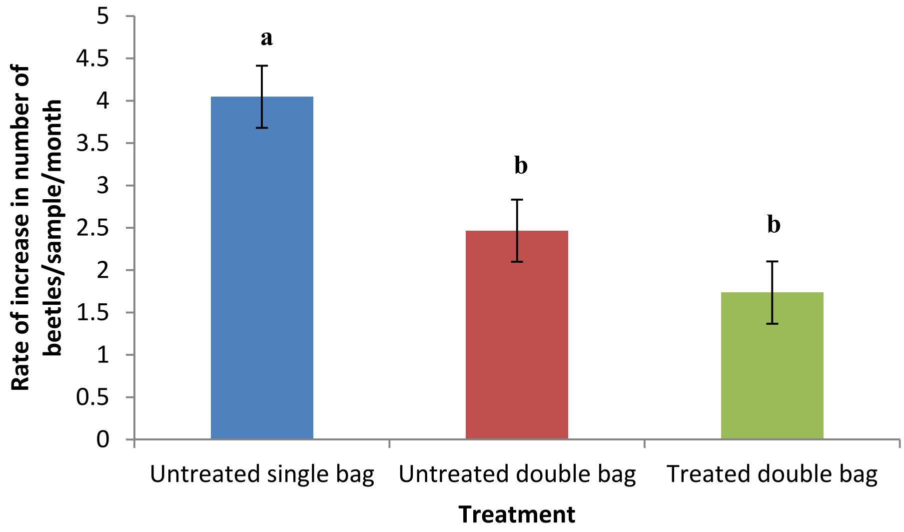



**Fig. 6.13b Mean (±SE) rate of monthly increase in total number *R. dominica* found in 100g samples of grain per store for each treatment (see text on Fig.6.12b for more details).** Overall, the treatment bags had a statistically significant effect on the monthly rate of increase in the number of beetles (ANOVA, $p<0.001$). Bars with different letters are significantly different (Tukey HSD, $p< 0.001$)

Figure 6.13c indicates that there was difference in the rate of increase in number of live adult *R. dominica*, which was found to be statistically significant (ANOVA; $F=13.96$, df=2 and 87, $p<0.001$). However, the difference between the means in each of the three treatments was only found to be significant between the untreated single and treated double bags, and untreated single and untreated double bags ($p<0.001$), but not between untreated double and treated double bags, ($p=0.454$), Tukey HSD. This suggests that addition of Lem-ocimum did not affect the number of live *R. dominica* penetrating into double bags, with or without plant material.

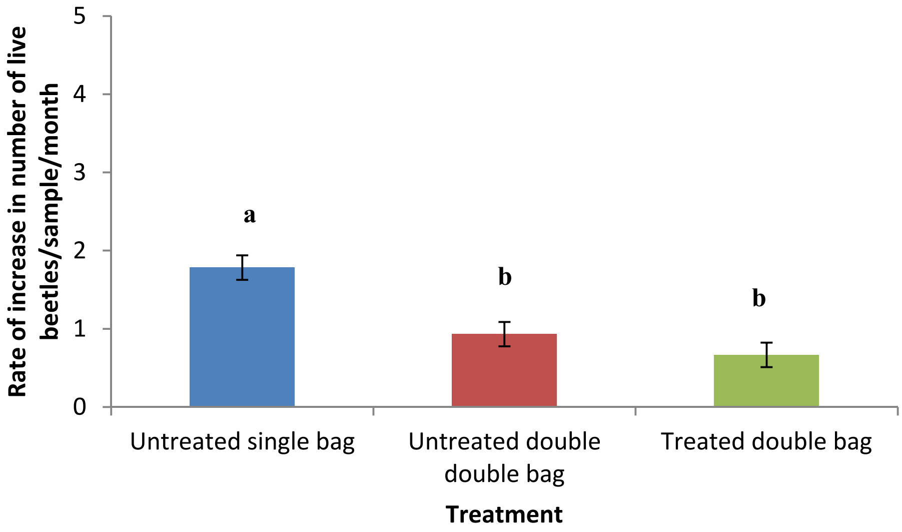



**Fig. 6.13c Mean (±SE) monthly increase in number of live *R. dominica* found in 100g samples of grain per store for each treatment (see text on Fig.6.12b for more details)**. Overall, the treatment bags had a statistically significant effect on the monthly rate of increase in the number of live beetles (ANOVA, $p<0.001$). Bars with different letters are significantly different (Tukey HSD, $p< 0.001$)

The results in Fig. 6.13d indicate that the rate of monthly increase in number of dead *R. dominica* differed between the treatments. These differences were found to be statistically significantly (ANOVA; F=7.07, df=2 and 87, $p<0.01$). However, the differences between the means of the three treatments was only found to be significant between treated double and untreated single (Tukey HSD; $p<0.001$). The low number of dead *R. dominica* found in treated double bags could be because the Lem-ocimum had either a low toxic effect or quite a high repellent effect on *R. dominica* compared to untreated bags, but this would require further investigation to determine the cause of the effect.

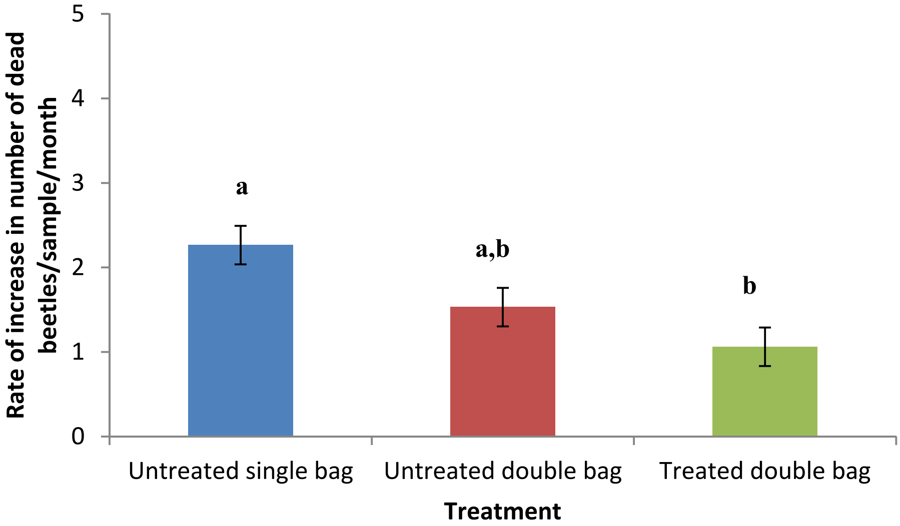



**Fig. 6.13d Mean (±SE) monthly increase in number of dead *R. dominica* found in 100g sample of grain per store for each treatment (see Fig6.12b for more details)**. Overall, the treatment bags had a statistically significant effect on the monthly rate of increase in the number of dead beetles (ANOVA, $p<0.01$). Bars with different letters are significantly different (Tukey HSD, $p< 0.001$).

### *Lasioderma serricorne*

Figure 6.14a shows the trend in the mean number of *L. serricorne* per sample per month obtained from each of the three bag treatments. There was a rapid increase in mean number of beetles in untreated single bags from the first month to the fifth month of storage, with similar slow increase in mean number of the beetles in untreated and treated double bags. The results in Fig. 6.14b indicate that this increase between the treatments was not found to be statistically significant (ANOVA; F=1.71, df =2 and 87, p=0.188). This suggests that the beetle has an equal chance of selecting each treatment, although the number of *L. serricorne* found during the experiment in many stores was very few.

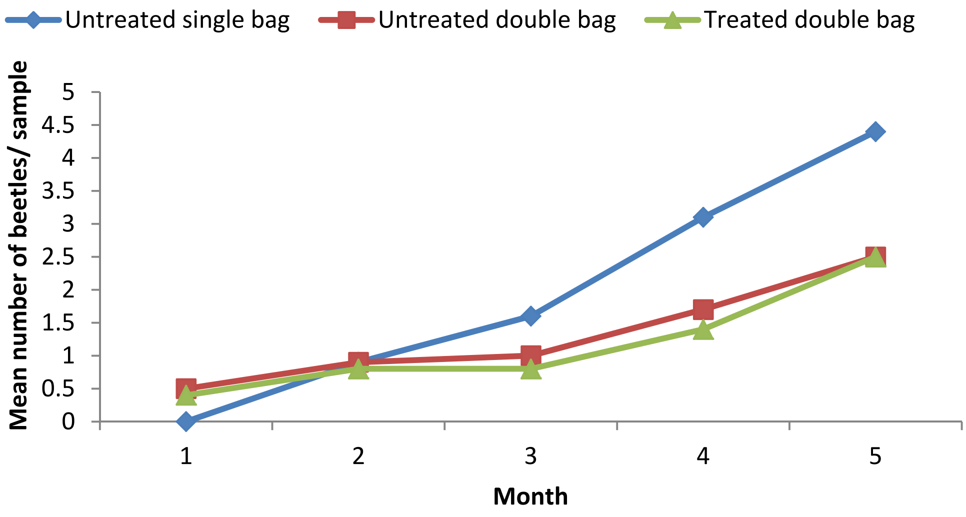



**Fig. 6.14a Mean number of *L. serricorne* /sample/month obtained from untreated single bags, untreated double bags and treated double bags (n=30 store-rooms/treatment).**

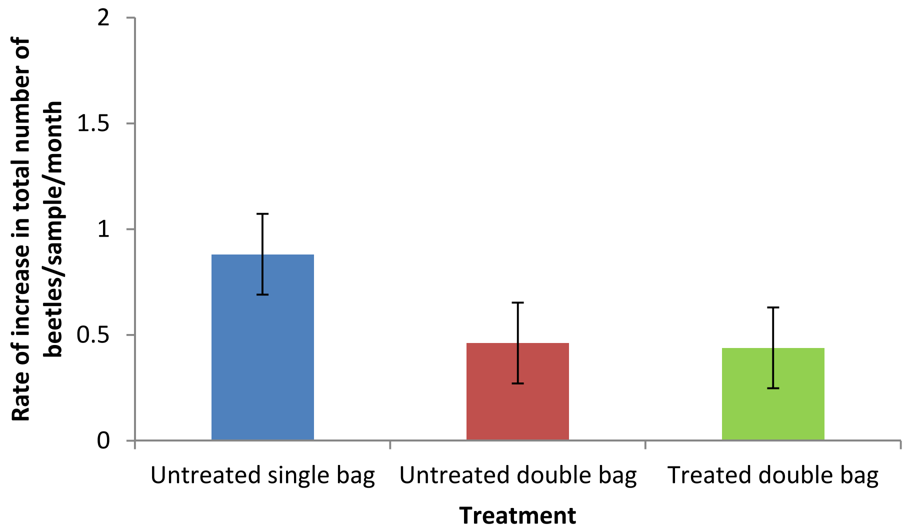



**Fig. 6.14b Mean (±SE) monthly increase in total number of *L. serricorne* found in 100g samples of grain per store for each treatment (see text on Fig.6.12b for more details)**. The difference in the monthly rate of increase in number of beetles between the treatments was not statistically significant ($p>0.05$).

### 6.3.1.1 Effect of adding 5kg double bag and plant material on grain weight loss due to beetle infestation

The results in Fig. 6.15a show the trend in the monthly mean amount of grain weight loss in each of the three bag treatments. The result shows a monthly increase in the mean grain weight loss between the treatments, with untreated single bags having the highest monthly increase in grain weight loss compared to the other bag treatments over the 5 months of storage. The results show a similar increase in weight loss from month 1 to month 2 for all the treated bags, however, from month 2 to month 3 shows a greater increase in weight loss in untreated single and untreated double bags, which later steadily shoots up from month 3 to month 5. The increase was slow for treated double bags until the fourth month when the slope increased continuously to fifth month. This may suggest an increase in the population and activity of the beetles. The results in Figure 6.15b indicate that the difference in the rate of monthly increase in weight loss between the samples of the three bag treatments was found to be statistically significant (ANOVA; $F=23.5$, $df=2$ and $87$, $p<0.001$), and the differences between the means of the three treatments were found to be significant (Tukey HSD; $p<0.001$). The double bag treated with Lem-ocimum had the lowest monthly increase in weight loss. Thus, addition of Lem-ocimum in between the layers of a double bag appears to repel pest species from infesting the grain in the bag.

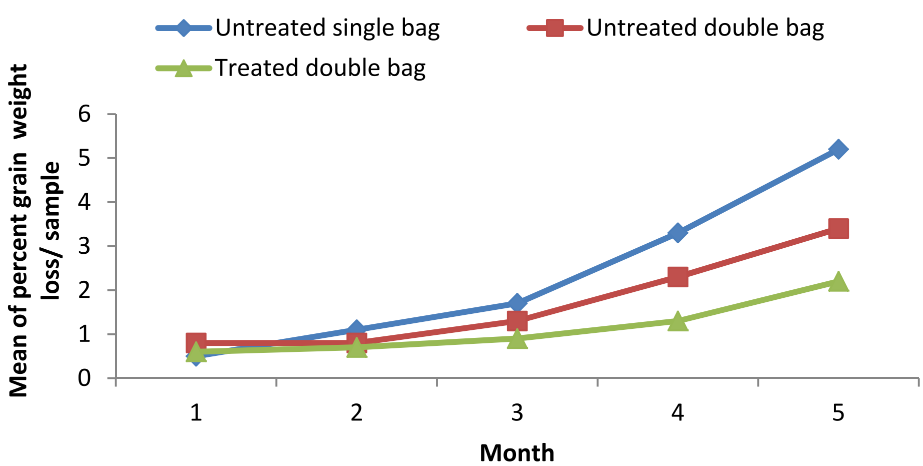



**Fig. 6.15a Mean percent weight loss/sample/month obtained from untreated single bags, untreated double bags and double bags treated with plant materials (n=30 store-rooms/treatment).**

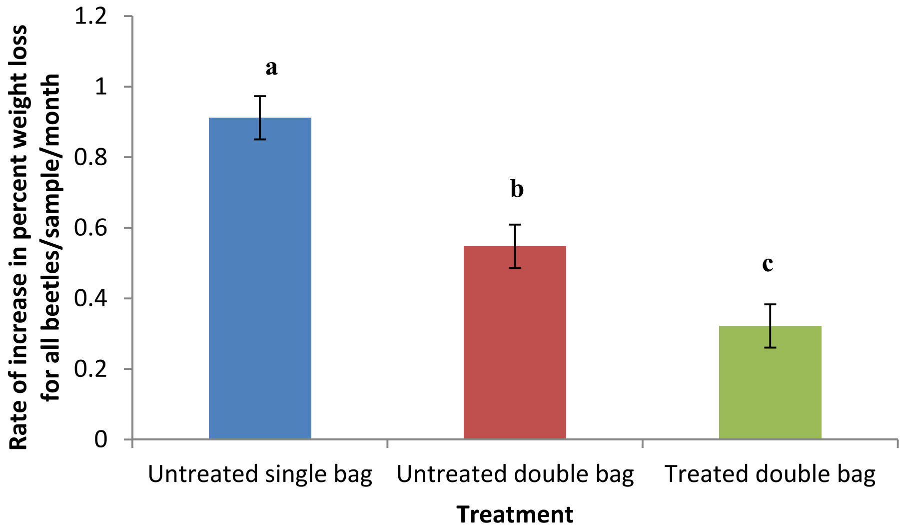



**Fig. 6.15b Mean (±SE) monthly increase in weight loss due to all beetles in 100g samples of grain per store for each treatment (see text on Fig.6.12b for more details)**. Overall, the treatment bags had a statistically significant effect on the monthly rate of increase in weight loss (ANOVA, $p<0.001$). Bars with different letters are significantly different (Tukey HSD, $p< 0.001$).

### 6.3.2 Experiment 2: The effect of storing a variable number of 5kg treated double bags in sorghum store-rooms on the rate of growth of insect infestations for three species; *T. castaneum, R. dominica* and *L. serricorne*

The main aim of this experiment was to determine how well the new double bag method (i.e., Lem-ocimum placed between the two bags) protected sorghum that was initially clean from infestation by the three target species. The untreated bags of sorghum in the farmers' store-rooms were considered to be the primary source of insect infestations, and the aim was to determine how well the treated bags could protect grain from insects migrating into the treated bags mainly from the untreated bags. The two variables tested for their effect on infestation levels and grain weight loss in treated bags were: 1) three levels of treated bags (small, medium or high) in a store-room, and 2) variable numbers of untreated bags in the store-rooms.

Double bags treated with Lem-ocimum were added to specific farmers' store-rooms at three treatment levels: small (2 -3 treated double bags per store-room), medium (5-8) or high (9 – 18). The store-rooms used for this experiment were chosen because the untreated bags of grain in them were known to have an initial moderate level of *T. castaneum* infestation at a density of 26-50 adults per spear sample (see section

6.2.2, above). In each of the 40 store-rooms selected, the participating farmers had anywhere from 15–36 untreated 60kg bags of threshed sorghum. The farmers had agreed not to protect their grain from insect pests, and, therefore, the untreated bags were considered to present a reasonably standardized level of infestation pressure on the treated double bags.

#### 6.3.2.1 The effect of levels of treated bags on absolute insect numbers and the rate of increase in insect numbers over time

***Tribolium castaneum***

The results in Fig. 6.16a show the trend in the mean rate of monthly increase in number of *T. castaneum* per month in store-rooms with three levels of treated bags (small, medium or high). It shows that there was a continuous increase in the mean number of beetles collected over the study period in all the stores.. The increase in mean number of the beetles started very slow at the beginning and then rapidly increased from month 4 to month 5 with a distinct difference between the three levels of treated bags. The results in Fig. 6.16b indicate that the level of treated bags placed in each store-room had a statistically significant effect on the rate of monthly increase in numbers of beetles found in the treated bags (ANOVA; F=16.13, df=2 and 117, p>0.001): the higher the level of treated bags added to a store-room, the lower the rate of increase in numbers of beetles found in the treated bags. The difference between the means of the three treatments (small, medium and high) was found to be statistically significant (Tukey; p<0.01).

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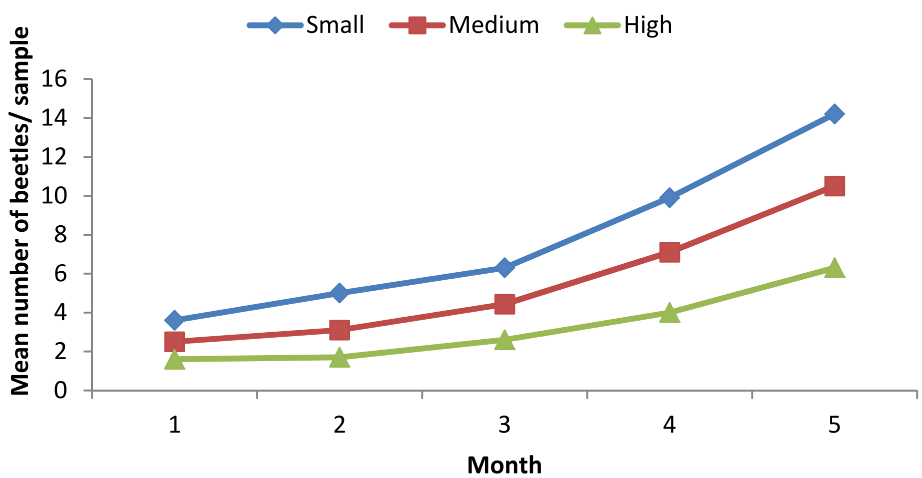


**Fig. 6.16a Trend in the mean number of *T. castaneum*/sample/month obtained from store-rooms with three levels of treated bags (small, medium or high).**

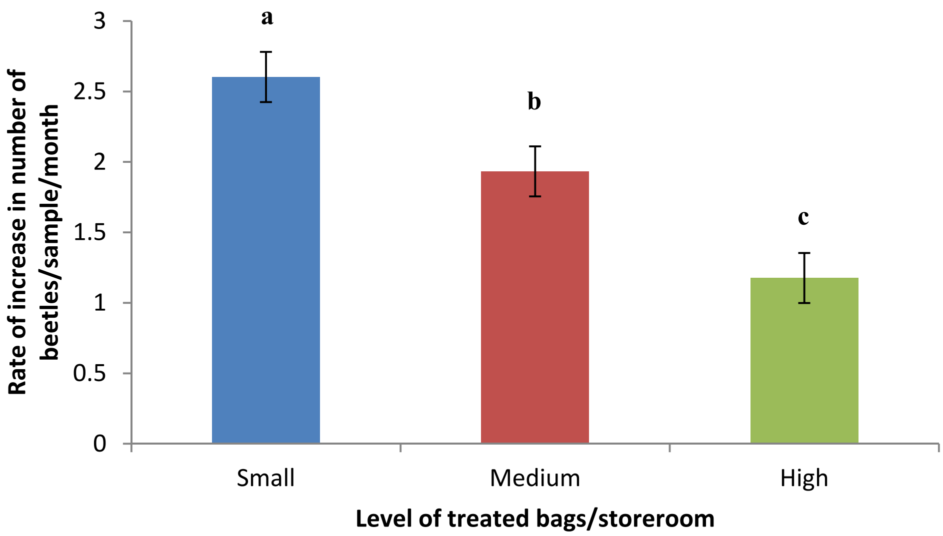


**Fig. 6.16b Mean (±SE) rate of monthly increase in number (i.e., slope) of *T. castaneum* per 100g samples of grain per store-room with a small, medium or high level of treated bags per untreated bags.** The slope was obtained by measuring the rate of change in beetle numbers from the beginning of the experiment to the 5th month for each store-room. Standard error bars were calculated from the residuals of the ANOVA; n=40 stores, which were each sampled monthly over five months. The treatments had a statistically significant ($p<0.001$) effect on the monthly rate of change in numbers of beetles. Bars with different letters are significantly different (Tukey HSD, $p< 0.01$).

The data for numbers of beetles found in treated bags was analyzed in greater detail by analyzing 'live' and 'dead' beetles separately to determine whether the beetles established colonies within the treated bags, or tended to move through the bags without laying eggs. Figure 6.16c shows the mean monthly change in the number of live adult *T. castaneum* per store-room per treatment. The level of treated bags (small, medium or high) had a significant effect on the rate of monthly increase in numbers of live *T. castaneum* (ANOVA; $F=14.36$, df = 2 and 117, $p<0.001$). The difference between the means of each of the three treatments was found to be significant only between high and medium levels of treated bags, and high and small levels of treated bags ($p<0.001$), but not between small and medium levels of treated bags ($p=0.104$), Tukey HSD. Hence, there were fewest live adults *T. castaneum* found in treated bags when the level of treated bags was highest. This suggests that there may have been a 'mass effect' of the presence of the Lem-ocimum repellent plant volatiles in store-rooms with the highest levels of treated bags; overall, the higher the level of Lem-ocimum in a store-room, the fewer *T. castaneum* were found in the treated bags.

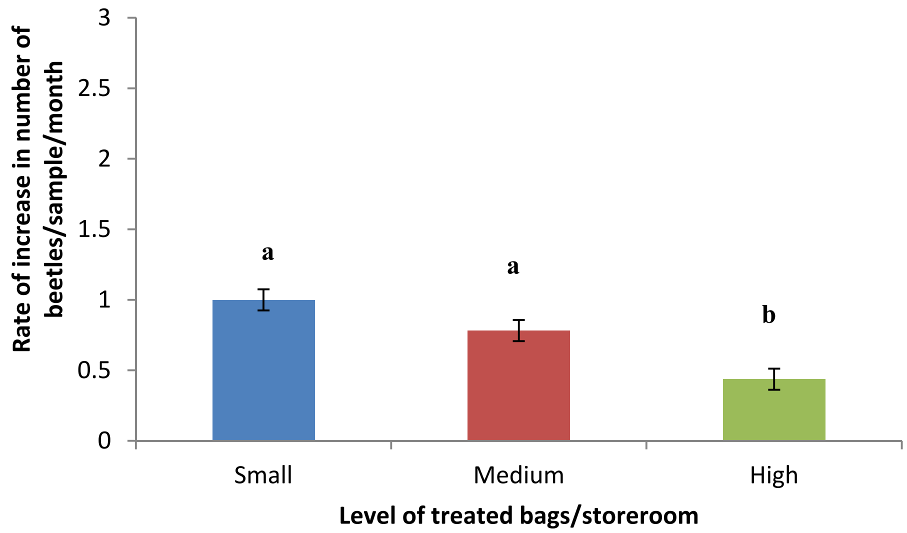


**Fig. 6.16c Mean (±SE) monthly increase in numbers of live adult *T. castaneum* per 100g samples of grain per store-room for each level of treated bags.** Overall, the level of treated bags had a statistically significant effect on the monthly rate of increase in the number of live beetles (ANOVA, $p<0.001$). Bars with different letters are significantly different (Tukey HSD, $p< 0.001$).

Figure 6.16d shows that there was also a mean monthly increase in the number of dead adult *T. castaneum* per store-room per treatment. This difference in the rate of increase in number of dead *T. castaneum* between the three treatments was found to be significant (ANOVA; $F=15.92$, $df=2$, and 117, $p<0.001$), and the difference between the means in each of the three treatments was also significant (Tukey HDS; $p<0.001$).

Overall, the rate of increase in live and dead *T. castaneum* was lowest when the level of treated bags was highest, which is what one might expect, since this treatment added the most repellent plant material to the store-rooms of the three treatments. It is interesting to note that the monthly rate of increase in live beetles was less than for dead beetles for every level of treated bags, and this increase was found to be statistically significant (ANOVA; $F=27.4$, $df=1$ and 234, $p<0.0001$), which suggests that the live beetles did not establish colonies (i.e. lay eggs) in the treated bags.

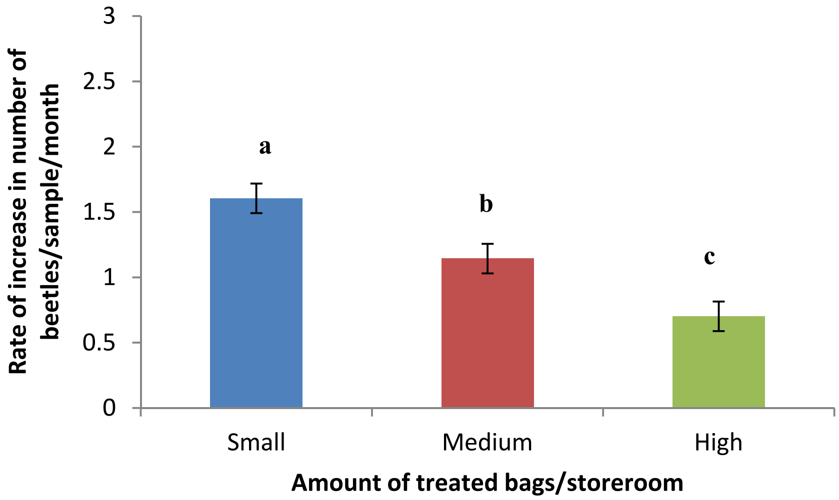


**Fig. 6.16d Mean (±SE) monthly increase in numbers of dead adult *T. castaneum* per 100g samples of grain per store-room for each level of treated bags.** Overall, the amount of treated bags had a statistically significant effect on the monthly rate of increase in the number of dead beetles (ANOVA, $p<0.001$). Bars with different letters are significantly different (Tukey HSD, $p< 0.001$).

***Rhyzopertha dominica***

The results in Fig. 6.17a show a trend in the mean rate of monthly increase in the number of *R. dominica* in store-rooms with all three levels of treated bags (small, medium or high). It shows that there was a continuous increase in the number of beetles collected over the study period in all the stores with three levels of treated bags. The increase in the mean number of the beetles started very slowly at the beginning and then rapidly increased to the fifth month. The results in figure 6.17b indicate that the levels of treated bags placed in each store-room had a statistically significant effect on the rate of monthly increase in the number of the beetles found in the treated bags (ANOVA; $F=5.52$, $df=2$, and 117, $p<0.01$). The differences between the means of each of the three treatments was found to be significant only between stores with high, and between stores with medium and small number of treated bags ($p<0.01$), but not between stores with medium and small number of treated double bags ($p>0.05$), Tukey HSD. This suggests that the level of treated bags in store-rooms was more effective in store-rooms with high levels of treated bags.

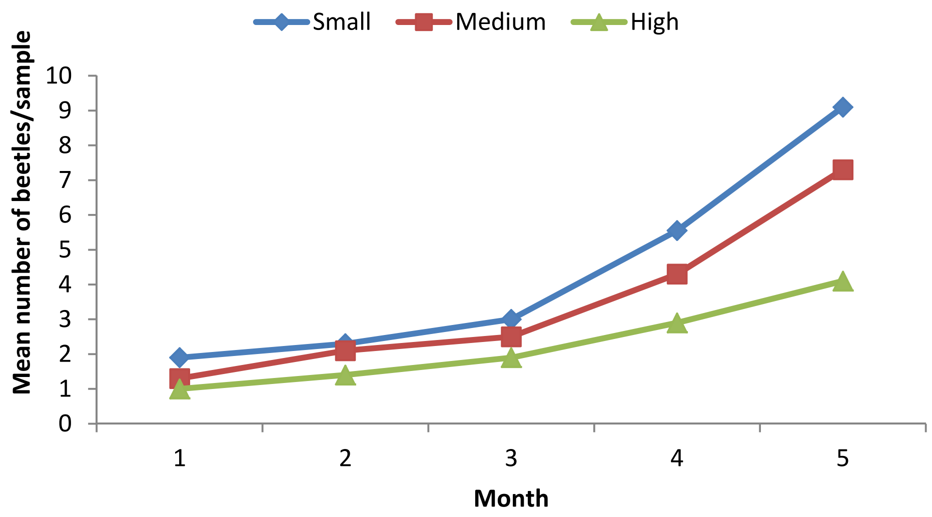


**Fig. 6.17a Mean number of *R. dominica*/sample/month/store-rooms for each level of treated bags (n=40 store-rooms/treatment).**

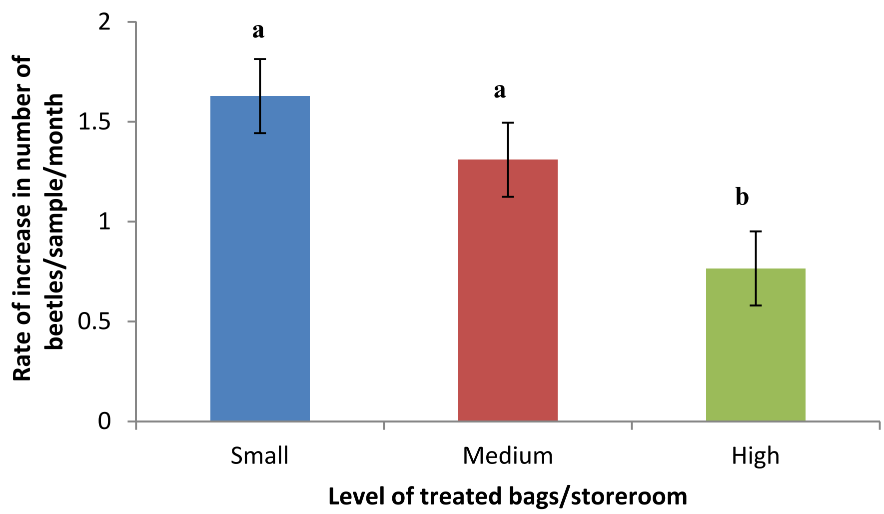


**Fig. 6.17b Mean (±SE) monthly rate of increase in total number of adult *R. dominica* per 100g samples of grain per store-room for each level of treated bags.** Overall, the level of treated bags had a statistically significant effect on the monthly rate of increase in the number of beetles (ANOVA, $p<0.001$). Bars with different letters are significantly different (Tukey HSD, $p< 0.001$).

Figure 6.17c shows the mean monthly change in number of live adult *R. dominica* per store-room per treatment. The level of treated bags (small, medium or high) had a significant effect on the rate of monthly increase in number of live adult *R. dominica* (ANOVA; F=3.53, df=2, and 117, p<0.05). The difference between the means of each of the three treatments was found to be significant only between high and small levels of treated double bags (p<0.01), but not between small and medium, and medium and high levels of treated double bags (p>0.05), Tukey HSD. Hence, there were fewest live adults *R. dominica* found in treated bags when the level of treated bags was highest.

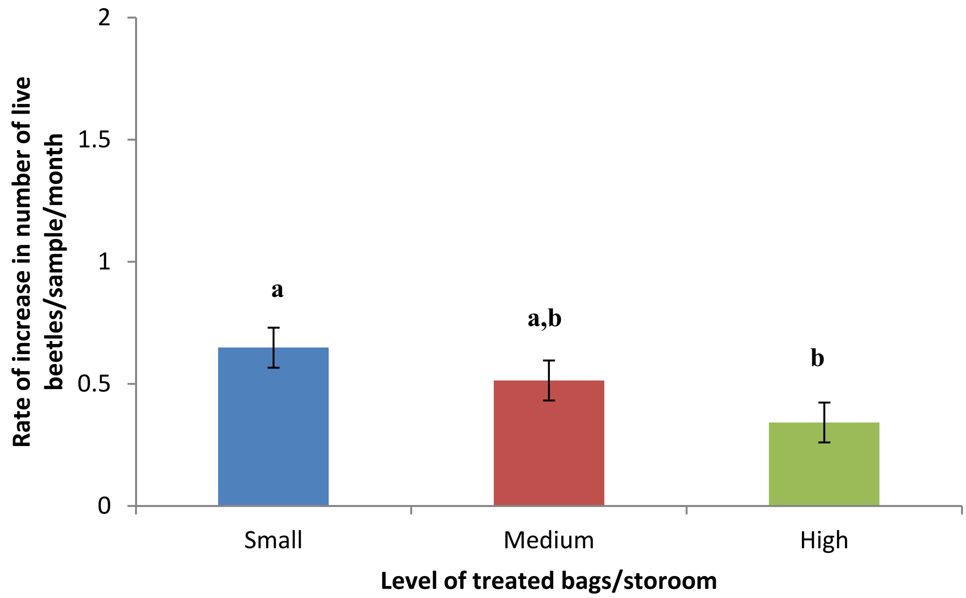


**Fig. 6.17c Mean (±SE) monthly increase in number of live adult of *R. dominica* per 100g samples of grain per store-room for each level of treated bags.** Overall, the level of treated bags had a statistically significant effect on the monthly rate of increase in the number of live beetles (ANOVA, p<0.05). Bars with different letters are significantly different (Tukey HSD, p< 0.01).

The results in Fig. 6.17d show that there was a mean monthly increase in the number of dead adult *R. dominica* per store per treatment. The difference in the rate of increase between the treatments was found to be significant (ANOVA; F=5.28, df=2 and 117, $p<0.01$). However, the difference in the rate of monthly increase in the number of dead *R. dominica* between the means of each of the treatments was only significant between high and small levels of treated bags ($p<0.01$), but not, between small and medium, and high and medium levels of treated bags ($p>0.05$), Tukey HSD.

Overall, the rate of increase in live and dead *R. dominica* was lowest when the level of treated bags was highest, and the rate of monthly increase in live beetles was less than for dead beetles for every level of treated bags, which suggests that the live beetles did not establish colonies (i.e. lay eggs) in the treated bags.

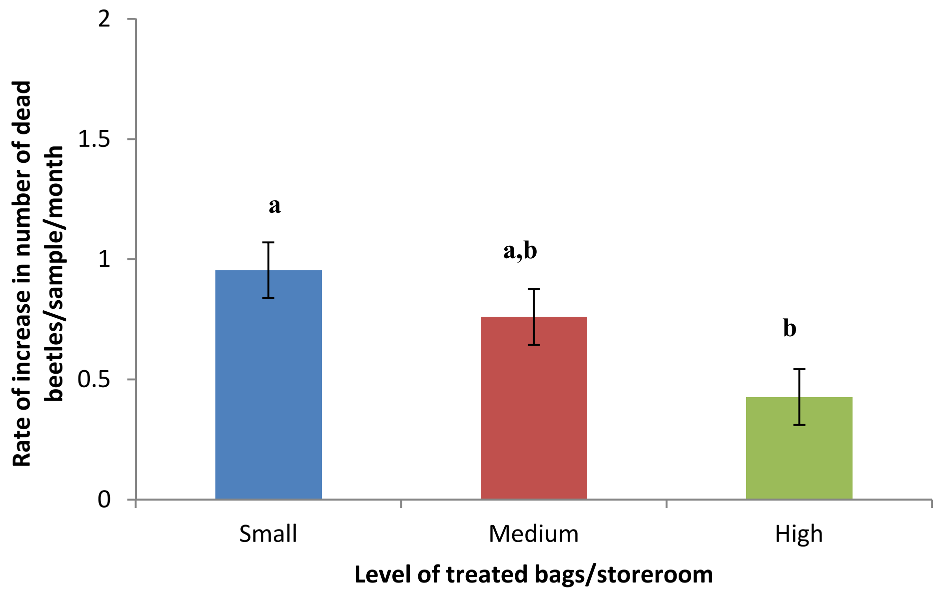


**Fig. 6.17d Mean (±SE) monthly increase in number of dead adult of *R. dominica* per 100g samples of grain per store-room for each level of treated bags**. Overall, the level of treated bags had a statistically significant effect on the monthly rate of increase in the number of dead beetles (ANOVA, $p<0.01$). Bars with different letters are significantly different (Tukey HSD, $p< 0.01$).

*Lasioderma serricorne*

Figure 6.18a shows a trend in the mean rate of monthly increase in number of *L. serricorne* per month in store-rooms with three levels of treated bags. It shows that there was a continuous increase in the mean number of beetles collected over the study period in all the store-rooms with the three levels of treated bags. However, the result in Fig. 18b indicates the levels of treated bags placed in each store-rooms had no statistical significant effect on the rate of monthly increase in the number of beetles found in the treated bags (ANOVA; $F=0.28$, $df=2$ and $117$, $p>0.05$). This suggests that the beetles were not affected by the deterrence property of double bag and Lem-ocimum, although, this may relate to the low numbers of beetles infesting the farmers' stores.

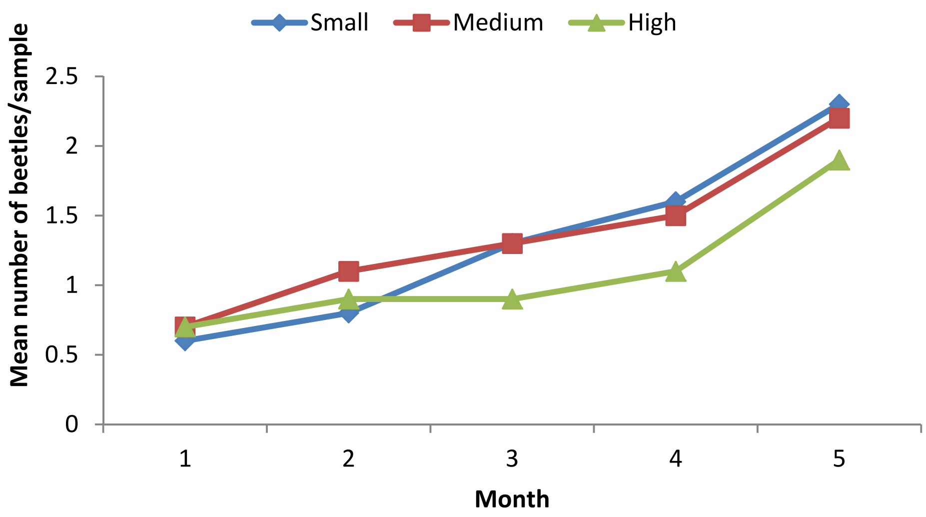


**Fig. 6.18a Mean number of *L. serricorne*/sample/month/store-rooms for each level of treated bags (n=40 store-rooms/treatment).**

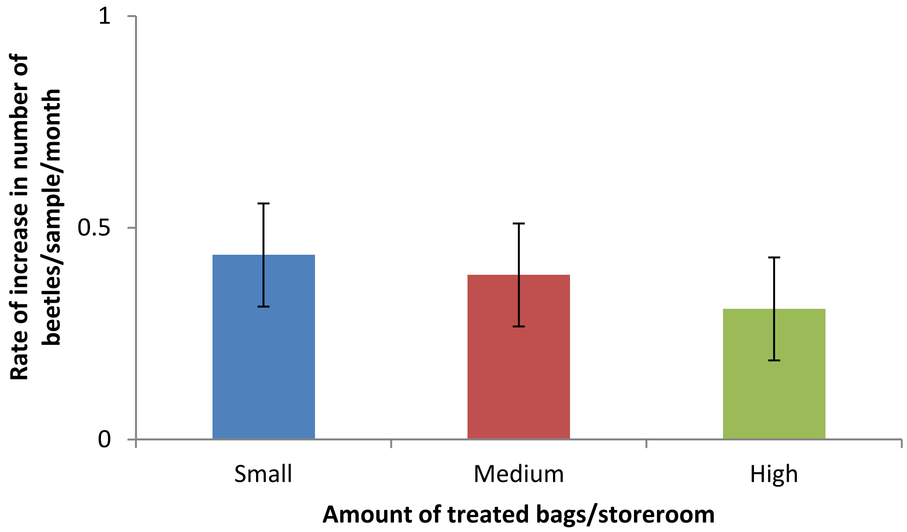


**Fig. 6.18b Mean (±SE) rate of monthly increase in number of adult *L. serricorne* per 100g samples of grain per store-room for each level of treated bags.** Overall, the level of treated bags had no statistically significant effect on the monthly rate of increase in the number of beetles (ANOVA, $p>0.05$).

### 6.3.2.2 Effect of adding different levels of treated double bags to store-rooms on grain weight loss due to insect infestations

Figure 6.19a shows the trend in the amount of mean monthly weight loss of grain due to insect species in store-rooms containing small, medium or high levels of treated bags. These results show that, overall, the mean monthly increase in the mean grain weight loss in all the stores with all three treatment levels of treated bags was relatively low. However, there was a continuous monthly increase in weight loss from the beginning to the fifth month for store-rooms with all three levels of treated bags, although the rate of increase is slower in store-rooms with high levels of treated bags. The results in Figure 6.19b indicate clearly that the monthly increase in weight loss demonstrated in Fig.6.19a was always positive, irrespective of the level of treated bags that were added to the store-rooms. The results show that the level of treated bags had a significant effect on the rate of increase in weight loss over time (ANOVA; $F=44.77$, df =2 and 117, $p<0.001$). Moreover, the difference between the means of each of the treatment levels was significant only between store-rooms with high and small levels of treated bags, and high and medium levels of treated bags ($p< 0.01$), but not between store-rooms with small and medium levels of treated bags ($p>0.05$), Tukey HSD.

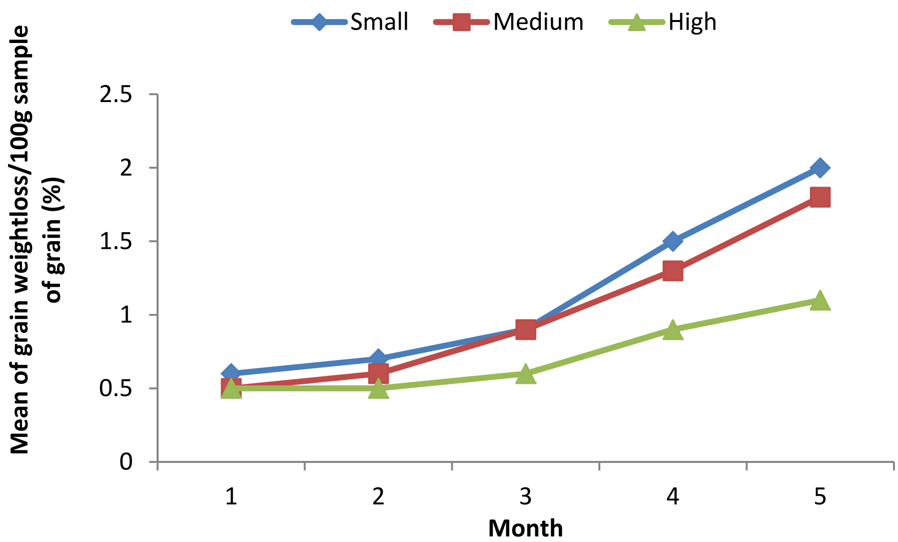


**Fig. 6.19a Mean percent amount of grain weight loss/month/100g samples of grain per store-room for each level treated bags (n=40 store-rooms/treatment).**

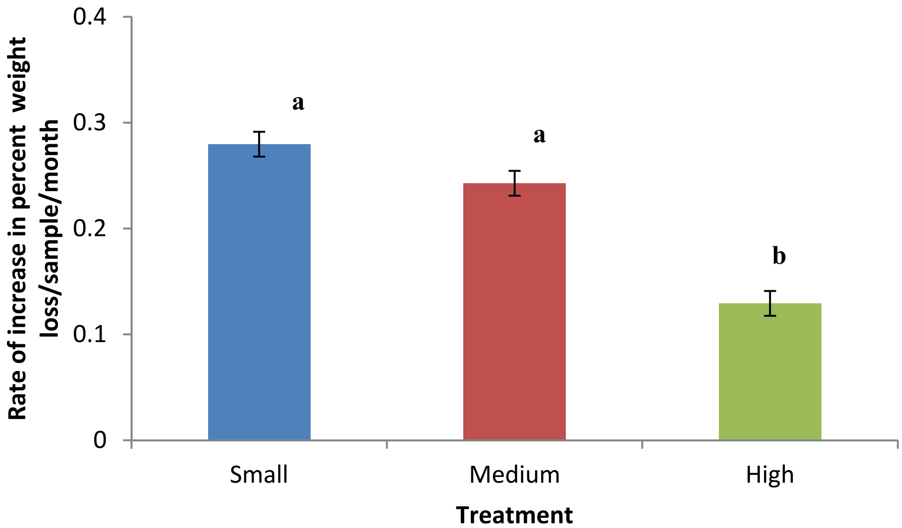


**Fig. 6.19b Mean (±SE) rate of monthly increase in amount of weight loss by insects per 100g samples of grain per store-room for each level of treated bags.** Overall, the level of treated bags had a statistically significant effect on the monthly rate of increase in the amount of weight loss (ANOVA, $p<0.001$). Bars with different letters are significantly different (Tukey HSD, $p< 0.01$).

### 6.3.2.3 Effect of the number of untreated bags in a store-room on the rate of increase in number of insects in the treated bags over time

The untreated bags of grain in the farmers' store-rooms were considered to be the main source of insect infestations. Their baseline levels of infestation were measured at the beginning of the experiment, and only storerooms with moderate infestations in the untreated bags were used (see section 6.2.2). The relationship between insect infestation in the treated bags and the number of untreated bags stored in the same store-room was investigated.

Figure 6.20 shows that, surprisingly, there was an **inverse** linear relationship between the numbers of untreated bags in a store-room and the rate of increase of *T. castaneum* in the treated bags, and that this relationship is dependent on the level of treated bags as a percentage of all the bags present, only reaching statistical significance for high and medium levels (Table 6.2). For larger stores, a given proportion of treated bags means a larger actual number of treated bags, i.e., absolute amount of Lem-ocimum present, and this may be the root cause of the increased effectiveness of treated bags in large stores.

**Table 6.3 Levels of treated bags**

| Level of treated bags | Number of treated bags added to a store-room as a percentage of total number of untreated bags in the store | Slope of regression line | t statistic for slope parameter in ancova | p-value |
|---|---|---|---|---|
| High (50%) | 9-17 | -0.15178 | 3.5755 | p<0.01 |
| Medium (33%) | 5-8 | -0.16939 | 3.99034 | p<0.01 |
| Small (10%) | 2-3 | -0.01119 | 0.2636 | NS |

The greater the number of untreated bags in a store-room the lower the rate of increase in the number of beetles in the treated bags for each store-room. An analysis of covariance had significant main effects for the untreated bag number covariate ($F=10.5$, $df=1$ and 114, $p=0.0016$) and for the treated bag level factor ($F=21.4$,$df=2$ and 114, $p<0.0001$). The interaction term was also significant ($F=6.0$, $df=2$ and 114, $p<0.01$) showing that the best fit model had different slope parameters for the different levels of treated bags (Table 6.2).

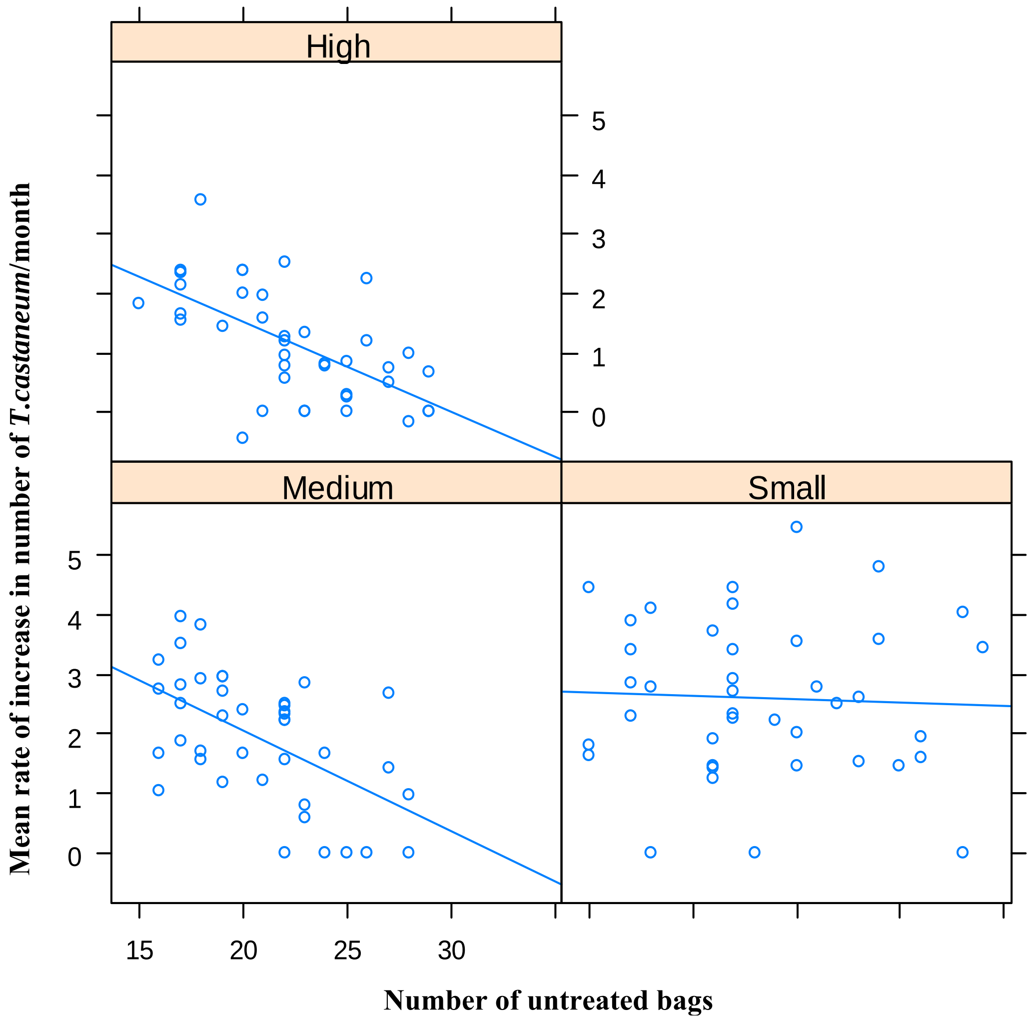


**Fig.6.20 The interaction between levels of treated bags and numbers of untreated bags in store-rooms on the mean monthly rate of increase in numbers of adult *T. castaneum* found per 100g sample of grain over the five month experiment.** Overall there was a significant inverse relationship ($p<0.001$) in the rate of increase in number of beetles in the treated bags as the number of untreated bags increased. There was significant main effect for untreated bag number, treated bag levels and their interaction ($p<0.01$, ANCOVA).

Figure 6.21 shows that there was an inverse linear relationship between the numbers of untreated bags in a store-room and the rate of increase of *R. dominica* in treated bags. This relationship was found to have no significant effect for all the store-rooms with small ($p=0.528$), medium ($p=0.641$) and high levels of treated bags ($p=0.315$). However, the ANCOVA had significant main effect for treated bags level factor ($F=6.023$, df=2 and 114, $p=0.003$), but not for the untreated bags number covariate ($F=3.204$, df=1 and 114, $p=0.076$). The interaction term was also not significant ($F=0.071$, df=2 and 114, $p=0.974$), showing that the best fit line for the model had the same slope parameters for the different levels of treated bags.

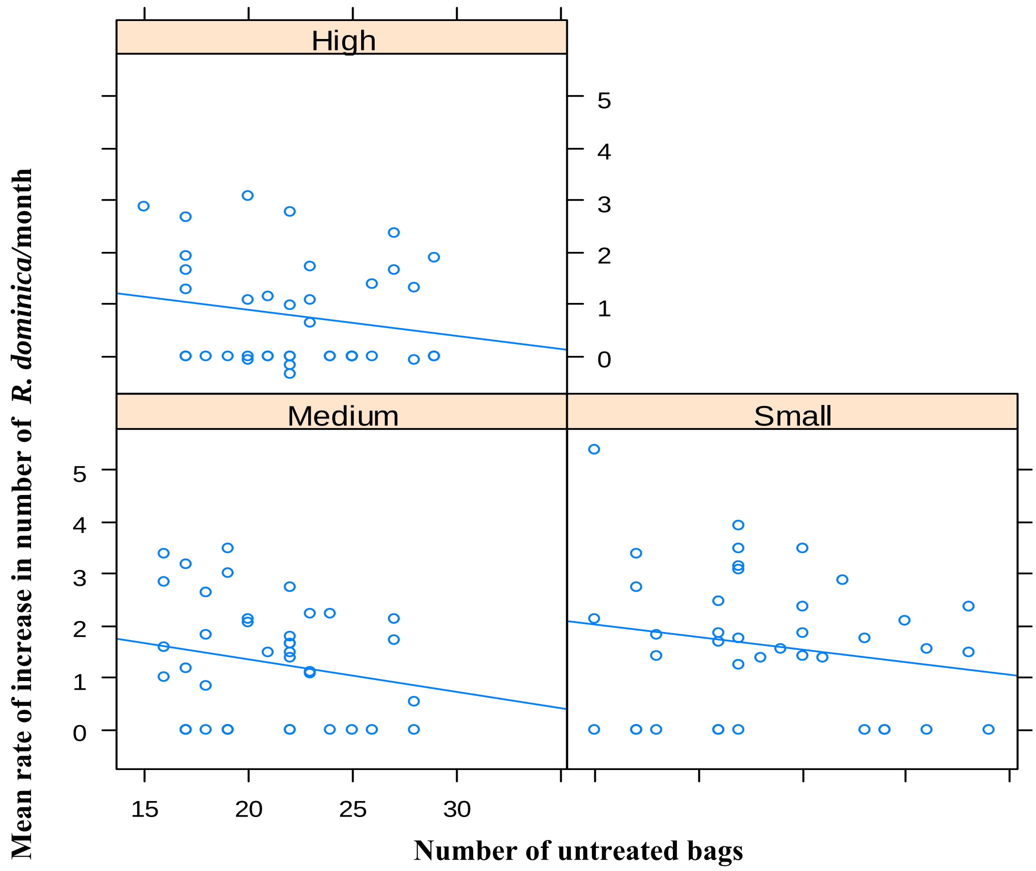


**Fig.6.21 The interaction between levels of treated bags and numbers of untreated bags in store-rooms on the mean monthly rate of increase in numbers of adult *R. dominica* found per 100g sample of grain over the five month experiment**. Overall there was no significant effect ($p>0.05$) in the rate of increase in number of beetles in the treated bags as the number of untreated bags increased. The ANCOVA found no interaction between untreated bag number and treated bag levels.

Figure 6.22 shows the relationship between the numbers of untreated bags in a store-room and the rate of increase of *L. serricorne* in treated bags. This relationship was found not significant for all store-rooms with small (p=0.742), medium (p=0.143) and high levels of treated bags (p=0.33). The ANCOVA also had no significant main effect for the untreated bags number covariate (F=0.316, df=1 and 114, p=0.575), and for treated bags level factor (F=0.266, df=2 and 114, p=0.766). The interaction term was also not significant (F=1.387, df=2 and 114, p=0.254), showing that the best fit line of the model for the different levels of treated bags were not different and hence the beetles were not affected by the proportions and deterrence effect of the treated bags.

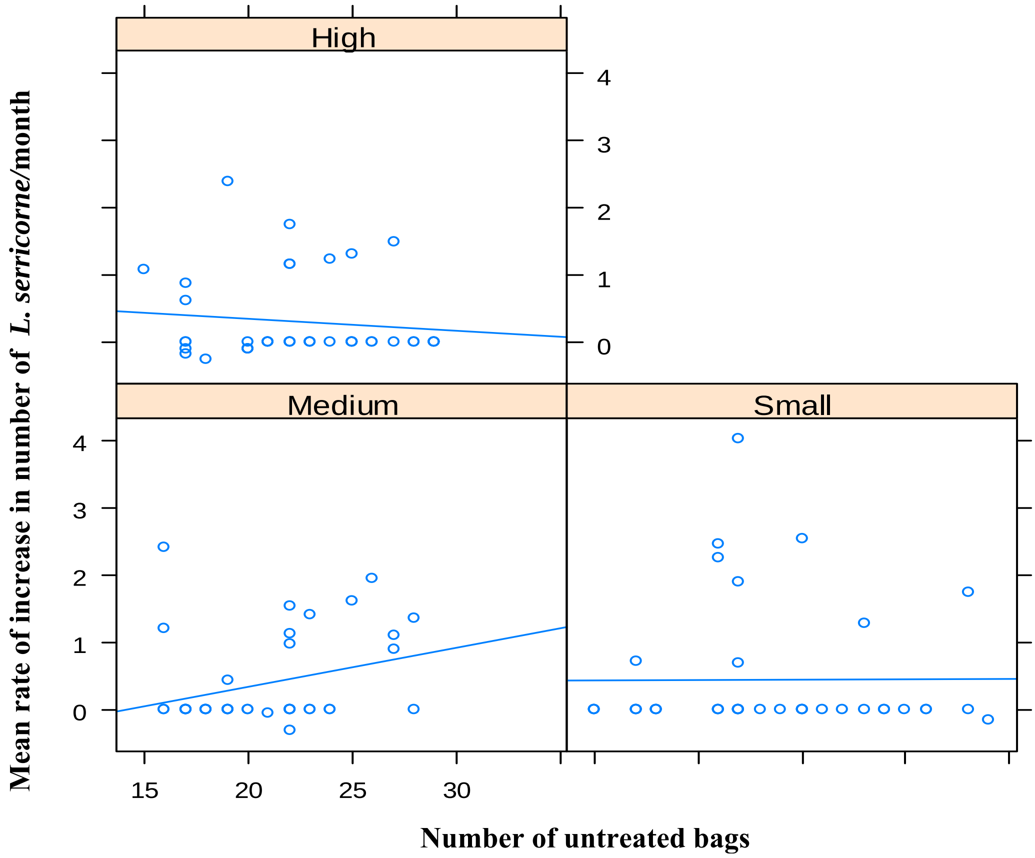


**Fig.6.22 The interaction between levels of treated bags and numbers of untreated bags in store-rooms on the mean monthly rate of increase in numbers of adult *L. serricorne* found per 100g sample of grain over the five month experiment**. There was no significant effect (p>0.05) in the rate of increase in number of beetles in the treated bags as the number of untreated bags increased. The ANCOVA found no interaction (p>0.05) between untreated bag number and treated bag levels.

**6.3.2.4 Effect of the number of untreated bags stored by farmers on the rate of infestation by *T. castaneum* in the treated bags kept in the same store-room.**

The untreated bags of grain in the farmers' store-rooms were considered to be the main source of insect infestations. In each store-room, the participating farmers had anywhere from 15–34 untreated 60kg bags of sorghum. Baseline levels of infestation were measured at the beginning of the experiment, and only store-rooms with moderate infestations in the untreated bags were used (see 6.2.2 Methods section).

The results in Fig. 6.23 show how the numbers of *T. castaneum* in samples from treated bags increased over time for every category of levels of treated bags and mean numbers of untreated bags. Results for each of the three levels of treated bags (labels in green) are given for four bins representing the mean numbers of untreated bags in each store-room (light brown labels). The result shows that overall there was a linear increase in the number of beetles in the treated bags from month 1 to month 5, irrespective of the levels of treated and untreated bags present. However, a pattern is evident; overall, the numbers of beetles in treated bags decreases from the first row of the plot to the bottom row, i.e., as level of treated bags increases, but there is little evidence of changes across the columns. The effect of month (ANCOVA; $F=1510.90$, $df=1$ and 55, $p<0.001$) and the level of treated bags (ANCOVA; $F=360.81$, $df=2$ and 55 $p>0.001$) on the number of beetles in treated bags were found be significant, but the number of untreated bags was not (ANCOVA; $F=0.3018$, $df=1$ and 55, $p=0.585$). This suggests that the increase in number of beetles in treated bags did not depend on the number of untreated bags in the storerooms, rather, the length of time the treated bags were stored and the level of treated bags in the storerooms affected the numbers of insects in treated bag.

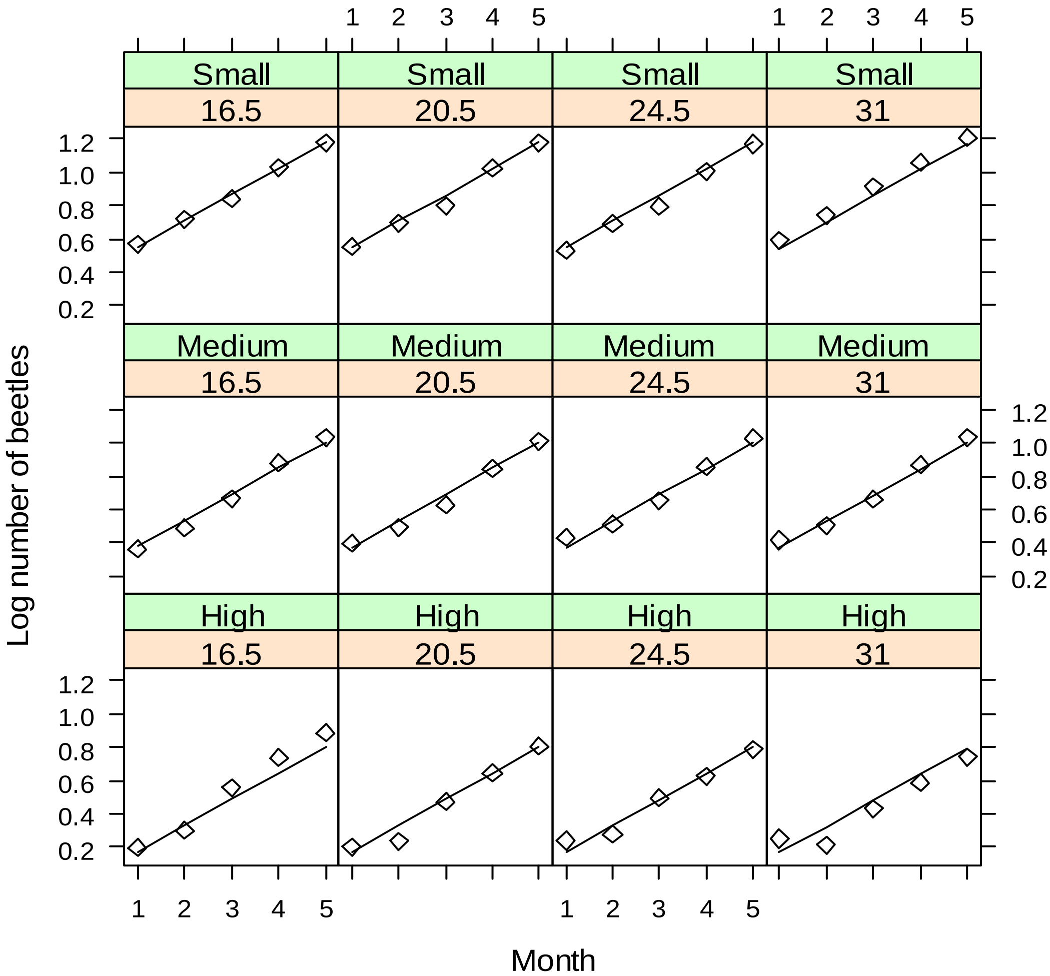


**Fig. 6.23 Multiple regression predicting the effect on numbers of *T. castaneum* of four levels of treated bags (green) matched against numbers of untreated bags (grouped into four bins; light brown) in each store-room over time.** Numbers of beetles were converted to log(x+1) to improve the linearity of the residuals. N= 40 store-rooms for each level of treated bags. ANCOVA indicates significant effect on increase in number of beetles only for month ($p<0.001$) and stores with different levels of treated bags ($p<0.001$), but no effect of number of untreated bags ($p>0.05$).

The results in Fig.6.24 indicate that the increase in number of beetles in the treated bags was matched by a similar corresponding increase in grain weight loss due to the same factors; the ANCOVA test showed that months of storage (F=732.78, df=1 and 55, p<0.001) and level of treated bags (F=42.67, df=2 and 55, p<0.001) had a significant effect on weight loss. This suggests that the increase in weight loss in treated bags was related to the increase in numbers of beetles, which were both affected by months of storage and the levels of treated bags in the store-rooms, but not by the number of untreated bags in the store-rooms.

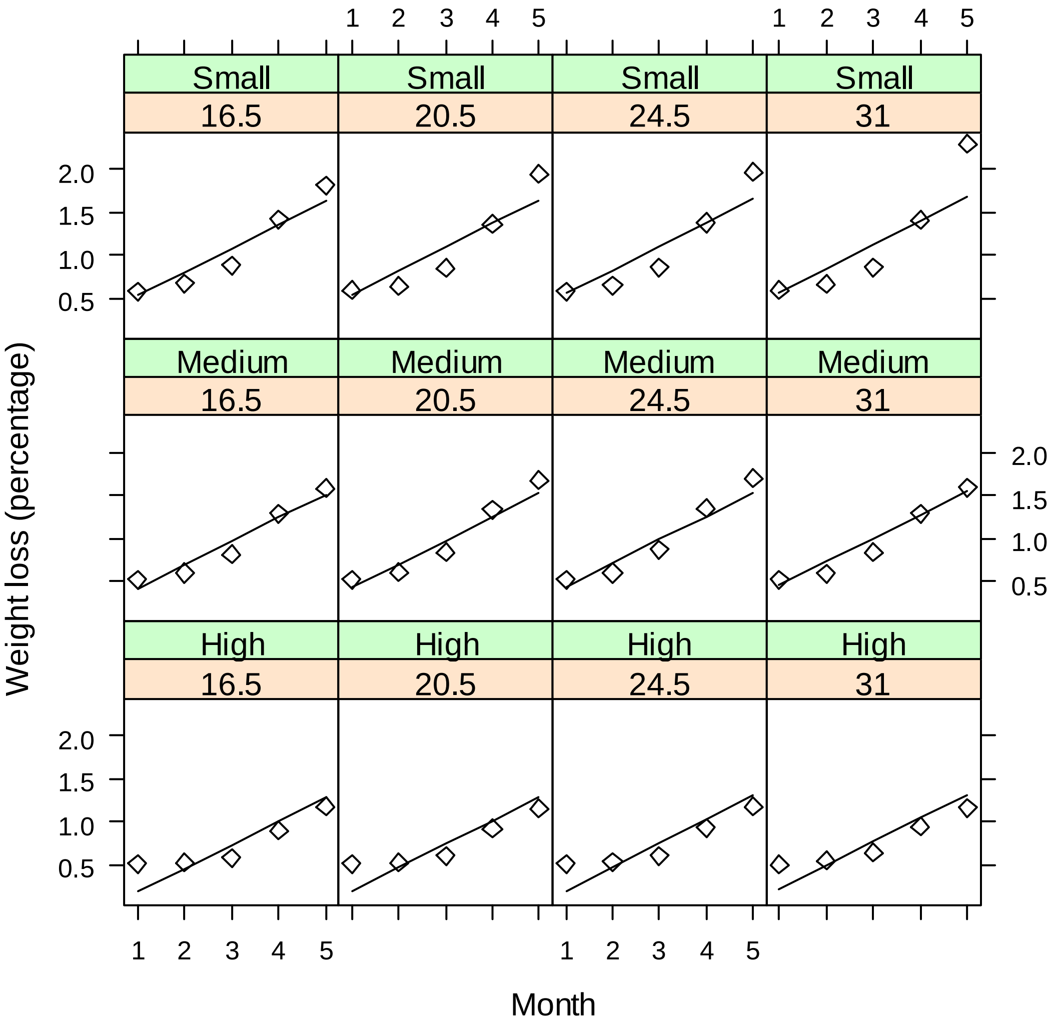


**Fig. 6.24 Multiple regressions predicting the effect on grain weight loss of four levels of treated bags (green) matched against numbers of untreated bags (grouped into four bins; light brown) in each store-room over time.**. N= 40 store-rooms for each level of treated store. ANCOVA indicates significant effect on increase in weight loss only from month (p<0.001), stores with different levels of treated bags (p<0.001), but no effect of the number of untreated bags (p>0.05).

### 6.3.3 Evaluation of the perceptions of the participating farmers on the effect of the new method of protecting stored grain in repellent double bags against insect infestations

Table 6.4 summarises the perceptions of the participating farmers on the effectiveness of the new method tested in their store-rooms compared to their existing methods of admixing dried repellent plant materials with their grain in single bags (section 6.1). The results indicate that the farmers, who participated in the experiments generally, had a positive impression that the new method was more effective than their existing methods. This view was given by 100% of respondents who tested a high level of treated double bags in their stores, followed by those that tested a medium or a small level of treated bags. However, some respondents who tested a medium or a small level of treated bags indicated that the effect of the new method was similar to their existing methods. A minority of the respondents were not able to discern any differences between the methods. This difference in perception between participants that used different levels of treated bags suggests that the respondents experienced a range of effectiveness based on the level of treated bags used in their store-rooms and respondents who tested a higher level of treated bags experienced better efficacy. There was a significant difference in the perception of respondents on the effectiveness of the different level of treated bags tested ($\chi^2$=12.69, df=4, $p<0.05$).

**Table 6.4 Distribution of responses according to the treatment used and the effectiveness compared to their local methods** (n=42 respondents)

| | Cannot say | | Similar | | Very effective | | Total | |
|---|---|---|---|---|---|---|---|---|
| Treatment | Freq | Percent | Freq | Percent | Freq | Percent | Freq | Percent |
| Small | 4 | 26.6% | 6 | 40.0% | 5 | 33.3% | 15 | 100.0% |
| Medium | 2 | 13.3% | 4 | 26.6% | 9 | 60.0% | 15 | 100.0% |
| High | 0 | 0.0% | 0 | 0.0% | 12 | 100.0% | 12 | 100.0% |

There was a significant difference in the perception of respondents on the effectiveness of the different level of treated bags tested ($\chi^2$=12.69, df=4, $p<0.05$).

The results in Table 6.5 indicate what the respondents thought about the effectiveness of the new method in reducing damage to grain in order to have more food to eat or for increased sales of grain. The respondents generally believed that the new method could give them more food to eat or sell. All the respondents who tested a high level of treated bags in their store-rooms reported this. However, some respondents who tested a small and medium level of treated bags said the method was inefficient in providing enough food to eat or sell. This suggests that having different levels of treated bags in store-rooms can confer different levels of efficacy in each farmer's store-rooms, consistent with the results on rates of insect infestation and grain weight loss described above (section 6.3.2). The respondents who tested a high level of treated bags were more confident in the efficacy of the new method in having a better and cleaner grain than the respondents who tested small or medium levels of treated bags. There was a significant difference in the perception of respondents tested different levels of treated bags on the effectiveness of the new method on the quality of grain obtained ($\chi^2$=10.64, df=4, p<0.05).

**Table 6.5 Perception of respondents on the effectiveness of the new method in reducing damage to grain for food or sales** (n=42 respondents)

| | No | | Not sure | | Yes | | Total | |
|---|---|---|---|---|---|---|---|---|
| Treatment | Freq | Percent | Freq | Percent | Freq | Percent | Freq | Percent |
| Small | 6 | 40.0% | 3 | 20.0% | 6 | 40.0% | 15 | 100.0% |
| Medium | 4 | 26.6% | 2 | 13.3% | 9 | 60.0% | 15 | 100.0% |
| High | 0 | 0.0% | 0 | 0.0% | 12 | 100.0% | 12 | 100.0% |

Chi-square test indicates a significant difference in perception of farmers on the effectiveness of the different levels of treated bags tested in their stores ($\chi^2$=10.64, df=4, p<0.05).

Table 6.6 indicates how the respondents perceived the relative simplicity or difficulty in the preparation and application of the new method compared to their existing methods. There was no significant difference in perception of respondents on the relative difficulty of implementing the new method compared to their existing methods ($\chi^2$=1.4, df=4, p=0.734).

**Table 6.6 Perception of respondents on the relative difficulty of implementing the new method compared to their existing methods** (n=42 respondents).

| | A bit easier | | Similar | | A bit harder | | Total | |
|---|---|---|---|---|---|---|---|---|
| Treatment | Freq | Percent | Freq | Percent | Freq | Percent | Freq | Percent |
| Small | 6 | 40.0% | 7 | 46.7% | 2 | 13.3% | 15 | 100.0% |
| Medium | 6 | 40.0% | 8 | 53.3% | 1 | 6.7% | 15 | 100.0% |
| High | 6 | 50.0% | 4 | 33.3% | 2 | 16.7% | 12 | 100.0% |

There was no significant difference in perception of respondents in difficulty of implementing the new method compared to their existing methods ($\chi^2$=1.4, df=4, p=0.734).

Table 6.6 above shows that more than half of the respondents who tested a high level of treated bags and about 40% who tested a small or a medium level of treated bags said the method was a bit easier than their existing methods. The results in Table 6.7 indicate a difference in perception among men and women on the easiness of the new method compared to their existing methods. The results indicate that among the respondents that said the new method was easier; more than 50% were female and only a few indicated that the method was harder or similar to their existing method. However, the largest percentage group of the male respondents (48%) said that the new method was a bit harder than their existing methods. There was a significant difference between the perception of men and women on the easiness of the new method ($\chi^2$=6.47, df=2, p<0.05). There are several possible reasons for this. It may be linked to differences in the roles of men and women in post-harvest management of grain. For example, if repellent plant materials are mixed with the grain to protect it from infestation, women are responsible for winnowing the grain to remove the plant materials before the grain is used for food. In the new method, there is little, if any need to winnow the grain before using it because the plant material is kept separate from the grain. Also, the survey results (Chapter 3) indicate that men are responsible for the preparation of bags for storage, and may consider the extra time and cost of preparing treated double bags to be a noticeable increase in difficulty of the method.

**Table 6.7 Perception on the easiness of the new method by gender** (n =42 respondents).

| | A bit easier | | Similar | | A bit harder | | Total | |
|---|---|---|---|---|---|---|---|---|
| Gender | Freq | Percent | Freq | Percent | Freq | Percent | Freq | Percent |
| Female | 10 | 58.8% | 5 | 29.4% | 2 | 11.7% | 17 | 100.0% |
| Male | 7 | 28.0% | 6 | 24.0% | 12 | 48.0% | 25 | 100.0% |

The Chi-square test indicates a significant difference between the perception of men and women on easiness of the new method ($\chi^2$=6.47, df=2, p<0.05).

The results in Table 6.8 show what the respondents thought about the extra work involved in the new method and how it might affect uptake of the new technology. The result indicate that, overall, the majority believed that, irrespective of the extra work involved by the new method, it would gain acceptance if could be proved to reduce infestations to the level where there was perceptibly more food and more grain to sell in their store-rooms. However, a greater percent of the respondents who tested a small level of treated bags in their store-rooms expressed a greater level of concern about the extra work. There was a significant difference in perception of respondents who tested different levels of treated bags on the effect of extra work on the likely uptake of the new method ($\chi^2$=16.55, df=4, p<0.01). This suggests that if the benefits of the new method can be demonstrated, this could motivate farmers to over-look the extra work involved. The respondents who did not see much benefit from the method as it was tested in their store-rooms were the most concerned about the extra work. Thus, the relative time costs and real benefits of the new method are likely to have implications for the rate of uptake.

**Table 6.8 Perception of respondents as to the effect of extra work on the likely uptake of the new method** (n=42 respondents).

| | People won't want to do all that work even if it means they have more food or money as a result | | People will do the new method if they are sure of having more food or money as a result | | People will wait and see if the effort is worth it for other people before they try | | Don't know | |
|---|---|---|---|---|---|---|---|---|
| | Freq | Percent | Freq | Percent | Freq | Percent | Freq | Percent |
| Small | 8 | 53.3% | 5 | 33.3% | 2 | 13.3% | 0 | 0.0% |
| Med | 0 | 0.0% | 11 | 73.3% | 1 | 6.7% | 3 | 20.0% |
| High | 0 | 0.0% | 8 | 66.7% | 0 | 0.0% | 4 | 33.3% |

Chi-square test indicate a significant difference in the perception of respondent who tested different levels of treated bags on the effect of the extra work on uptake of the new method ($\chi^2$=16.55, df=4, p<0.01).

The results in Table 6.9 generally indicate that the respondents were happy with the amount of plant residues found in the new method compared to their existing methods, which is to mix plant material with the grain. There was an almost equal distribution of respondents reporting no residue to a small quantity of residues in the treated double bags across all three treatment levels (small, medium and high numbers of treated bags per store-room), and no one reported higher levels of residue. The difference in perception of respondents on the amount of residues left in the grain across all the level of treated bags was statistically significant ($\chi^2$=9.52, df =1, p<0.05). This suggests that the new method was effective in keeping plant residues separate from the treated grain, hence reducing the extra work of winnowing compared to the direct method of admixing grain with plant material. This could have important gender implications, since women are mainly responsible for winnowing.

**Table 6.9 Perception of respondents on the level of plant residues left in the grain after using the new method** (n=42 respondents).

| | Small quantity of residue | | No residue | | Total | |
|---|---|---|---|---|---|---|
| Treatment | Freq | Percent | Freq | Percent | Freq | Percent |
| Small | 10 | 66.7% | 5 | 33.3% | 15 | 100.0% |
| Medium | 12 | 80.0% | 3 | 20.0% | 15 | 100.0% |
| High | 9 | 75.0% | 3 | 25.0% | 12 | 100.0% |

Chi-square test indicate a significant difference in perception of respondents on the amount of residues left in the treated grain after the experiment ($\chi^2$=9.52, df=1, p<0.05).

Table 6.10 shows that respondents had quite varied ideas about what could persuade them to change from using their existing methods to adopting the new method. More than half who tested a small level of treated double bags in their stores believed that the new method should be easier to implement than the existing methods. Very few considered effectiveness or cost of the new method to be factors that would persuade them to change to the new method. There was an equal distribution of respondents who believed that the new method should be either easy to implement, more cost effective or receive a higher price for the higher quality grain, and this formed the majority of respondents' views of those that had tested a medium or high level of treated bags. However, more than half of the respondents who tested a high level of treated bags said that they would be convinced to change to the new method for its effectiveness. There was a significant difference in the perception of respondents who tested different level on what might persuade them to change to the new method ($\chi^2$=13.0, df=6, p<0.05).

**Table 6.10 shows the views of the respondents on what might persuade them to change to the new method** (n=42 respondents).

| | Treatment | | | | | |
|---|---|---|---|---|---|---|
| | Small | | Medium | | High | |
| Respondents in each category | Freq | Percent | Freq | Percent | Freq | Percent |
| Existing method not effective | 7 | 26.6% | 1 | 6.7% | 7 | 8.3% |
| New method was easy to implement | 6 | 40.0% | 5 | 33.3% | 3 | 25.0% |
| New method is more cost effective | 5 | 33.3% | 5 | 33.3% | 1 | 8.3% |
| Expect to receive higher price for better quality | 0 | 0.0% | 4 | 26.7% | 1 | 58.3% |
| Total | 15 | 100.0% | 15 | 100.0% | 12 | 100.0% |

Chi-square test indicate a significant difference in the view of respondents who tested different level of treated bags on what might persuade them to change the new method ($\chi^2$=13.0, df=6, p<0.05).

The results in Table 6.11 show the difference in perception of men and women on what could persuade them to change the new method.  The results indicate that there was a statistically significant difference in the perception of men and women about the factors that could convince them to change to a new method of pest control ($\chi^2$=11.29, df=3, p<0.01). The results indicate most of the female respondents said that the new method should be cost effective and effective in controlling pests. However, this was not the case for the male respondents who emphasised effectiveness more and the easiness of the new method. However, very few among either the male or female respondents considered that the new method should be able to give high price for better quality as the most important factor.

**Table 6.11 Differences in perception of gender on the factors that could persuade farmers to change to the new method** (n=42 respondents).

| | Female | | Male | |
|---|---|---|---|---|
| Respondents in each category | Freq | Percent | Freq | Percent |
| Effectiveness of current method | 5 | 29.4% | 12 | 48.0% |
| New method found easy to practice | 3 | 17.6% | 8 | 32.0% |
| New method is more cost effective | 8 | 47.1% | 1 | 4.0% |
| Receive high price for better quality | 1 | 5.9% | 4 | 16.0% |
| Total | 17 | 100.0% | 25 | 100.0% |

Chi-square test indicates that there was a statistically significant difference in the perception of men and women about the factors that could convince them to change to a new method of pest control ($\chi^2$=11.29, df=3, p<0.01).

The results in Table 6.12 show the perception of men and women on who treat their grain with existing method of grain protection. The difference in perception of men and women on who treat their grain with existing method of pest control was not statistically significant ($\chi^2$=5.15, df=2, p=0.09).

**Table 6.12 Perception of respondents as to who treats their grain with the existing method** (n=42 respondents).

| | Head of household | | Men and women | | Other men of household | | Total | |
|---|---|---|---|---|---|---|---|---|
| Treatment | Freq | percent | Freq | Percent | Freq | Percent | Freq | Percent |
| Female | 12 | 71.0% | 3 | 17.6% | 2 | 11.8% | 17 | 100.0% |
| Male | 14 | 56.0% | 1 | 4.0% | 10 | 40.00% | 25 | 100.0% |

There was no statistical significant in the perception of men and women on who treat their grain with existing method ($\chi^2$=5.15, df=2, p=0.09).

The results in Table 6.13 show the perception of men and women on who would treat their grain with new method. There was no significant difference in perception of men and women on who treat there grain with new method ($\chi^2$=0.005, df=2 p=0.174).

**Table 6.13 Perception of respondents on who would treat their grain with the new method** (n=42 respondents).

| | Head of household | | Other men of household | | Total | |
|---|---|---|---|---|---|---|
| Gender | Freq | percent | Freq | Percent | Freq | Percent |
| Female | 15 | 88.2% | 2 | 11.7% | 17 | 100.0% |
| Male | 22 | 88.0% | 3 | 12.0% | 25 | 100.0% |

There was no statistical significant in the perception of men and women on who would treat their grain with new method ($\chi^2$=0.005, df=2 p=0.174).

Table 6.14 indicates the view expressed by the respondents as to whether they would keep using the new method as it was during the experiment or change the number of treated bags in their store-rooms. The results show that all of the respondents that had been in the medium and high groups said they would keep using the same method, but the majority of respondents who had a small number of treated bags stated that they would use more treated bags. There was a statistically significant difference in the perception of respondents who tested different levels of treated bags in their stores on the decision of level of treated bags to continue with ($\chi^2$=20.62, df =2, p<0.001). The view of more of the respondents who tested medium or high levels of treated bags that they will keep the number of treated bags suggests that farmers observed the high efficacy of treating stores higher level of treated bags, which the opposite was true for respondents that tested a small number of treated bags.

**Table 6.14 Views of respondents as to whether they would continue to use the treated double bag method** (n=42 respondents)

| | Higher number of treated bags | | Same number of treated bags | | Total | |
|---|---|---|---|---|---|---|
| Treatment | Freq | percent | Freq | Percent | Freq | Percent |
| Small | 9 | 60.0% | 6 | 40.0% | 15 | 100.0% |
| Medium | 0 | 0.0% | 15 | 100.0% | 15 | 100.0% |
| High | 0 | 0.0% | 12 | 100.0% | 12 | 100.0% |

Chi-square test indicate a significant difference in perception of respondents on the decision of what level of treated bags to keep using ($\chi^2$=20.62, df =2, p<0.001).

## 6.4 Discussion

The purpose of this field study was to test if infestation by beetles could be reduced when double bags are treated with Lem-ocimum at a dose of 1% w/w of 5kg sorghum, and if a number of treated bags in store-rooms could be used to reduce beetle infestations and increase the amount of clean grain in the treated bags. The farmers' infested single (60 kg) untreated bags in the 120 store-rooms, which were assessed at the beginning of the experiments to have moderate infestations (26-50 *T. castaneum*, section 6.2.2) were considered to be the main source of infestation.

This field study demonstrates that double bags treated with Lem-ocimum can deter beetles away from treated grain and reduce the rate of infestations in stored grain. Thus, the null hypothesis that treating double bags with repellent plant materials does not reduce infestations in treated bags is rejected. Experiment 2 demonstrated that high levels of treated bags in store-rooms can keep beetle infestations low (6 *T. castaneum* per sample) compared to the high levels of infestation in the surrounding single bags (baseline 26-50 *T. castaneum* per sample).

The results for Experiment 1 (comparing single, double and treated double bags) demonstrate that of all the treatments tested, grain in the single untreated bag had the highest rate of monthly increase in infestation by *T. castaneum*. More importantly and interestingly, the addition of Lem-ocimum to double bags resulted in a significantly lower (2.77±0.25) monthly increase in infestation of about 3 times less than that found for single (7.88±0.25), and 1.5x untreated double bags (4.36±0.25). The repellent efficacy of treated double bags used in this experiment are similar tot the findings of Anwar *et al*. (2005) who demonstrated that treatment of jute bags with Neem seed oil to have a significant effect in repelling and protecting stored wheat from infestation by *T. castaneum* and other storage beetles for over 4 months in a warehouse experiment. Mikhaiel (2011) reported that coating the surface of a gunny plastic bag and damour cloth bag with essential oils from *O. basilicum* could provide protection for stored grain from *T. castaneum* and *Ephestia kuehniella* for over 45 days of storage. In similar research studies, Anwar *et al*. (2005), Hou *et al*. (2004) found that Neem and Deet oils are effective insect repellents, preventing them from penetrating or invading packaged food materials. It should be noted that all these methods involved treatment of a single bag with the plants oils or essential oils, which may evaporate quickly, and because of the technology involved in the extraction of oils, this approach may not be feasible in practice by local farmers. Farmers need methods that are simple and easy to practice; hence, for quick and easy adoption a method should be similar to what they are already practicing. The new method in this study is an improvement of what farmers currently practice of mixing grain directly with dried repellent plant materials. Dried whole plant material is easier to process and the active repellent compounds may last longer than their essential oils which volatilize quickly due to their high evaporation pressure (Sarah *et al*., 2006), particularly in areas of high ambient temperatures, such as northern Nigeria. In Chapter 5, section 5.3.5 of this study, when citronella or methyl salicylate and ground plant material from *C. nardus* were used to treat a double bag against *T. castaneum*, more beetles were found in the bags treated with citronella, methyl salicylate than in the bags treated with plant materials. This may be explained by the higher rate of evaporation of the active compounds in the two chemical compounds than in the plant materials.

The studies presented in this chapter demonstrate that treatment of packaging materials such as polypropylene bags with dried repellent plant material between the layers of a double bag has potential for improving protection of stored grain. The efficacy of the double bag method is likely due to: a) the combination of the two types of repellent plant materials in Lem-ocimum (*C. nardus* plus *O. basilicum*) benefiting from a wider range of active compounds, and b) the paste of dried plants applied in a layer between the two bags formed a more concentrated barrier than if the same amount of material had been scattered throughout the 5 kg of grain inside the inner bag. Unfortunately, the efficacy of the particular mixture used varied with insect species. Wong *et al*. (2005) reported citronella, an important compound in *C. nardus,* and essential oils rich in camphor and methyl chavicol from *O. basilicum* (Mikhaiel, 2011; Mishra *et al*., 2012) to be repellent to *T. castaneum* and other stored product pests. Cline & Highland (1981) reported that storage pests such as *R. dominica*, *L. serricorne*, and *T. castaneum* could enter packaging through openings less than 1.35 mm and their larvae can enter even smaller openings. However, double bagging appears to be more difficult for beetles to penetrate according to Mullen & Mowery, 2002 and the results presented here. Significantly fewer beetles were found in untreated double bags than in single bags in Experiment 1. Thus, the combination of a double barrier of potent repellent plant material and a double layer of woven plastic may explain the significant reduction in infestation by *T. castaneum*. The effect was much less impressive for *R. dominica* and *L. serricorne,* but these species occurred at much lower levels and are not considered to cause as much damage as *T. castaneum.*

The high number of beetles found in the untreated single bags should not be a surprise, since the bags present the least barrier to the beetles. In the treated bag, the plant materials may have a double effect; as repellents and by masking the grain odour making it more difficult for beetles to detect the presence of grain.

Experiment 1 demonstrated that the efficacy of the different treatments varied with insect species. For instance, *T. castaneum* was found to be more susceptible to the deterrent properties of the double bag itself and the plant materials than *R. dominica,* which was equally affected by the double bag, with or without plant materials; and for *L. serricorne,* which was least affected by double bagging and repellent plant material, although numbers were low (<1 beetle per sample per month for all treatments). This may suggest that some insect species are more susceptible to certain treatments than other, which may be due to differences in responses to physical barriers and chemical compounds in plants. Isman (2006) reported that some plant substances that deter one pest can be tolerated or even an attractant to other pests. The infestation by *L. serricorne* was generally very low in the study area, and where infestations were found, the number of beetles was negligible except in a few stores with moderate infestations. Hence, the results of the experiments presented here are probably not adequate to reach any conclusions about this species. However, Baker *et al*. (2010) reported that the vapor of essential oils of *O. basilicum* has a significant repellent effect on *L. serricorne*, and therefore further investigation may be worthwhile.

The monthly increase in the number of live adult beetles of both species in the bags suggests the possibility of a population build-up; however, the rate of increase was low in the double bags treated with plant materials compared to untreated single bags and untreated double bags. This may suggest that the effect of double bagging and plant materials deterred the live beetles from egg-laying, and diverted them to a single untreated bag.

Despite the reports of Sule & Ahmed (2009), Mikhaiel (2011) and Nenaah & Ibrahim (2011) that essential oils of *O. basilicum* have toxic effects on *T. castaneum* adults and larvae and *R. dominica* (Kumawata, 2009), on the present work, the monthly increase in number of dead adults *T. castaneum* and *R. dominica* was found to be higher in the untreated single bags than in both the untreated and treated double bags. This may suggests that the effective dose of the the plant combination used (Lem-ocimum) was not high enough to cause toxic effects on the beetles or that most of the beetles affected by the repellent substances caused the beetles to find their way to the untreated bags, where they may have multiplied faster, hence producing more dead beetles over the 5 month observation period. Low toxicity could also be related to differences in plant species and their chemical compositions. Isman (2006) reported that the insecticidal effects of plant materials on insects depend to a high degree on the plant species and their chemical compositions.

Generally, the rate of monthly increase in the grain weight loss encountered during Experiment 1 was very low. However, as expected, untreated single bags had a significantly greater rate of increase in grain weight loss. The higher grain weight loss was expected because untreated single bags also had a significantly highest number of both live and dead beetles than the treated double bags. In addition to a having a significantly lower number of beetles, treated double bags also had the lowest grain weight loss, which may have been caused in part by the anti-feedant activity of essential oils in *O. basilicum and C. nardus* (Yogita *et al*. 2001; Sule & Ahmed, 2009; Stefanazzi *et al*., 2011). Overall, the higher monthly increase in number of beetles and grain weight loss found in single bags suggests that when untreated and treated bags are kept in the same place, beetles that are affected by the treatment (repellent plant material) may move to the untreated bags.

Experiment 2, the study on the effects of various levels of treated bags on beetles and grain weight loss, rejects the null hypothesis that the addition of varying levels of treated double bags to a grain store-room has no effect on infestations. This was confirmed by testing the effect of three treatments (addition of a small, medium or high number of 5kg treated double bags to store-rooms containing a variable number of 60 kg untreated single bags of grain each containing a known moderate level of beetle infestations). Experiment 1 demonstrated that treated double bags repelled beetles, significantly more *T. castaneum* and *R. dominica* than untreated double bags, however in Experiment 2, it was demonstrated further, that the degree of repellency of beetles depends on level of treated bags added in to a store-room. The stores with the highest level of treated double bags had the significantly lowest monthly increase in infestation by beetles compared to stores with medium or small levels of treated double bags. The numbers of *L. serricorne* collected were so low that it is not possible to come to any conclusions about the repellent efficacy of the treated bags for this species. However, further laboratory research should be conducted with this species and the repellent plant combination to ascertain the effects more accurately.

The greater repellent efficacy demonstrated by the stores treated with a high level of treated double bags could be as a result of having more treated double bags placed next to each other; the release of repellent volatiles from numerous bags treated with repellent plant materials could increase the deterrence of insect pests. Mikhaiel, (2011) and Mishra *et al*. (2012) reported that more repellent volatiles from many sources lead to more deterrence of insects. This may not be the case in store-rooms with small or medium levels of treated bags, where the bags were sparse and at distance from each other. The mechanism could be that when there is a greater number of treated bags placed next to each other in a store-room, there would be increase in the amount of the repellent compounds released from many sources (treated bags), that may seeps down into the untreated bags beneath the treated bags and cause a local decrease in beetles even in the untreated bags.

The low numbers of beetles found in treated bags in store-rooms with a high level of treated double bags was found to be accompanied by a low monthly increase in weight loss of grain in the treated double bags for both Experiment 1 and 2. Experiment 2 demonstrates that, overall, the monthly increase in grain weight loss was generally low compared to the monthly increase in grain weight loss in Experiment 1. Even though the amount of grain protected by the treated bags was very small (45kg-90kg) compared to amount of untreated bags in the same storeroom (1020kg-2100kg). However the results of this study shows the possible protection could be obtain with bigger farmers bag size (60kg). This study suggests that with this method a farmer could ensure a certain proportion of his grain is relatively free of infestation and damage from insects if as much as bag of grain are placed close to each other in his store-room. Moreover, it appears that, irrespective of the level of treated double bags used, a significant reduction in the expected level of beetle infestation and corresponding grain weight loss is measureable after only 3-4 months;

which implies that farmers could store their grain for immediate use (within 1-3 months) without any treatment. Even though the level of infestation found in the store-rooms with high levels of treated 5kg bags after five months of storage was reasonably low, however, the impact of this method is important for a long period of storage i.e. at least a year with size of bags similar to what famer are using (60kg).

To evaluate if the beetle infestations and their corresponding effect on grain weight loss in the treated double bags were affected by the number of untreated bags of famers grain in store-rooms, farmers' stores were assessed based on the stores with few, medium, high and very high numbers of untreated bags, in each of the store-rooms with small, medium and high levels of treated double bags. The results show that the number of beetles found in the treated double bags was not affected by the number of the famers' untreated single bags. This suggests that, irrespective of the number of farmers' untreated bags in store-rooms, if a relatively high number of bags are treated with repellent compounds and kept in double bags, the build-up of beetles in treated bags will be significantly slower than in untreated bags. However, this may be different in highly infested stores. The experiments reported here were done with farmers' stores that had a moderate level of beetle infestations in their untreated single bags.

When a new method of grain protection is developed and tested among local participants, it is important to evaluate the perception of the participants about the new method tested. This may provide information about what participants think about the method, what they appreciate most and where there are needs for improvement for better acceptance and uptake.

Generally, the perception of the respondents of the new method, as tested in their respective stores, was positive based on the efficacy, easiness and cost effectiveness; apart from a few participants who expressed concern about the low efficacy, difficulty and cost of additional materials. These views were influenced by the number of treated double bags that were tested in their own store-rooms and the gender of the respondents. The positive impression given by all the respondents who tested high levels of 5kg treated double bags in their store-rooms indicates that they were impressed by how efficacious the method was. The impression of a few respondents of those who tested small or medium levels of treated double bags, that the new method produced similar results to their own methods confirms that the method was less effective in their store-rooms than in those with a high level of treated double bags. This suggests that farmers' interests can be influenced by the demonstrated efficacy of a control method. In reports by Belmain & Stevenson (2001), Mugisha-Kamatenesi *et al*. (2008) and Deng, *et al*. (2009) that farmers' perceptions and choices of botanical pesticides as control agents are influenced by efficacy, availability and cost effectiveness, indicating that these could affect farmer acceptance and uptake. Hence, when introducing a new method of grain protection to farmers, the efficacy, cost and availability should be considered for easy acceptance by farmers, although this may depend on the particular circumstances of farmers and their locality.

The perception of the respondents based on the extra work required by the new method varied depending on the level of treatment that was tested in their store-rooms. All of the respondents testing high levels of treated bags and a few who tested small or medium levels of treated bags in their store-rooms believed that the extra work was of less importance provided the method can offer good food to eat or to sell. A few of the respondents who tested small or medium levels of treated bags raised a concern about the difficulty of the method. This suggests that a few respondents did not benefit from the efficacy of the method tested; hence, they compared it unfavorably with their own method. More than half of the respondents thought that the new method was a bit easier and were satisfied with the efficacy despite all the extra work involved. This suggests that no matter how difficult a method is, if it can result in a higher quality of food to eat or extra to grain to sell, farmers may adopt it. The perception of men and women also varied in terms of the extra work required by the new method in comparison with their existing methods. Most of the men expressed the view that the new method was harder to practice, whereas the women considered that the new method was easier to practice. Nevertheless, the perception of women on the cost of the new method is something of a major concern. The majority of the women respondents expressed more concern about the cost effectiveness and effect of the new method as factors that can affect their willingness to adopt the new method. Their views differed from the men who preferred methods that were effective and easy to implement.

Some direct quotes from interviewed farmers reveal their concerns about the cost and labour of additional bagging:

> (a farmer) expressed that she was happy with the new method; however, she needed more money to buy additional bags.
>
> (a farmer) observed that "the method is good as we see less plant residue and the grain looks nice, but one needs extra time for preparation".

Generally, the respondents reported there was low level of plant residue in the grain stored with the new method than their existing method of directly mixing the grain with plant material, which had to be winnowed out before the grain could be sold or eaten.

This survey demonstrates that acceptance and uptake of the new method may be affected by the extra work, additional costs, and its efficacy, and also depends on gender because of the difference between men and women in their access to resources such as time, cash, responsibilities and the plant materials. The impression of respondents who tested high levels of treated bags was that the new method can give them more food to eat or sell, irrespective of the extra work, which demonstrates how effective the method is. This study has shown that the use of plant materials combined with the double bagging method can provide an improved method of on-farm grain storage, particularly if farmers can afford to treat their store-rooms with high numbers of treated double bags. However, the cost of the additional bags is a challenge, which would need to be addressed in future studies.

Another aspect of the new method is that the plant combination has little toxic effect on the insect; this can be confirmed by the low numbers of dead beetles found in the treated grain when compared with untreated grain. However, it may be that untreated bags had higher numbers of dead beetles because they also had higher numbers of live beetles, due to having established breeding populations within the single bags. Both *O. basilicum* and *C. nardus* species have been reported to be toxic to *T. castaneum*, but more research may be required on the toxicity and fumigation effect of the plant combination on all the developmental stages of the beetles. It may be worthwhile to test a wider range of plant species. Inclusion of a repellent plant which is safe to use and has a greater toxic effect to insects could enhance the efficacy of this new method, because some of the beetles that resist the treatment or escape pre-storage treatment and get into the bags later can multiply and survive in them. However if the plant material used is toxic to beetles and kills them or delays their development (Isman, 2006; Mahmoudvand *et al*., 2011; Talukder & Khanam, 2011), then a combination of this effect with repellency may give more cost-effective control over long-term storage.

Farmers can benefit from this new technology since it reduces the burden of winnowing required by their traditional method of mixing grains with plant materials. Farmers are already conversant with the use of bags and plant materials and the material are all available in the study area. Applying repellent plant materials to the outside of the inner double bag suggests that plant materials that taste bitter and can be toxic when mixed with grain could be used with double bagging method since grain will have no direct contact with plant materials. However, future research needs to be conducted on the effect of plant residues that may remain in the grain when stored using this double bag method.

# CHAPTER 7

## Perspectives of small-scale farmers on sorghum storage and the use of botanical repellents as grain protectants in their locality

### 7.1 Background

Botanical pesticides have a long history of use in Africa, but the introduction of synthetic pesticides reduced their usage until recently. The increasing costs and environmental impacts of pesticides have encouraged farmers and researchers to return to traditional protection methods (Poswell & Akpa, 1991; Mathenge, 2001; Banwo & Adamu, 2003). In many developing countries, including Nigeria, there is renewed interest in traditional botanical pest control agents to repel pest insects from infesting stored grain (Banjo *et al.,* 2003; Isman, 2008). In the survey reported in Chapter 3, ~30% of 240 farmers in Kebbi use botanical repellents to protect their stored sorghum from insect infestations.

Although botanical repellents are a cheap and easily accessible means of pest control (Morse, *et al*., 2002; Prakash et *al*., 2008; Salako *et al.,* 2008), farmers frequently experience low efficacy, which poses a challenge to their wider uptake. Farmers might make more use of botanical repellents if their efficacy and ease of use could be improved. However, factors such as low efficacy, high cost, uncertain availability, time and labour costs of preparation, and limitations of traditional knowledge currently constrain the widespread uptake of botanicals as a grain protectants (Belmain & Stevenson, 2001; Morse et al., 2002; Mugisha-Kamatenesi *et al*., 2008). For instance, in northern Ghana cost was found to be more important than efficacy in the choice of botanical repellents by small-scale farmers (Belmain & Stevenson, 2001). However, the opposite was found in Uganda, where farmers perceived efficacy and availability to be most important (Mugisha-Kamatenesi *et al.,* 2008). In Nigeria, the uptake of tobacco-based products for spraying against pests of maize and cowpea was affected by cost and availability (Morse *et al*., 2002). Until farmers' perceptions of the reason for selection and uptake of botanical repellents are well understood, efforts to promote and sustain the use or acceptance of botanical repellents may not be fully achieved.

Gender differences in perceptions of, and experiences with, grain production and post-harvest management are also important to consider (Ogunlela & Mukhtar, 2009; Manda & Mvumi, 2010). Understanding differing perceptions is vital for improved grain storage and pest management practices. Manda & Mvumi (2010) found that men and women differed in their contribution to household store management and grain sales. Botanical repellents have been one of the control measures used by men and women to protect their stored grain. However, no publications have been identified that focus on gender differences in the perception of the use of botanicals as grain protectants and the factors that influence these differences. Although botanical repellents are used as grain protectants in Kebbi, farmers' views on their use are not well understood.

The main aims of the work presented in this chapter are:
* To gain greater understanding of how small-scale farmers in Kebbi use and assess the effectiveness of botanical repellents as grain protectants, particularly for sorghum.
*To identify factors that influence and facilitate choice and acceptance of particular types of plants.
*To confirm whether there are gender differences in perceptions of grain storage methods and the choice and use of botanical repellents.

## 7.2 Methodology

### 7.2.1 Study area

A second major survey was conducted in five villages (Tondi, Tungan-doro, Wasagu, Kimo, and Sabongarin-rumu) of Kebbi South. Kebbi South was chosen because the first survey found that botanical repellents were most used in this district.

### 7.2.2 Data collection

Information was collected using both qualitative and quantitative research methods. Initially, a key informant interview was conducted with a group consisting of the King of Farmers[1], the extension agent, village head and other important people in each of the five villages (Figures 7.1 & 7.2). They were asked in detail about the

[1] An honorific title awarded to the most experienced and influential person in faming activities in a given locality.

family organization in their villages, the main grain crops grown, plant materials used as grain protectants, and the way in which they are used. A questionnaire (Appendix 1.3) was designed, based on the information obtained from the key informant interviews, and a survey of 100 households was undertaken in the five villages, over a period of 24 weeks, between August and March 2012. Two enumerators, who were staff of Kebbi Ministry of Agriculture, were trained to interview farmers using the questionnaire and to record their responses.

Farmers were asked about the main grain crops grown and the most important pests attacking their stored grain. They were asked to identify the plant species used as grain protectants, the time and place of collection of the plant materials, details of the processing method and impact on stored grain and what difficulties they encountered in the collection of the plants. In addition, they were asked about socio-economic factors, such as age, family size and educational level, to enable correlation of these with their views about the efficacy of botanical grain protection. Storage insects were identified using a poster of storage insect species (Fig. 3.2). The questionnaire was carried out with 20 heads of households, who were either currently using botanicals or had used them before, in each village and selected to ensure gender balance, i.e., ten men and ten women from five villages, for a total of 100 heads of household. The head of household was selected based on a meeting organized in each village (Figures 7.4-7.6). Unfortunately, the participation rate was low because the survey was conducted during the busy period of farming activities. Some farmers had to be approached on their farmland for a short interview and to inform them of the next visit for the main interview. All data were summarised using simple percentages and, where necessary, subjected to statistical analysis as outlined in section 7.2.3.

**Figure 7.1 Village informants interviewed in Sabongarin-rumu village,** with the village head (the King of Farmers) and an extension agent, March 2012.

**Figure 7.2 Village informants interviewed in Tungan-doro village**, March 2012.

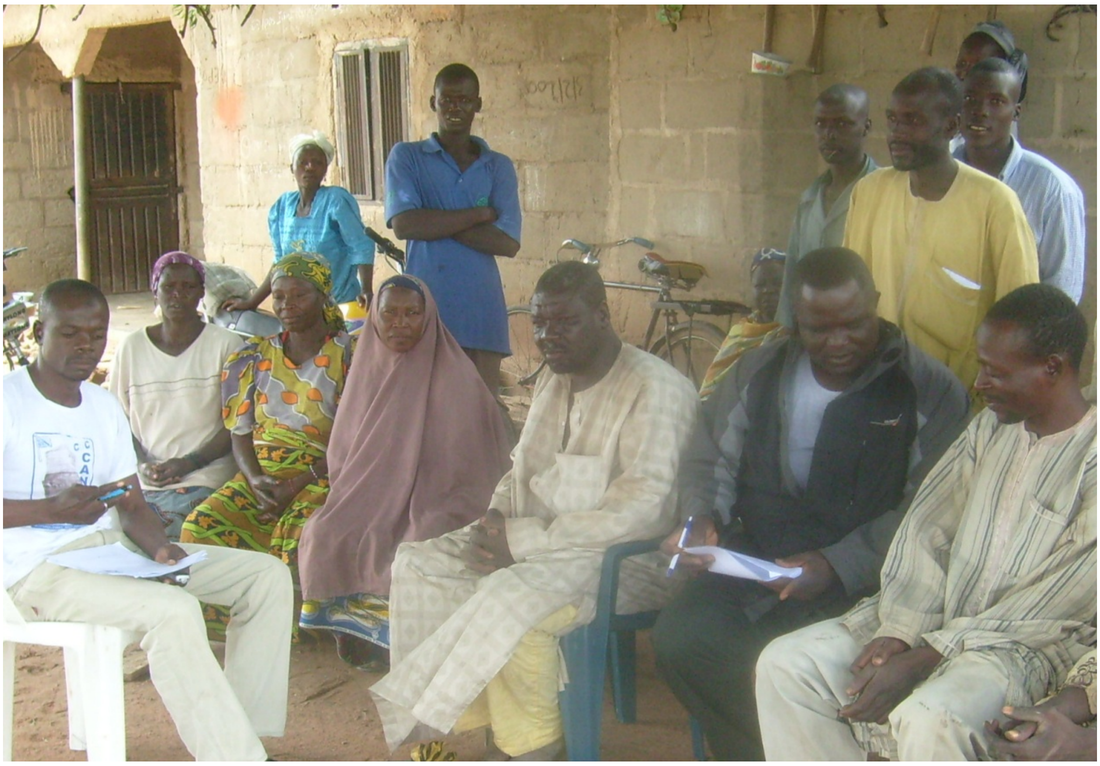

**Figure 7.4 Cross-section of the men and women farmers in Tungan-doro** at a meeting to understand their storage practices and to choose the appropriate respondents for the main interview, March 2012.

**Figure 7.5 Some of the women respondents in Tungan-doro village,** March 2012.

**Figure7.6 Respondents at a meeting in Sabon garin rumu,** held to gain an understanding of their storage systems and to choose appropriate respondents for the main interviews.

**Figure 7.7 Storeroom with polypropylene bags and plastic containers used for storing grain.**

### 7.2.3 Analysis of results

Data from the questionnaires were summarised using frequency and simple percentages; then the percentage responses in each category were correlated with gender and other variables, such as level of infestation and plant material or level of infestation and storage method. The chi-square test of independence was used to test for significant differences. T-tests were used to determine whether there were any significant difference between men and women for the scale variables (household size, crop production).

## 7.3 Results

### 7.3.1 Information obtained from village informants

The results obtained from informants included general information on family makeup, the types of crops grown, the types of botanical protectants used and how these were used within their community. Based on the information provided by key informants in all the villages, it was found that the main grain crop was sorghum, which, depending on the family, was intercropped with either millet, maize or rice and some potatoes and vegetables. There were two types of family identified in the villages: monogamous and polygamous. The majority of families were monogamous in all the villages, with either a male or female head of household, although most monogamous households were headed by a man.

In the polygamous families, which were usually headed by a male, the household consisted of his wives and children (either unmarried or married) living in the same compound. Farming activities were carried on the family land, although the children and wives have their own separate plots, which they worked on after the work on the family plot was completed. The grain from the family land was used for the family's daily food and to meet important household needs through sale, barter or gift.

Most of the female heads of household were widows with either no or few dependents. They possessed their own land where they grew a range of crops used to feed their dependents or meet other needs.

Threshed or unthreshed grain was stored either loose in a granary or in polypropylene bags, plastic containers or loose in a store-room of the main dwelling (Figure 7.7).

In all the villages, depending on what knowledge the farmers had inherited from their forefathers or from relatives, farmers used the following plant materials for post-harvest management; *Ocimum basilicum* (Sweet basil), wood ash, ground pepper, *Erythropleum guineeses* (Sassy bark), *Nauclea diderrichii* (Yellow three) and *Vernonia amygdalina* (Bitter leaf). *Ocimum basilicum* was the most common and widely used plant material due to its efficacy and availability. The stem bark of *E. guineeses* and *N. diderrichii* were collected from forests, but were used by only a few farmers.

### 7.3.2 Socio-economic background of the respondents

The results in Table 7.1 highlight the socio-economic profiles of the respondents, and show that the main gender difference between male and female heads of households was in the distribution of levels of education attained. Level of education can influence farmers' interests in new ideas (Rosenzweing, 1995). The majority of respondents had not completed any education level, and most of these were women. Of the few who had attended primary and post-primary education, a significant majority was male ($p<0.05$: Table 7.1).

Although the age distributions of men and women was not significantly different ($p>0.05$), there was a trend for women to be older than men (46% women and 58% of men were <41 years). Of the 100 respondents interviewed who either used botanical repellents or had experience of using them, the majority were between 31 and 50 years for both male and female-headed households. Few respondents were aged between 20 - 30 or 51 - 60 years and the fewest were in the age group of over 60 years. There was a statistically significant difference in family size of the male and female respondents (t-test assuming non equal varience, $t=2.01$, $df=48$, $p=0.0003$).

**Table 7.1 Socio-economic profile of the respondents, from survey data** (n =100 respondents).

| | Female | | Male | | Chi-sq test |
|---|---|---|---|---|---|
| Socio-economic characteristics | Freq. | Percent | Freq. | Percent | |
| Age | | | | | |
| 20-30 | 4 | 8 | 12 | 24 | |
| 31-40 | 19 | 38 | 17 | 34 | $\chi^2$ =5.5 |
| 41-50 | 17 | 34 | 12 | 24 | df=4 |
| 51-60 | 8 | 16 | 6 | 12 | p>0.05 |
| 61-70 | 2 | 4 | 3 | 6 | |
| Total | 50 | 100 | 50 | 100 | |
| Level of education completed | | | | | |
| No education | 43 | 86.0 | 31 | 62.0 | $\chi^2$ =8.1 |
| Primary | 6 | 12.0 | 13 | 26.0 | df=2 |
| Secondary | 1 | 2.0 | 6 | 12.0 | p<0.05 |
| Total | 50 | 100 | 50 | 100 | |
| Average family size | 5.4 | | 8.4 | | t=2.01, df=49, p=0.0003 |

Chi square test or t-test for difference in socio-economic characteristics for male and female respondents is significant where p<0.05.

Sorghum was the grain grown most, followed, by millet, maize and rice (Table 7.2). Male respondents grew all the types of grain more than female respondents. The difference in the quantity of type of grain grown between male and female respondents was found to be statistically significant; sorghum (t-test assuming non equal varience; t=5.01, df=49, p<0.001); millet (t=4.86, d4=49, p<0.001); maize (t=3.15, df=49, p<0.01); and rice (t=2.0, df=49, p<0.05). The sorghum yield for male respondents (mean = 45 x 60 kg bags/yr = 2700 kg/yr) was more than for female respondents (1650 kg/yr). The reason given most frequently for growing sorghum was that it was their staple food, with any surplus sold to meet household expenditures. This reason was given more frequently by women than by men, which suggests that women have more concerns about food security than men, although overall the differences in reasons for growing sorghum were not significantly different between men and women (p>0.05). The reason for growing excess and selling the surplus was given mostly by men. This could be because more men than women consider market value to be important, possibly because male heads of households generally have larger families than women heads of households, and may need more cash income to cover other expenses.

**Table 7.2 Main crops grown by respondents and purpose for growing crop** (n = 100).

| | Female | | Male | | |
|---|---|---|---|---|---|
| Average quantity of crop grown (number 60 kg bag /respondent/yr) | | | | | |
| Sorghum | 27.5 | | 44.9 | t=5.01, | df=49 p<0.001 |
| Millet | 8.0 | | 21.1 | t=4.86, | df=49, p=0.001 |
| Maize | 6.6 | | 14.9 | t=3.15, | df=49 p=0.01 |
| Rice | 0.5 | | 4.6 | t=2.0, | df=49, p<0.05 |
| Main purpose for growing crop | Freq. | Percent | Freq. | Percent | |
| It is a staple food | 41 | 82 | 33 | 66 | $\chi^2$ =2.5 df=1 p>0.05 |
| For its market value | 9 | 18 | 17 | 34 | |
| Total | 50 | 100 | 50 | 100 | |

Chi square test or t-test for difference in socio-economic characteristics for male and female respondents is significant where p<0.05.

### 7.3.3 Sorghum processing and storage

The results in Table 7.3 show the differences in perception of respondents on sorghum processing before storage: threshed[2] or un-threshed[3] sorghum, storage materials and duration of storage. There were significant differences between male and female respondents in all three aspects of storage methods. All women respondents stored sorghum threshed and winnowed.

Threshing was done by either the powerful young men in the household or the village youth were rewarded with as much food as they could eat throughout the activity. Winnowing was generally done by women. A female could invite help from community self-help group members (who join to help each other and reduce the cost of labour during faming activities) and other relatives, depending on the size of the harvest. However, for a man, if his wives are not group members, he has to invite laborers and other relatives to assist. This self-help group work among women is done only among the group members and help is given in rotation among the members, depending on who harvests first.

Although store-rooms were used by all respondents to store sorghum, there was a significant effect of gender ($p<0.001$); 90 % of women used store-rooms and 72% of women packed their grain in 60 kg bags and kept most of the rest in plastic containers, whereas only 68% of men used store-rooms but they packed 100% of their grain in bags.

[2] Sorghum grain separated from the head of the sorghum stem.
[3] Sorghum grain kept attached to the head of the sorghum stem.

**Table 7.3 Difference in perception of respondents on sorghum storage** (n =100).

| | Female | | Male | | Chi-sq test |
|---|---|---|---|---|---|
| How sorghum is processed before storage | Freq. | Percent | Freq. | Percent | |
| Threshed | 50 | 100 | 33 | 66 | $\chi^2$ =20.5 df=2 p<0.001 |
| Threshed/unthreshed | - | - | 16 | 32 | |
| Unthreshed | - | - | 1 | | |
| Location used for grain storage | | | | | $\chi^2$ =18.8 df=2 p<0.001 |
| Granary | 4 | 8 | - | - | |
| Store-room | 45 | 90 | 34 | 68 | |
| Granary/store-room | 1 | 2 | 16 | 32 | |
| Container used to stored grain | | | | | $\chi^2$ =16.3 df=2 p<0.001 |
| Loose grain | 4 | 8 | - | - | |
| Polypropylene bag | 36 | 72 | 50 | 100 | |
| Bag/plastic container | 10 | 20 | - | - | |

Chi-square test for difference in sorghum storage for male and female respondents is significant when $p<0.05$.

The results in Table 7.4 indicate that the duration of storage differed significantly between men and women ($p<0.05$); the majority of women (48%) stored sorghum for 2-6 months, although 32% stored their grain up to 1 year, whereas the majority of male respondents stored grain for 6-12 months (48%), followed by those that stored grain for up to 6 months (30%) and more than a year (18%). The difference in duration of storage among male and female respondents could be due to differences in the size of their land holdings (Ogunlela & Mukhtar, 2009).

**Table 7.4 Length of time grain stored** (n =100).

| | Female | | Male | | |
|---|---|---|---|---|---|
| Duration of storage | Freq. | Percent | Freq. | Percent | |
| Up to 2 months | 7 | 14 | 2 | 4 | |
| 2-6 months | 24 | 48 | 15 | 30 | $\chi^2=9.5$ |
| 6-12months | 16 | 32 | 24 | 48 | df=3 |
| More than a year | 3 | 6 | 9 | 18 | $p<0.05$ |

Chi-square test for difference in length of sorghum storage for male and female respondents is significant when $p<0.05$.

### 7.3.4 Perceptions of respondents on sorghum insect pest infestation and grain damage.

The results in Table 7.5 show trends in the differences in perceptions of male and female respondents to the main pests infesting their stored sorghum and the relative effect on quality or quantity of grain. Although a few respondents reported having no pests in their stores, the majority (64% of women and 48% of men) reported having an infestation every year, although there were no significant difference overall between men and women in the data presented in Table 7.5. With the help of a storage pest poster (Fig. 7.3), respondents identified some of the insect species infesting their stored sorghum. All the respondents reported *Tribolium castaneum* as the main pest of their stored sorghum, followed by *Rhyzopertha dominica* and *Sitophilus zeamais*. There was a tendency for male respondents to report higher levels of infestation than female respondents. When the respondents were asked about the effect of the pest damage on the grain, women tended to be more concerned about loss of food quality, and men were more concerned about loss of market value, but the gender differences were not significant ($p>0.05$).

**Table 7.5 Perceptions of respondents on sorghum infestation (n=100).**

| | Female | | Male | | Chisq test |
|---|---|---|---|---|---|
| Pest infestation in stored sorghum | Freq. | Percent | Freq. | Percent | |
| Never | 5 | 10 | 8 | 16 | $\chi^2$ =4.9 |
| Occasionally | 13 | 26 | 15 | 30 | df=3 |
| Sometimes | - | - | 3 | 6 | p>0.05 |
| Every year | 32 | 64 | 24 | 48 | |
| Type of insect infesting sorghum | | | | | |
| Never | 6 | 12 | 6 | 12 | $\chi^2$ =6.2 |
| *Tribolium castaneum* | 21 | 42 | 26 | 52 | df=3 |
| *Rhyzopertha dominica* | 13 | 26 | 16 | 32 | p>0.05 |
| *Sitophilus zeamais* | 10 | 20 | 2 | 4 | |
| Current level of pest infestation | | | | | |
| No pest | 10 | 20 | 13 | 26 | $\chi^2$ =3.3 |
| Low pest | 28 | 56 | 19 | 38 | df=2 |
| High pest | 12 | 24 | 18 | 36 | p>0.05 |
| Effect of pest damage on food quality | | | | | $\chi^2$ =0.75 |
| Yes | 37 | 74 | 29 | 58 | df=1 |
| No | 13 | 26 | 21 | 42 | p>0.05 |
| Effect of pest damage on grain market | | | | | $\chi^2$ =2.1 |
| Yes | 27 | 54 | 36 | 72 | df=1 |
| No | 23 | 46 | 14 | 28 | p>0.05 |

Chi-square test for differences in perception of male and female respondents on sorghum infestation is significant where p<0.05.

### 7.3.5 Perceptions of respondents on plant materials used as sorghum protectants

Table 7.6 shows there were significant effects ($p<0.01$) of gender on the choice of plant materials used as grain protectants, the place of plant harvesting, the plant parts used and how they were dried. Less than 15% of respondents used no plant materials. The majority of women (74%) used an *Ocimum* preparation, whereas the plant most used by men was *N. diderrichii* (42%). Less than 10% of the respondents used *V. amygdalina* or *E. guineeses*. The majority of women (87%) cultivated their plants in their home gardens, while the majority of men (58%) collected their plants from the forest ($p<0.001$). This may be an indication of women's interest in propagating plants generally. These gender differences may play an important part in making future recommendations to farming communities; it may be easier to encourage women to use plant protectants if the recommendations are to use plants propagated in their gardens and men to harvest wild plants. However, it may be that women collect plants from their own gardens because only men are free to travel far from home to collect plants. This is an interesting point that deserves further research.

Although there were significant ($p<0.001$), if slight, differences in the plant parts (leaves, stem bark and whole plant) used by men and women, the reasons why are not obvious. Most women used the whole plant, whereas men used stem bark or the whole plant. Leaves alone were the least used by men or women (13%), which suggest leaves have limited effectiveness (Table 7.6).

Men and women varied significantly ($p<0.01$) in the methods they used to prepare the plants and apply them as grain protectants. The majority of respondents sun-dried their plants for 2-3 days and then ground them to a powder. This was done by the majority of the men. However, a few of the women shade-dried their plants and then ground them to powder. Only a few respondents sun-dried and used the whole plant, placing it between layers of grain. The majority of both men and women that treated their grain with ground plant materials ad-mixed[4] it with the grain to be stored.

[4] Mingle ground material with the grains

**Table 7.6 Perceptions of respondents on preparation and application of protectant plants** (n=100).

| | Female | | Male | | Chisq test |
|---|---|---|---|---|---|
| Plant materials used | Freq. | Percent | Freq. | Percent | |
| None | 4 | 8 | 7 | 14 | $\chi^2$ =16.8 |
| *Ocimum* spp | 25 | 50 | 11 | 22 | df=5 |
| *Nauclea diderrichii* | 6 | 12 | 21 | 42 | p<0.01 |
| *Ocimum*/ground pepper | 12 | 24 | 6 | 12 | |
| *Erythropleum guineeses* | - | - | 4 | 8 | |
| *Vernonia amygdalina* | 3 | 6 | 1 | 2 | |
| Place of collection | | | | | $\chi^2$ =17.8 |
| Backyard garden | 40 | 87 | 18 | 41 | df=1 |
| Forest far from village | 6 | 13 | 25 | 58 | p<0.001 |
| Part of plant collected/ used | | | | | |
| Leaves | 6 | 13 | 6 | 13 | $\chi^2$ =19.9 |
| Stem bark | 6 | 13 | 25 | 56 | df=2 |
| Whole plant | 34 | 74 | 14 | 31 | p<0.001 |
| Preparation method used | | | | | |
| Shade-dried/ ground powder | 11 | 24 | - | - | $\chi^2$ =12.1 df=2 |
| Sun-dried/ ground powder | 31 | 67 | 36 | 84 | p<0.01 |
| Sun–dried/ used whole | 4 | 9 | 7 | 16 | |
| Grain treatment method | | | | | $\chi^2$ =2.7 |
| Layered in-between grain | 4 | 8 | 8 | 18 | df=2 |
| Admixed with grain | 41 | 90 | 35 | 82 | p>0.05 |
| Treated container | 1 | 2 | - | - | |

Chi-square test for differences in perception of male and female respondents on choice of plant and preparation methods used is significant when p<0.05.

The results in Table 7.7 show where the respondents sourced information about botanical repellents. Although women tended to consult their grandparents more than the men, there was no significant effect of gender on source of information ($p>0.05$), Less than 10% received their information from extension agents and only male respondents received information this way. This suggests that interactions on botanical repellents with extension agents are rare and/or little information regarding traditional practices comes from an external source.

**Table 7.7 Sources of information about botanical repellents** (n=100).

| | Female | | Male | | |
|---|---|---|---|---|---|
| Sources of information | Freq. | Percent | Freq. | Percent | |
| Extension agent | - | - | 4 | 8 | $\chi^2=1.3$ |
| Friends | 15 | 30 | 17 | 34 | df=2 |
| Parents | 15 | 30 | 17 | 34 | $p>0.05$ |
| Grandparents | 20 | 40 | 12 | 24 | |

Chi-square test for difference in sources of information between male and female respondents is significant when $p<0.05$

Table 7.8 shows the effect of duration of storage, cleaning of the store-room before loading and the effect of plant materials on the level of infestation in the respondents' stores. The majority of respondents (56%) that stored sorghum for only 2 months reported low or no infestations. High infestations were mentioned by the majority of respondents (89%) who stored for 6 months to over 1 year. This suggests that levels of infestation depend on the length of time grain is stored. High infestations were reported by the majority of respondents (67%) who did not clean the insects out of their stores before they added fresh grain. Low or no infestations were reported by the majority of respondents (75%) who cleaned their store-rooms at the beginning of each new storage season. These results indicate that good storage hygiene can help reduce storage infestations.

High infestations were reported by respondents who did not apply any treatment and those who used *E. guineeses*. The majority of respondents using *Ocimum* alone (69%) or in combination with ground pepper (94%) reported either no or low infestations. This suggests that types of plant differ in their efficacy and that farmers have had experience of combining two different plant types to improve the efficacy of their control methods.

**Table 7.8 Levels of pest infestation of stored sorghum in relation to duration of sorghum storage of respondents** (n=100).

| Percentage of respondents in each category of infestation level | | | | Chisq test |
|---|---|---|---|---|
| | High | Low | No pests | |
| Duration of storage | | | | |
| 2-6 months | 17.0 | 56.4 | 25.6 | $\chi^2$ =21.2 |
| 6-12 months | 46.2 | 41.0 | 12.8 | df=6 |
| more than 1yr | 42.8 | 42.8 | 14.2 | p<0.01 |
| up to 2 months | | 25 | 75 | |
| Store-room cleaned before storage | | | | |
| No | 66.7 | 33.3 | - | $\chi^2$ =2.1 |
| Yes | 25.0 | 48.9 | 26.1 | df=2<br>p>0.05 |
| Plant materials used as grain protectants | | | | |
| No plant materials | 72.7 | 27.3 | - | |
| *E. guineeses* | 75.0 | 25.0 | - | |
| *N. diderrichii* | 37.0 | 44.4 | 18.5 | $\chi^2$ =13.0 |
| *O. basilicum* | 30.6 | 55.6 | 13.8 | df=8 |
| *O. basilicum*/ground pepper | 5.5 | 38.9 | 55.6 | p<0.05 |
| *V. amygdalina* | 50 | 50 | - | |

Chi-square test for difference in storage practices of farmers and level of infestations is significant where p<0.05

### 7.3.6 Perceptions of respondents on the effectiveness of plant material used as sorghum insect pest protectants.

Table 7.9 shows that men and women respondents differed in the factors they used to decide which plant materials to use as grain protectants ($p<0.05$). The majority of women (64%) were more concerned about availability of plant material than the men (40%). In contrast, the majority of men were more concerned about the effectiveness (60% of men, vs 36% of women) of plants. This difference in gender perception may affect the choice of plant materials as grain protectants.

Likewise, men and women differed significantly in their perceptions of how plant protectants work ($p<0.05$). Most women believed the insects were either killed (26%) or sent away (36%), whereas nearly half the men (48%) believed the insects were sent away. A few of the respondents believed the effect of plant materials acted as an anti-feedant. This suggests that respondents may have knowledge of plant repellency, toxicity and anti-feeding properties.

The majority of respondents (64% overall) were satisfied with the way plant materials worked as grain protectants. However, when the respondents were asked to give reasons for their preference of botanical repellents over synthetic pesticides, men and women gave cost effectiveness [5] and availability as their reasons. This suggests that the majority may be more likely to accept and use a new botanical repellent if it can be sourced locally and is shown to be cost effective.

When asked about the disadvantages of using plant materials as grain protectants, the main responses were; time taken to collect and prepare large quantities of plant materials, the altered taste of food contaminated by the repellent plant materials and the time needed to winnow the grain to remove the plant material. Conversely, obtaining clean grain, improvement in market value and reduced quantity and quality of losses were reported as the main advantages of treating grain with plant materials.

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[5] The nominal value of reduced damage to the stored grain was greater than the effort involved in collecting, preparing and adding the plant material.

**Table 7.9 Perceptions of respondents on the efficacy of the plant protectants they used** (n=100).

| Variable | Female | | Male | | Chisq test |
|---|---|---|---|---|---|
| Factors consider when choosing plant protectant | Freq. | Percent | Freq. | Percent | |
| Availability | 28 | 64 | 18 | 40 | $\chi^2$ =4.1 |
| Effectiveness | 16 | 36 | 27 | 60 | df=1 |
| | | | | | $p<0.05$ |
| How protectant works | | | | | |
| Don't know | 15 | 30 | 13 | 26 | $\chi^2$ =2.4 |
| Insects stay but cause no damage | 4 | 8 | 6 | 12 | df=3 |
| Kills insects | 13 | 26 | 7 | 14 | $p>0.05$ |
| Send insects away | 18 | 36 | 24 | 48 | |
| Satisfaction with way plant works | | | | | |
| Don't know | 1 | 2 | 3 | 6 | $\chi^2$ =1.6 |
| Yes | 31 | 62 | 33 | 66 | df=2 |
| No | 18 | 36 | 14 | 28 | $p>0.05$ |
| Reason for botanical rather than synthetics | | | | | |
| Availability | 20 | 44 | 19 | 40 | $\chi^2$ =0.03 |
| Cost effectiveness | 25 | 56 | 28 | 60 | df=1 |
| | | | | | $p>0.05$ |

Chi-square test for difference in perception male and female respondents in the plant efficacy is significant where $p<0.05$

### 7.3.7 Grain marketing

Table 7.10 gives the perceptions of respondents on the price received for stored grain treated with botanical repellents. Overall, > 80% of both men and women said they sell grain to consumers and other traders, and that traders were the major buyers of their grain. At least 80% of respondents overall believed that they were offered a good price for good quality grain stored with botanical repellents. The majority of respondents reported that they received a better price for their treated grain. Less than 15% of male and female respondents sold their grain to other farmers.

**Table 7.10 Perception of respondents on the marketing and price of treated grain** (n=100).

| Variable | Female | | Male | | Chisq test |
|---|---|---|---|---|---|
| Sales of grain | Freq. | Percent | Freq. | Percent | |
| Consumers | 6 | 12 | 8 | 16 | $\chi^2$=16.6 |
| Other farmers | 3 | 6 | 7 | 14 | df=2 |
| Traders | 41 | 82 | 35 | 70 | p<0.001 |
| Price offered as a result of good treatment | | | | | |
| Don't know | 7 | 14 | 10 | 20 | $\chi^2$=16.6 |
| Yes a good price | 42 | 82 | 40 | 80 | df=2 |
| No the same price | 1 | 2 | - | - | p<0.001 |

Chi-square test for difference in perception of male and female respondents on marketing of treated grain is significant where p<0.05.

## 7.4 Discussion

The analysis of the findings in this study have helped to establish a good understanding of the perceptions of farmers who use botanical repellents to protect their stored sorghum from beetle infestations and the underlying factors that influence their choices of plant type and method of preparing grain for storage.

The amount of sorghum produced and stored differed between male and female respondents, with men producing >1.6 times more sorghum than women, which could be related to the main reasons they grow and store grain; the majority of men want to sell grain, and the majority of women want to satisfy their household food needs first. This accords with views reported in the literature, that women are more concerned than men about household food security (DFID & CIDA, 2009; Ogunlela & Mukhtar, 2009; Manda & Mvumi, 2010).

Considering the size of a household, which determines the amount of food required, amongst other needs, male-headed households tend to be larger than those headed by women, which means more mouths to feed and hence the need to produce more food. Men have larger land-holdings on which to grow crops and their larger families provide access to more labour-power, which enables them to produce more grain than female-headed households. According to the report by DFID and CIDA (2009), in Nigeria, as in other African countries, women have limited access to inputs such as land, which contributes to their lower agricultural output. This reinforces the situation that women are mainly concerned about their household food security and have difficulty meeting other expenditure demands.

Sorghum is stored either threshed or un-threshed, depending on the locality and fear of pest attack (Adejumo & Raji, 2007). It has been argued that sorghum is stored unthreshed to reduce pest infestation (FAO, 1992). However, based on the first survey results in Chapter 3, minority of farmers in southern Kebbi have the opposite perception and store threshed grain to reduce infestation. This study found that all of the women respondents stored their sorghum threshed, while men stored both threshed and unthreshed sorghum, which could be why men lose less grain to beetle infestations (grain loss for men is 58%, and for women 74%). It would be good to disseminate the information that unthreshed sorghum is less at risk to infestations than threshed sorghum. It is important to note that grain threshing is not a one-person job; it involves the use of energetic people and has financial implications. Since female-headed households tend to have smaller families and less income than male-headed households, women in Kebbi who store threshed grain are at a financial disadvantage because either they have to pay for labour to help with threshing (Ogunlela & Mukhtar, 2009) or take advantage of the free self-help groups to thresh their grain in one go, rather than having to do this many times during the year.

The location where sorghum was stored differed among the respondents. The majority of respondents used a store-room rather than a granary, although granaries were used by more men than women. The construction of a granary requires experience and money (Udoh *et al*., 2000), hence women and men with less income and experience may prefer to convert an available room in the house or a part of sleeping room to be their storage area. Moreover, the amount of grain to be stored could also influence the type and size of storage area; hence some men use both granary and store-rooms.

Farmers' level of production and financial needs may determine the duration of grain storage, which may also be influenced by family size, the level of infestation problems and the amount of grain produced (Omideyi, 1988; Thamaga *et al*., 2004). The ability of ~12 % of men and women to store grain for over a year (Table 7.4) indicates they can produce and store enough to take care of their family and other financial needs across the storage season. However, the fact that the majority of women stored grain for up to only 6 months may mean they produced an insufficient amount to meet their needs for a whole year and that they may need to find other alternative sources of food and to pay for other financial costs through to the next season. This suggests that differences in basic infestations levels, income levels and experience play a role in the choices respondents made regarding the methods of grain processing, storage location and duration of storage.

Insect infestation in storage is inevitable, depending on how the grain is stored and for how long (Gwinner *et al*., 1990; Bekele *et al*., 1997). Due to regular contact with insect pests, farmers become aware of the species present in their store-rooms. With the help of the NRI insect poster, all respondents were able to identify *T. castaneum* as the major pest found in their stored sorghum. This pest has already been reported as a pest of sorghum and millet in Nigeria (Lale & Yusuf, 2000; Turaki *et al*., 2007), in Kebbi (KARDA, 2004) and confirmrd by the first survey reported in Chapter 3 of this study. The majority of respondents reported a high rate of infestation and damage in their stored sorghum by the pest. Although infestation rates and damage were perceived differently by male and female respondents, (more men reported higher levels of infestation and damage rates than women). This finding suggests that since men are more concerned with the market value and the market does not accept infested grain, they may put more attention on infestations. Moreover, as women's grain is mainly used for household consumption, the level of grain damage considered to be high by men could be considered acceptable by women since damaged grains can be removed and mixed with good grain. In Chapter 4 of this study it was found that good quality grain with low levels of infestation was considered to be marketable, whereas grain with moderate or high levels of infestation was separated out and mixed with clean grain for home consumption. This indicates that women may be more open to using botanical repellents since they would benefit more than men, in terms of feeding their families, from improved grain quality.

This study revealed that farmers experienced high infestation rates when their grain was stored for six months to over a year, which coincides with the view that storage infestation mostly starts after three months of storage and continues to increase, at a rate that depends on the intervention approach taken (Dobie *et al*., 1991). This suggests that grain to be stored for a longer period of time requires more attention than grain to be stored for less than 3 months.

More than 90% of respondents used plant protectants; 71% of these reported 'none/low' beetle infestations, whereas, of the 10% that did not use plant protectants, 76% had high infestations (Table 7.6), demonstrating that overall, the respondents had good results with using botanical repellents. *Ocimum basilicum* alone or in combination with ground pepper was the main plant protectant used by women (74% vs 34% of men) and *Nauclea diderrichii* was the main plant used by men (45% vs 12% by women; Table 7.6), which was associated with 63% of respondents overall reporting 'none/low' infestations (Table 7.8). Of the respondents who used only *O.*

*basilicum,* and, more importantly, *O. basilicum* in combination with ground pepper, 69% and 95% had 'none/low' infestations, respectively (Table 7.8). The importance of *O. basilicum* as a grain protectant was reported by Asawalam *et al*. (2007), Mikhaiel (2011) and Mishra *et al.* (2012) and reviewed in Chapter 2. Its efficacy against *T. castaneum* was confirmed by the bioassays reported in Chapter 5 and field work in Chapter 6 of this study.

Men and women differed in the location where they collected repellent plants. Women cultivated and collected their plant materials at home, whereas men collected them from the wild. This could indicate how important women could be in promoting botanicals.

Different parts of *Ocimum* species, such as leaves flower and seeds, were reported to contain compounds that affect insect behavior (Klimánková *et al*., 2008). More research is needed to identify which parts of the plants are most repellent to target species.

The efficacy of whole plant and their constituent parts can be affected by the way they are prepared, particularly during the drying processes (Díaz-Maroto *et al*., 2004; Dev *et al*., 2011). It was confirmed in this study that men and women mainly sun-dry their plants. The few respondents that shade–dried their plants were all female. It could be that women have a greater knowledge of using and conserving bioactive compounds from plants, but this knowledge needs to be reinforced and disseminated to all farmers. The wide range of responses to most of the questions asked in this study demonstrates that there is a long way to go to reach a common acceptance of best practice in the use of plant protectants.

The results of this study demonstrate that the choice and acceptance of the use of botanical repellents may be influenced by a number of factors including gender, education, availability of plant materials, effectiveness of plant materials, cost of control materials and the market value of treated grain. This study also observed that the use of botanical repellents was greatest for people aged between 30 and 50 years, irrespective of gender. This finding is consistent with that of Mugisha-Kamatenesi *et al*. (2008) who found that botanical repellents were used mainly by respondents aged between 30 and 50 years, which is probably the best group to work with when planning future developments of botanical repellents. Although this study demonstrates that older people, including parents and grandparents, were the main source of traditional knowledge, the number of people over 50 years using botanical repellents was found to be very few. However, this may be due to the observation of numerous research findings that a low number of older people in a society continue farming (Mugisha-Kamatenesi *et al*., 2008; Salau *et al*., 2011; Ebojei, *et al*., 2011), and once they are no longer heads of a household, they are excluded from interviews.

Access to education is reported to increase the awareness of farmers and their access to modern farming techniques (Rosenzweing, 1995; Deng, 2009). However, the level of education of the majority of respondents in this study was very low, especially amongst the women, with only a few having attended primary and post primary education. This may be a contributing factor to their interest in traditional methods of storage pest control with botanicals rather than the use of modern control methods. Although farmers with less education may be easier to convince on the use of botanical, those with more education need to be teach in the use of botanical repellents.

This study confirmed that farmers' perceptions were influenced by the availability and effectiveness of particular plants, and that this differed between the male and female respondents. Women were more concerned with availability of plant materials than their effectiveness, which may relate to their lack of freedom of movement and may be the reason they preferred to cultivate and harvest their plants at home. Men were more concerned with effectiveness and this may be related to their interest in marketability. Generally, the respondents expressed great satisfaction with the way the plant protectants worked in protecting their stored grain. However, the fact that some respondents expressed no satisfaction at all may be due to the efficacy of the plant type chosen, and the preparation and application methods used (Belmain *et al*., 2001). The results of the efficacy of botanicals against *T. castaneum* in Chapter 5 confirmed this. Generally, availability of plant material and the lack of availability and cost of synthetic pesticides were the factors that have encouraged farmers to use botanical repellents. This may mean that for repellent plants to gain acceptance among farmers it is important to focus on developing and improving plant materials available and known to them already.

The findings of this survey indicate that both men and women sell grain and were satisfied with the price offered to them as a result of the quality obtained after treatment with botanical repellents. This implies that existence of a quality-demanding market could motivate farmers to extend their use of botanical repellents. The respondents believed that grain treated with botanical repellents had low or no infestations, which resulted in a higher price being achieved than for infested grain.

Although the respondents agreed that the main advantage to using botanical repellents is the improved market value of uninfested grain, the collection of large quantities of plant material, changes in the flavor of treated grain, time taken to prepare plant materials and the need for winnowing the grain were reported to be the main disadvantages of using botanical repellents as control measures. Furthermore, according to the respondents, the plants used as botanical protectants have a variety of alternative uses in their localities, such as ingredients for soup, to deter snakes, for traditional healing and as a room freshener.

# Chapter 8

## Identification of *Ocimum* and *Cymbopogon* species used as grain protectants in Nigeria based on their chemotypes and the relative bioactivity against *Tribolium castaneum*

### 8.1 Background

The findings of the survey conducted in Kebbi at the end of the field experiments (Chapter 7) indicated that a range of cultivars of *Ocimum* have been used by farmers as grain protectants. However, little is known about the differences between cultivars. For instance, despite some obvious physical differences between the plants, farmers often referred to them as if they were just one species, using a common local name (Doddoya), meaning 'scented' plants. The cultivars of plants used by farmers varied, depending on the locality and what famers had inherited from their forefathers. Some were harvested from the wild, and others were cultivated in family gardens. Raseetha *et al.* (2009) and Švecová & Neugebauerova (2010) reported that plants of the same species that are grown in different locations can vary in the quantity and range of their chemical components. Hence, the different plants used by the farmers could look physically similar, but have very different chemical profiles, which could affect their anti-insect activity (Isman, 2006).

Similarly, farmers differed in the methods they used to dry plants (sun-dried vs shade-dried) before use, which in some cases is likely to affect the chemical content of the final product. UNIDO and FAO (2005) reported that weather and the amount of sunlight can have a major impact on the quality and quantity of active volatile compounds that are preserved in dry plant material. Diaz-Maroto *et al*. (2004) and Zhang & Zhezhi (2007) reported that plants vary in their concentrations of volatile compounds, depending on the drying methods used, environmental details of the region and the time of year the plants were collected (Belmain, 2002), which may also affect their bioactivity on insect pest species. Hence, it is not surprising that the survey of farmers presented in Chapters 3 and 7 found that many farmers in southern Kebbi did not have much success with using dry plants to protect their grain, since they used quite a wide range of sources for plant material and they did not all dry their plants in the same way. Hence, this suggests the need to determine how much of the variation in chemical compounds is linked to their bioactivity, which plant cultivars have the optimum profile of repellent compounds and to determine by which type of drying the plants retained the greatest amount of active compounds. It is against this background that work presented in this chapter was carried out.

The aims were to:

- Identify the species of samples of plants used by farmers in the study area, based on their physical and chemical characteristics;
- Compare the effect of sun and shade drying on the chemical variability of the same plant samples that had been dried locally before they were brought to the UK;
- Confirm if differences in the chemical compounds in the plant samples correlated with their biological activity against *T. castaneum* in laboratory assays;
- Develop recommendations for the farmers on the species of plants and drying methods that would give the best results for the new double bag treatment method.

### 8.1.1 Chemical variation in some *Ocimum* and *Cymbopogon* species

Evidence from the previous research discussed in Chapter 2 and 6 (Mohiuddin *et al*., 1987; Wong *et al*., 2005; Manzoor *et al*., 2011; Mishra *et al.,* 2012) indicated that both *Ocimum* and *Cymbopogon* species possess compounds that have bioactive repellent effects on storage pests. However, there can be considerable variability in the number and the relative composition of the bioactive compounds, depending on the plant species (Raseetha, *et al.,* 2009), region, and plant cultivar (Chagonda *et al*., 2000; Pascual-Villalobos & Ballesta-Acosta, 2003; Sajjadi, 2006), the stage of development of plants at harvest (Klimánkova *et al.,* 2008) and the method of plant preparation (Diaz-Maroto *et al*., 2004). There are several species of *Ocimum* that are closely related (*O. basilicum*, *O. gratissimum* and *O. sanctum),* similar in appearance and have quite a pungent, characteristic scent to humans, and, therefore, are often called the same name, e.g., Basil or Sweet Basil. Although in many cases they can be differentiated by physical and chemical attributes, since these attributes can vary widely within species it is important to investigate the status of these plants in each local area. For instance, the main constituents of oil extracts of *O. basilicum* collected from the republic of Guinea were linalool and eugenol, which differed from *O. gratissimum* and *O. suave* collected from the same region, which were rich in thymol and *p*-cymene, respectively (Kéita *et al*., 2000). Whereas *O. basilicum* grown in Malaysia was found to be highest in methyl chavicol, which differed from a sample of *O. sanctum* grown from the same region, which was highest in methyl eugenol (Raseetha *et al*., 2009). Different cultivars of *O. basilicum* grown in different parts of northern Italy varied in their compositions (Miele *et al*., 2001). Different cultivars of *O. basilicum* collected in different locations in Benin republic showed differences in composition and type of the major compounds such as linalool, eugenol and methyl chavicol (Moudachirou & Yayi, 1999). Methyl chavicol was found to be a major component of *O. basilicum* grown in southern Nigeria (Kasali *et al*., 2005). However, this was different for *O. gratissimum* grown in Nigeria, which was richer in thymol and cis-ocimene (Saliu *et al*., 2011), and thymol and paracymene (Asawalam *et al*., 2008). And more generally, *O.basilicum* and *O. sanctum,* were reported to vary in their bioactive compounds (Vińa & Murillo, 2003; Raseetha *et al*., 2009; Švecová & Neugebauerova, 2010). Similarly, the composition of bioactive compounds of different cultivated and wild species of *Cymbopogo*n has been found to differ significantly (Chagonda *et al*., 2000).

The above evidence demonstrates the strong possibility of wide variation in chemical type and composition of different *Ocimum* and *Cymbopogon* species, which may be related to plant type, geographical location and weather conditions. Since bioactivity of different repellent plants against pests is related to their active ingredients and their composition (Isman, 2006; Belmain, 2002) knowledge of the chemical profile of plants can help predict their potential efficacy as grain protectants. Therefore, knowledge of the activity of different plant compounds against particular insect pests can help in the selection of plant species that are most likely to be effective against those pest species. Hence, preliminary chemical and bioassay analysis of local plant species can reduce time and resources wasted in using plant species chosen by trial and error.

Some main compounds, such as linalool, estragole (methyl chavicol), eugenol, methyl eugenol, thymol, 4-terpineol, neral and 1,8-cineol, that are found in different *Ocimum* species (Grayer *et al*., 1996; Yayia *et al*., 2001) and geranial, neral, citronellal, that are found in *C. nardus* (Nakahara *et al*., 2003) were reported to be responsible for the bioactivity of several plants against particular insect pests. Some of these compounds are found to be toxic to some insects, particularly if used in a confined environment as fumigants (Rozman *et al*., 2007), however they could be used in more open environments (e.g. storerooms) to repel storage pests (Wong *et al.,* 2005). Therefore, the research presented in this thesis focused on only the potential of repellent compounds in plants as insect control agents. For instance, Kim *et al*. (2010) and Ukeh & Umoetok (2011) found linalool to be highly repellant (>90%) on *T. castaneum* over 24hr. Suthisut *et al*. (2011) reported a significant (>77%) repellent effect of 4-terpeneol on *T. castaneum* at a dose as low as 0.31µl/cm$^2$. This indicates there is promising potential for plants with different repellent compounds to be used as pest control agents.

## 8.2 Materials and Methods

### 8.2.1 Plant materials used

Samples of *Ocimum* species from four sites and *Cymbopogon* species from three sites were collected from six villages (Yauri, Danko, Tondi, Kimo, Zuru and Wasagu, Map 3.1 chapter 3) in southern Kebbi during the peak period of the dry season. Each plant sample was named after the village where it was collected from as follow; OCT (*Ocimum* collected from Tondi), OCK (Kimo), OCZ (Zuru), OCW (Wasagu) and *Cymbopogon nardus* LGY (lemongrass collected from Yauri), LGZ (Zuru) and LGD (Danko). The farmers were asked to select plants that were called 'Doddoya' (which they associate with *Ocimum*-type plants) in each village. Although the plants they chose had obvious differences in leaf shape, it was decided to accept their own taxonomy, to understand better the difficulties associated with recommending the use of local plants as grain protectants at the village level. Farmers were also asked to select *Cymbopogon* plants, but these were more uniform in appearance. The sample of OCW was collected from an area of wild vegetation near Wasagu village and the other *Ocimum* samples OCT, OCZ and OCK were cultivated by famers of Tondi, Zuru and Kimo, respectively. The samples of *C. nardus* were collected from cultivated fields by farmers in Yauri, Danko and Zuru. Each plant sample was dried in the sun or shade dried for three days as used by local farmers, packed in plastic bags, and transported to the UK. In addition, for each plant sampled, specimens were dried according to the method of Stevenson's herbarium techniques (http://www.nri.org/projects/adappt/mcknight.htm). One herbarium specimen of each plant sampled was deposited in the college of Agriculture, Zuru herbarium and another was deposited at Kew Gardens for their identification and to add to their collection.

Based on the physical attributes of the samples, experts at Kew Gardens identified the samples as follows, but not until after the chemical identifications and repellency bioassays described below, had been completed. Therefore, the chemical and bioassay investigations were done ‘blind’.

OCT: *Ocimum basilicum*

OCK: *O. basilicum*

OCZ: *O. gratissimum*

OCW *O. africanum*

All three samples of Cymbopogon were identified as *Cymbopogon nardus*

### 8.2.2 Sample preparation and extraction methods used for chemical identification

Dried leaves from sun dried and shade dried samples of the four different plant samples of *Ocimum* (OCT, OCK, OCW and OCZ) and *Cymbopogon nardus* (LGY, LGD and LGZ) were ground to powder using a mini laboratory mortar and pestle. A 50mg sample of each ground plant was accurately weighed and mixed with 5ml of hexane containing 1mg of decyl acetate as an internal standard in a glass vial. Samples were left to extract overnight before the analysis.

### 8.2.3 Gas chromatography/ mass spectrometry analysis

Gas chromatography linked to mass spectrometry (GC-MS) analysis was used to identify the main compounds in the *Ocimum* and *Cymbopogon* samples. The samples were analysed by the GC-MS using a 6890 GC and 5973 MSD (Agilent). Samples were analysed on a non-polar (DB Wax; Agilent) column (30 m x 0.25 mm i.d. x 0.25 μm film thickness). The carrier gas was helium (1 ml/min) and the oven temperature was programmed for 60°C for 2 min, then increasing by 6°C/min to 240°C. Compound identification was confirmed by comparison to the NIST 05 mass spectral library. This analysis was performed at the NRI.

### 8.2.4 Bioassay preparation to compare the repellent effect of different *Ocimum* species

A choice bioassay using the thigmotactic method (open arena with stones and pits) described in Chapter 5.2.1 and 5.2.5 was used to compare the effect of each of the four *Ocimum* samples and the two drying methods on their biological activity against *T. castaneum*. Bags of wheat (20 g) were mixed with ground dry leaves of one of each of the *Ocimum* samples (OCT, OCW and OCZ) for which there were samples that had been sun dried and samples that had been shade dried, at each of three different doses (0.25, 1% and 2% w/w). One treated bag and one control bag (20 g wheat with no dried leaves) were placed at opposite ends of the tray, and 30, 7-10 day old adult beetles were released in the centre and left for 4 hrs (for details see Chapter 5). The numbers of beetles in each bag and in the rest of the arena were counted. This was repeated eight times for each of the three plant samples x two drying methods x three doses of plant material to test the relative repellency of each plant type.

Based on the findings of the experimental comparisons of different bioassay methods (Chapter 5), it was decided that the new thigmotactic bioassay should be tested for efficiency and efficacy against one more standard assay – the dual choice tube assay. The relative repellency of two samples of *Ocimum* plants, one that was found to be highly repellent (OCT) and one that was significantly less repellent (OCW) in the experiment above were tested by these two methods.

The dual choice assay consisted of a transparent round plastic tube, 80cm long x 12.5cm in diameter (Fig. 8.1). A treatment and a control bag of grain (20g) were placed at either end of the tube. The two ends of the open tube were then covered with white mesh cloth (0.5mm holes size) to prevent the beetles from escaping. There was an opening at top of the middle of the tube, through which the beetles were placed on the floor in the middle of the tube. Soft clear plastic tubing (35cm long x 7.0mm x 10.5mm in diameter) was then inserted into the same opening, and connected to a pump that sucked air gently out of the bioassay tube, effectively drawing air from the netting ends of the tube toward the centre of the tube where the beetles had been placed and out through the opening in the main tube. This flow of air brings the odours from the control and treatments bags to the centre of the tube.

In each run of the two bioassays 30, 7-10-day old beetles were introduced to the center of the test arenas and allowed a free choice between treated grain (1% w/w of 20g wheat) and untreated grain placed at either end of the test arenas. The bioassay was left for 4 h, after which the numbers of beetles in the treated and untreated bags and the rest in the arenas were recorded. This was repeated eight times for each of the two plant samples in each of the bioassay arenas.

**Fig. 8.1 Dual choice tube bioassay arena (80cm long x 12cm diameter);** with soft clear plastic tubing (35cm long x 7.0mm x 10.5mm) connected from the opening at the top-centre of the tube to a fan for sucking out air from the tube. A 20g bag of treated wheat was placed at one end and a 20g untreated bag of wheat at the other end, with beetles introduced in the center of the tube.

**8.2.5 Statatistical analysis**

The ratio of the different chemical compounds identified in the GC analysis for each sample of plants was compared visually by histogram to give a clear picture of the difference between the different plant samples. Cluster analysis with R software was used to identify plant samples with similar chemical characteristics. Bioassay result were added to the cluster analysis to test how well clusters of plants based on biological activity correlated with the clusters of plants based on chemical composition. Data on the proportion of beetles responding from the bioassays were analysed using a General linear model (GLM) with binomial error and a logit function to describe appropriately the set of data obtained. The statistical significance in the responses of beetles to different plant samples were tested using analyses of deviance. Multiple comparisons of means for the different plant samples were made using Bonferroni method. The tests were run using the 'R' statistic software package (R Development Core Team, 2012).

## 8.3 Results

### 8.3.1 Identification of volatile compounds in *Ocimum* species and *Cymbopogon nardus*

The results from the GC-MS analysis presented in Table 8.1 indicate that the different samples of *Ocimum* and *Cymbopogon* plants from Kebbi possessed different profiles of volatile compounds that have the potential to be used as bioactive materials against insect pests. The GC analysis identified ~ 15 compounds from the four samples of *Ocimum* species collected from different locations (OCT, OCK, OCW and OCZ) and the three samples of *Cymbopogon nardus* (LGY, LGZ and LGD) collected from different villages. The major compounds (defined as > ~ 10% of the maximum; 2.0 mg/g, Table 8.1, see data in bold) found in the *Ocimum* samples were, in descending rank order, 4-terpineol > linalool > caryophyllene > estragole > thymol > eugenol > o-cymene. However, for *Cymbopogon nardus,* caryophyllene and geranial were the dominant volatile compounds; all other compounds were found in negligible amounts.

The results show that OCT had greater amounts of the major compounds than the other three samples. The main compounds found in OCT, in descending rank order, included 4-terpineol > linalool > caryophyllene > estragole > eugenol. However, OCK had no 4-terpinol, but it did have estragole and linalool as the main compounds, although in less amounts than found in OCT. OCW had estragole and 4-terpineol, as the main compounds, but in less amounts than found for OCK and OCT. Thymol and o-Cymene were the main compounds found in OCZ, which were completely different than the main compounds found in the other *Ocimum* samples.

**Table 8.1 Volatile chemical compounds identified in samples of shade dried *Ocimum* species and *Cymbopogon nardus*** in mg/g of plant samples, extracted by methanol (except for o-cymene which was only found in the hexane extract), analysed by GC-MS. Data in bold indicate amounts > 2.0 mg/g, considered to be the major compounds present.

| | *Ocimum* species | | | | *Cymbopogon nardus* | | |
|---|---|---|---|---|---|---|---|
| Compound | OCT | OCK | OCW | OCZ | LGY | LGZ | LGD |
| 3-carene | 0.60 | 0.13 | 0.08 | 0.18 | 0.06 | 0.07 | 0.05 |
| Linalool | **23.20** | **10.00** | 1.64 | 0.69 | 0.48 | 0.72 | 0.56 |
| Camphor | 0.60 | 0.35 | 0.04 | 0.03 | 0.02 | 0.04 | 0.07 |
| 4-terpineol | **26.10** | 0.00 | **2.39** | 0.09 | 0.03 | 0.13 | 0.10 |
| Estragole | **19.40** | **11.69** | **2.69** | 0.05 | 0.04 | 0.13 | 0.08 |
| Neral | 0.24 | 0.03 | 0.06 | 0.40 | 0.50 | 0.25 | 0.99 |
| Nerol | 0.34 | 0.05 | 0.35 | 0.14 | 0.58 | 0.25 | 0.37 |
| Geranial | 0.17 | 0.09 | 0.34 | 0.00 | 0.78 | **2.88** | 1.29 |
| Thymol | 0.29 | 0.35 | 0.02 | **8.37** | 0.00 | 0.00 | 0.00 |
| Eugenol | **6.49** | 0.22 | 0.61 | 0.00 | 0.42 | 1.52 | 0.31 |
| Caryophyllene | **20.10** | 1.08 | 1.38 | 0.66 | **6.86** | **10.80** | **10.64** |
| O-cymene | 0.00 | 0.03 | 0.10 | **2.06** | 0.01 | 0.01 | 0.01 |
| á-Penene | 0.23 | 0.19 | 0.12 | 0.33 | 0.56 | 0.41 | 0.39 |
| a-terpineol | 0.22 | 0.12 | 1.86 | 0.03 | 0.04 | 0.01 | 0.03 |
| a-Bergamotene | 1.50 | 1.12 | 1.12 | 0.67 | 0.07 | 0.08 | 0.06 |
| Sample wt. (g) | 0.044 | 0.068 | 0.088 | 0.095 | 0.127 | 0.086 | 0.088 |

**8.3.2 Variation in the chemistry of *Ocimum* species extracted using different extraction solvents (methanol and hexane)**

The results in Fig. 8.2 and 8.3 show the variation in type and quantity of the major volatile compounds between the samples of *Ocimum* extracted using methanol or hexane. The results show that these extraction solvents dissolved a different profile of compounds from the plants tested. Samples extracted with methanol (Fig.8.1) had greater amounts of the different compounds and some compounds found were completely absent from hexane extracts, i.e caryophyllene (Fig. 8.1). Similarly, o-cymene was found in samples extracted with hexane in relatively high amounts in OCZ samples, compared to the other *Ocimum* samples, but absent in the samples extracted with methanol.

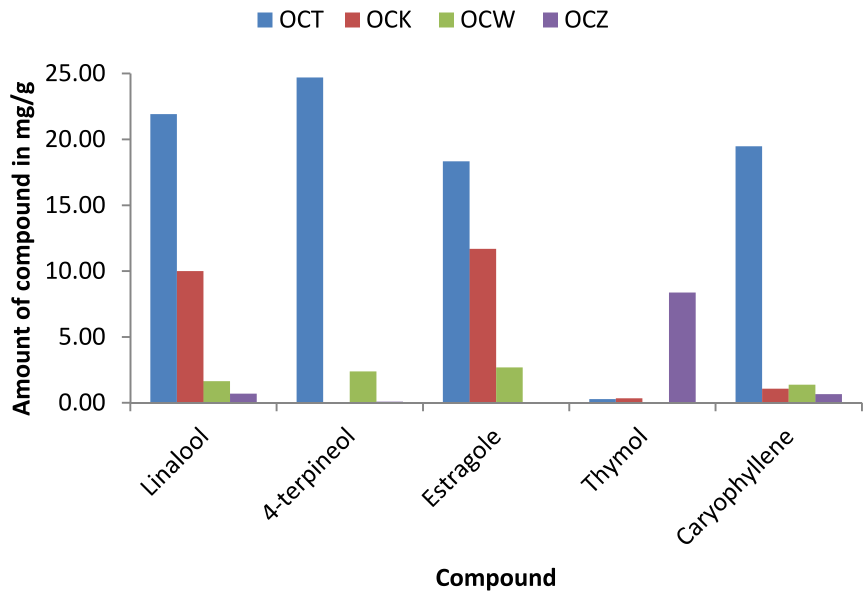


**Fig. 8.2 Methanol extraction of the main volatile compounds found in samples of *Ocimum***. The plant samples were analyzed by GC-MS. OCT = sample of *Ocimum* collected from Tondi, OCK from Kimo, OCW from Wasagu and OCZ from Zuru.

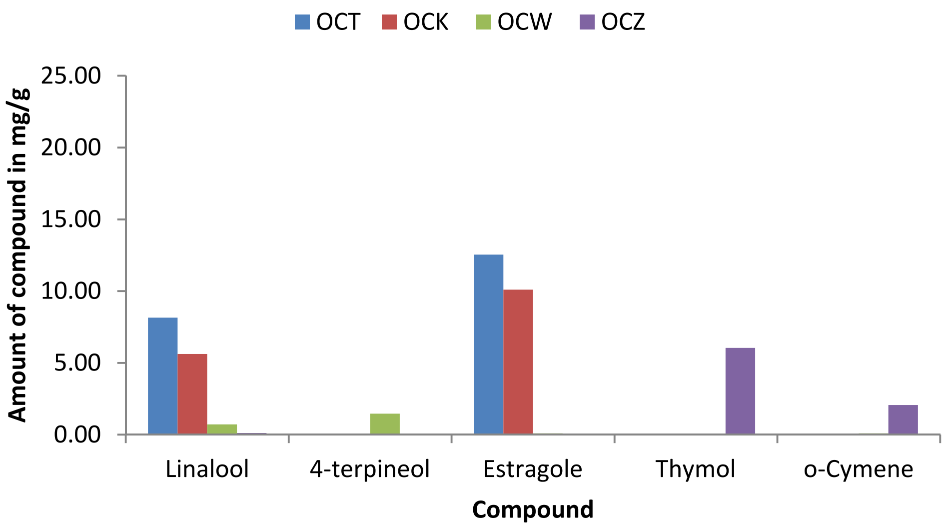


**Fig. 8.3 Hexane extraction of the main volatile compounds found in samples of *Ocimum***. The plant samples were analyzed by GC-MS. OCT = sample of *Ocimum* collected from Tondi, OCK from Kimo, OCW from Wasagu and OCZ from Zuru.

### 8.3.3 Variation in the chemistry of *Cymbopogon nardus* samples extracted with methanol and hexane

The results in Figure 8.4 indicates that all the samples of lemongrass (*Cymbopogon nardus*) (LGY, LGD and LGD) show a similar profile of the major volatile chemical compounds (caryophyllene, geranial and neral), but differed in the relative amounts of these chemicals. Caryophyllene was found in samples extracted with methanol, but absent in the samples extracted with hexane, and was found in relatively high amounts in LGD and LGZ, but in lower amounts in LGY. Although geranial and neral occurred in relatively small amounts in all the samples of LG, the amount found in LGZ was greater than that found in LGD and LGY. However, in further comparisons, the amount of neral and geranial found in all the samples of LG extracted with methanol was less than that found in hexane (Fig. 8.5). This may suggest that caryophyllene was more soluble in methanol than in hexane, and neral and geranial dissolved better in hexane than in methanol.

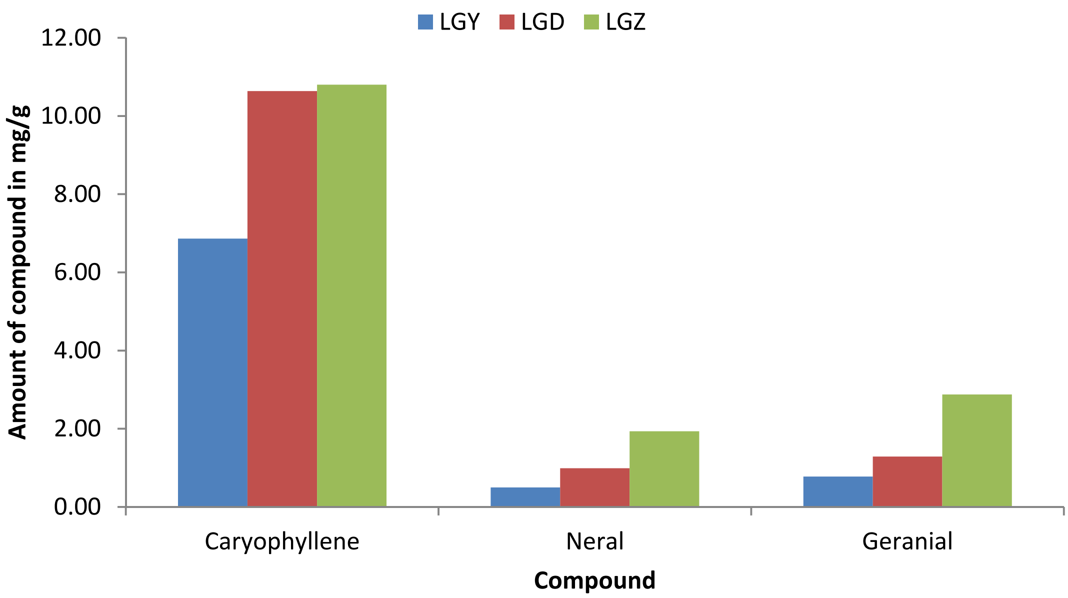


**Fig. 8.4 Methanol extract of the main volatile compounds in samples of *Cymbopogon nardus* (lemongrass).** The plant samples were analyzed by GC-MS. LGY = sample of lemongrass collected from Yauri, LGD from Danko and LGZ from Zuru.

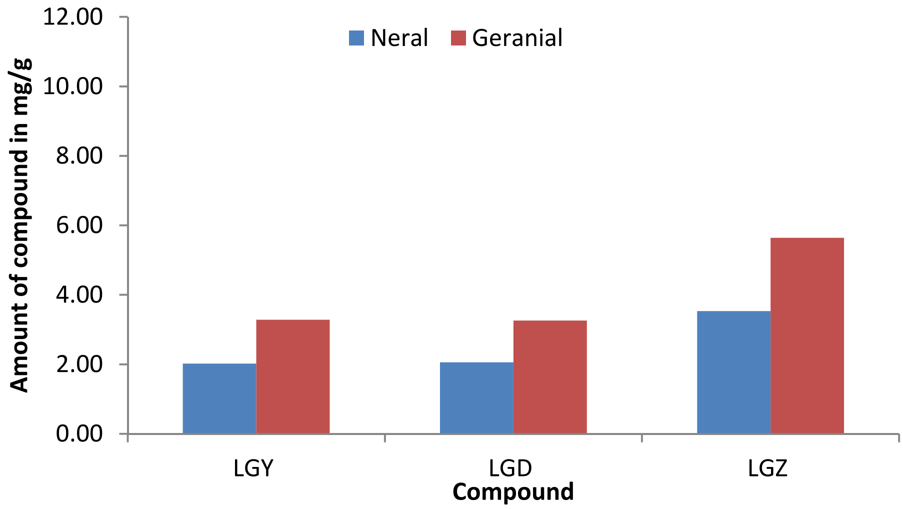


**Fig. 8.5 Hexane extract of the main volatile compounds in samples of *Cymbopogon nardus* (lemongrass).** The plant samples were analyzed by GC-MS. LGY = sample of lemongrass collected from Yauri, LGD from Danko and LGZ from Zuru.

### 8.3.4 Effect of drying methods on the main compounds extracted from samples of *Ocimum* and *Cymbopogon*

The effects of the method of drying plants on the chemical composition of the *Ocimum* samples are shown in Table 8.2. *Ocimum* species dried in the shade had greater amounts of the main chemical compounds than found in sun dried *Ocimum*. The variability depends on the *Ocimum* sample. For instance, the shade-dried samples of OCT contained higher amounts of all compounds than the sun dried samples. In particular, more thymol and o-cymene were found in the shade dried samples of OCZ than in the sun-dried samples. There is not much difference, however, between the amounts of compounds found in the samples of shade dried and sun dried OCK, OCW or LG, which may suggest the loss of plant compounds may be influenced by the type of plant.

**Table 8.2 Effect of drying methods on the main compounds of the different samples of *Ocimum* and *Cymbopogon* (in mg/g of plant) extracted using methanol.**

| | *Ocimum* | | | | | | | | *Cymbopogon* | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | OCT | | OCK | | OCW | | OCZ | | LGY | | LGZ | | LGD | |
| | shade | sun | shade | sun | shade | sun | shade | sun | shade | sun | shade | sun | shade | sun |
| Compound | Relative composition mg/g | | | | | | | | | | | | | |
| Linalool | **23.20** | **12.31** | **10.00** | **12.93** | 1.67 | 1.64 | 0.69 | 0.52 | 0.48 | 0.77 | 0.72 | 0.74 | 0.56 | 0.55 |
| 4-terpineol | **26.10** | 0.00 | 0.00 | 0.00 | **2.91** | **2.39** | 0.09 | 0.06 | 0.03 | 0.02 | 0.13 | 0.18 | 0.10 | 0.10 |
| Estragole | **19.40** | **13.40** | **11.69** | **13.65** | **2.69** | 1.58 | 0.05 | 0.04 | 0.04 | 0.10 | 0.13 | 0.03 | 0.08 | 0.03 |
| Thymol | 0.31 | 0.43 | 0.35 | 0.42 | 0.02 | 0.07 | **8.37** | **3.11** | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Caryo-phyllene | **20.10** | 1.01 | 1.08 | 0.98 | 1.38 | 1.06 | 0.66 | **13.06** | **6.86** | **10.83** | **10.80** | **11.62** | **10.64** | **8.66** |
| Eugenol | **6.49** | 0.00 | 0.22 | 0.10 | 0.61 | 0.23 | 0.00 | 0.02 | 0.42 | 0.33 | 1.53 | 0.61 | 0.31 | 0.47 |
| o-Cymene | 0.00 | 0.01 | 0.03 | 0.01 | 0.10 | 0.10 | **2.06** | 0.84 | 0.01 | 0.00 | 0.01 | 0.00 | 0.01 | 0.00 |
| Geranial | | | | | | | | | **3.29** | **3.38** | **5.64** | **2.35** | **3.26** | **4.81** |
| Neral | | | | | | | | | **2.02** | 1.97 | **3.53** | 1.50 | **2.06** | **3.00** |

### 8.3.5 Cluster analysis to identify plant samples with similar chemical characteristics

A cluster analysis, using Ward's method, was applied to the chemical composition data shown in Table 8.2. This resulted in four clear clusters, whose average profiles are in Fig. 8.6.

All the samples of lemongrass, including sun dried and shade dried, are in cluster 3, showing that they were chemically similar, differing only in the relative amount of each chemical compound, which may be due to the different environments, especially soil types, in which they were grown.

Samples of *Ocimum* varied, spanning three groups. For instance, Cluster 1 consists of shade-dried OCT on its own, which had the greatest proportion of important compounds known to be repellents i.e., linalool, 4- terpineol, estragole, eugenol and caryo-phyllene. Sun-dried OCT and sun- and shade-dried OCK were placed together in Cluster 2, which all had similar amounts of linalool and estragole in methanol extracts. Shade- and sun-dried OCW and OCZ were placed in Cluster 4. In this cluster, shade-dried OCW differed from Clusters 1 & 2 in not having particularly high amounts of the five important repellents found in OCT and OCK, and OCZ was the most different to all the other *Ocimum* samples, with low levels of the five important repellents found in OCT and OCK, and it was the only sample to have relatively high levels of thymol and o-cymene.

The species of each plant sample had not yet been identified by Kew Gardens when the chemical and cluster analysis was done, so the finding that the three clusters of *Ocimum* samples differed in the profile of their volatile chemical compounds was the first indication that the different samples might not be of the same species of *Ocimum*. Consequently, the next step was to determine whether the chemical differences between *Ocimum* samples might be reflected in relative differences in their biological activity on the insect pests, which is presented in the next section.

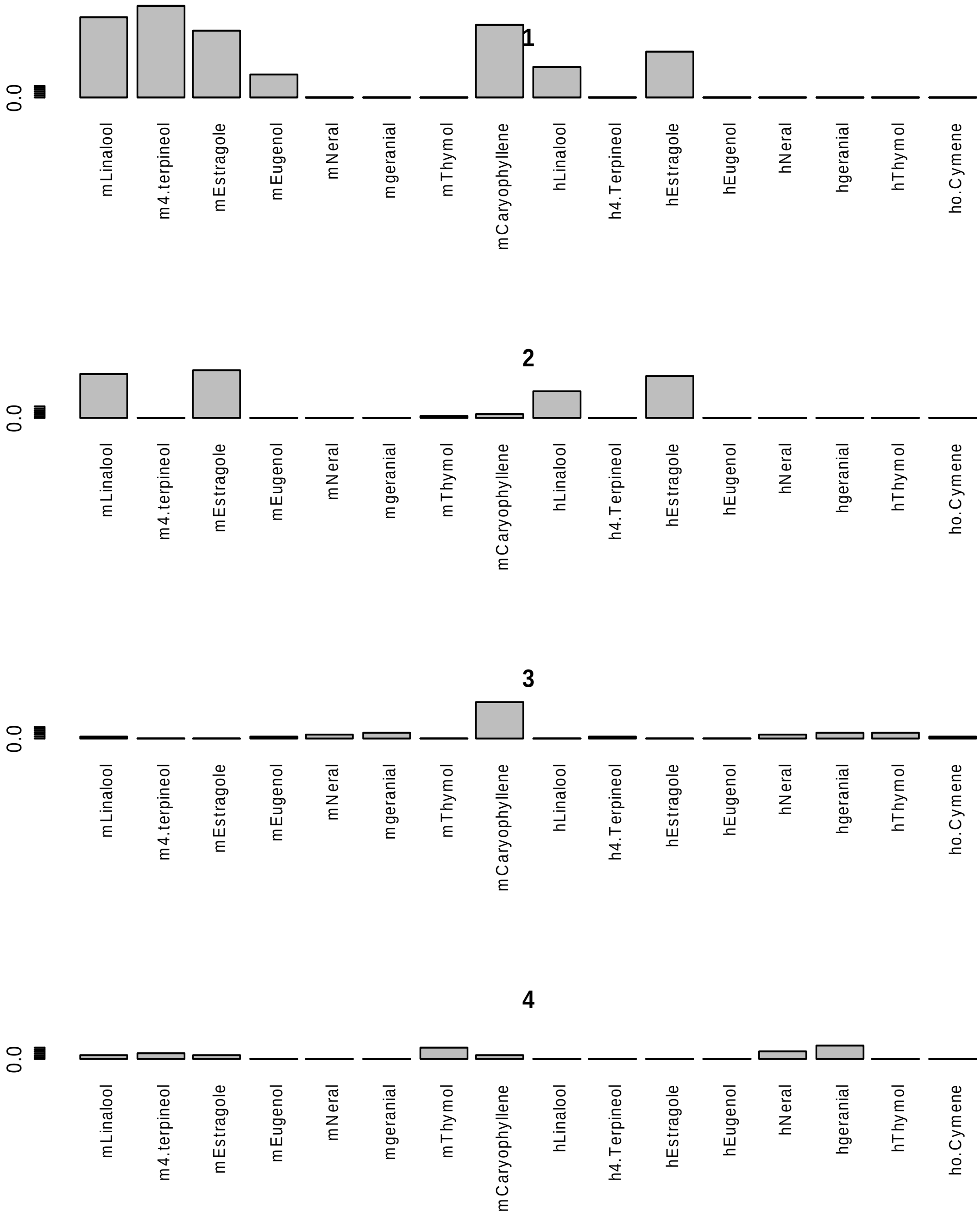


**Fig. 8.6 Results of cluster analysis placed the plant samples into different groups based on their volatile chemical compounds.** Cluster 1 = OCT shade alone, Cluster 2 = OCT sun, OCK sun, OCK shade, Cluster 3 = LGY, LGZ and LGD shade and sun, and Cluster 4 = OCZ and OCW sun and shade dried.

**8.3.6 Thigmotactic bioassay to compare the bioactivity of the four different shade-dried *Ocimum* samples on *T. castaneum***

The results in Fig. 8.7 show the relative repellency of *T. castaneum* to the different *Ocimum* samples (OCT, OCK, OCZ and OCW) over a range of doses (0.25%, 1% and 2% w/w). Only shade-dried samples were tested, because the chemical analysis (above) had shown that these samples had higher amounts of compounds known to be bioactive in *T. castaneum* than the sun-dried samples.

The findings indicate that all the *Ocimum* samples demonstrated a progressive dose effect, which increased in repellency with increasing dose from 0.25% w/w to 2% w/w, except for OCW, which appears to be attractive at the lowest dose. The main effect of dose was statistically significant (Analysis of variance; $\chi^2$=78.41, df =2, $p<0.001$), showing that the dose/response relationship was significant.

The 2% w/w doses had the greatest effect for all the plant samples (Fig. 8.7) and the effect of the dose on the proportion of beetles repelled was statistically significant (Analysis of deviance; $\chi^2$=127.29, df =3, $p < 0.001$). The Multiple comparison tests using the Bonferroni correction shows that OCT significantly repelled a higher proportion of beetles (0.88±0.015, $p<0.001$) than OCW (0.62±0.020) and OCZ (0.73±0.022). The proportion of beetles repelled by the effect of OCK was not significantly different (0.76±0.022, p=0.09) from OCT (0.88±0.015) or OCZ (0.73±0.060, p=2.25), but differed significantly ($p<0.01$) from OCW. This suggests that these four *Ocimum* samples have a different repellent effect on *T. castaneum,* with OCT having the greatest repellent effect of the four.

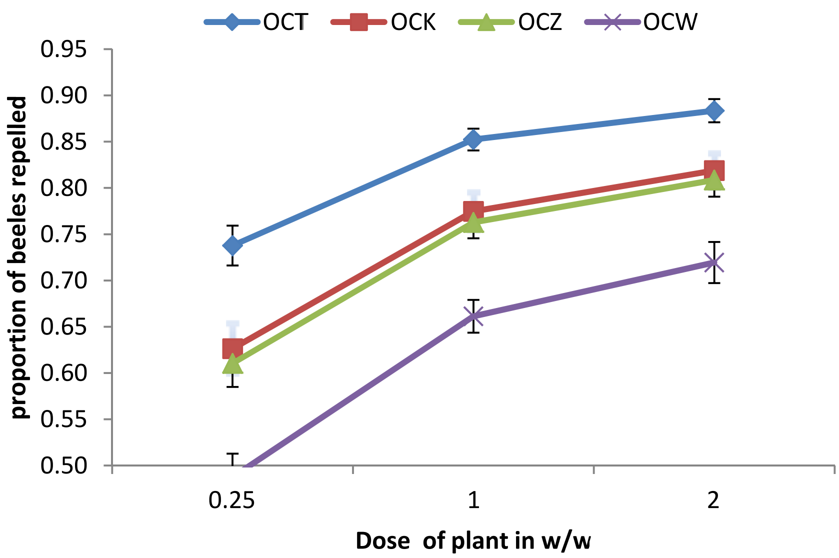


**Fig. 8.7 Thigmotactic bioassay results, comparing the mean±SE proportion of beetles repelled by grain treated by four different types of *Ocimum* species (OCT, OCW, OCK and OCZ)** compared to a control, at three doses (0.25%, 1% and 2% w/w wheat). Standard error bars were calculated from the analysis of deviance residuals in a binomial test. N=30 beetles, the experiment was replicated eight times, with a duration of 4 hours/bioassay. Increase in concentration of plant materials had significant effect ($p<0.001$) on the proportion of beetles repelled (Analysis of deviance).

The results in Fig. 8.8 show the efficacy of different methods of drying *Ocimum* (sun or shade dried) on the proportion of beetles repelled. The results show that the proportion of beetles repelled by shade dried plants was greater (0.76±0.039) than that of sun dried plants (0.60±0.034) and the control (0.55±0.037). Overall, the differences in the proportion of beetles repelled between the *Ocimum* samples dried either by shade or sun was found to be statistically significant (Chi sq=34.33, df =1, $p<0.001$). The Multiple comparison tests using the Bonferroni correction shows that the difference between the mean proportion of beetles repelled by shade dried OCT to be significantly greater ($p<0.001$) than OCZ (shade and sun), OCW (shade and sun) ($p<0.001$) and the control ($p<0.001$), but not different ($p=0.09$) than OCK (shade). OCK was shown to be significantly more repellent ($p<0.001$) than OCW (shade and sun) and the control, but not different ($p=2.25$) to OCZ (shade). The effect of OCZ (shade) was significantly greater ($p<0.001$) than OCW (shade and sun) and OCT (sun), but OCW was not different ($p=0.055$) from the control. In summary, shade dried plant material was always significantly more repellent than sun-dried material of the same plant type and the control, and OCT, OCK and OCZ shade-dried plant material were similarly repellent.

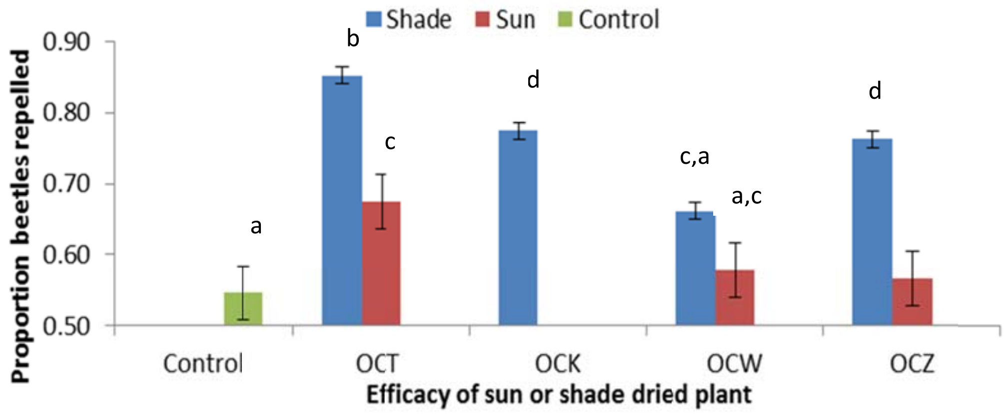


**Fig 8.8 Thigmotactic bioassay results, comparing the mean proportion (±SE) of beetles repelled by treated grain of four types of *Ocimum* species (OCT, OCW, OCK and OCZ), either sun dried or shade dried, at 1% w/w wheat against a control (untreated grain).** Standard error bars were calculated from the analysis of deviance residuals using in a binomial test. N=30 beetles for each run, test was replicated eight times, and the duration of each run was 4 h. Overall, the method of drying plants had a significant effect on the proportion of beetles repelled (Analysis of deviance; $p<0.001$, see text). Bars with different letters are significantly different (Bonferroni test, $p< 0.05$), see text**).**

A comparison of the bioassay results with those of the cluster analysis of the chemical composition of the *Ocimum* samples indicates that the relative repellency of the *Ocimum* samples was consistent with the cluster groups based on their profiles of volatile chemicals. For instance, the fact that OCT shade (on its own in Cluster 1) had the highest number and amount of the active compounds was reflected in the bioassay results, which showed the highest proportion of beetles, were repelled by OCT (shade). The results for the bioassay indicating that the effect of OCT (sun) and OCK (sun) were not different, is reflected in their chemical similarity as members of Cluster 2. The results for the bioassay experiment indicating that the effect of OCW (sun and shade) and OCZ (sun) on beetles is consistent with these three samples being in the same chemical Cluster, except for OCZ (shade), which differed from the others by having a high amount of thymol.

### 8.3.7 Comparison of the efficacy and efficiency of two bioassay methods (Thigmotactic vs Dual choice) for testing the repellent effect of different *Ocimum* samples

As in Chapter 5, the aim of comparing two types of bioassay is to determine which assay is more effective and more efficient in identifying bioactive compounds. The more 'effective' assay gives a more precise estimate of the difference between the number of beetles that choose the control or the treatment options. The more 'efficient' assay gives a smaller proportion of insects that never arrive at the control or treatment option.

Figure 8.9 shows the results for the two bioassay methods (thigmotactic tray and dual-choice tube) used to test the repellency of *Ocimum* samples (OCT and OCW). Overall, the results show that there was a significant difference between the proportions of beetles repelled by the plants in the thigmotactic assay than the tube assay (Analysis of deviance, GLM; Chisq=4.02, df =1, $p<0.05$). Significantly more beetles were repelled by OCT in the thigmotactic assay than in the tube assay (Analysis of deviance, GLM; Chisq=24.11, df=2, $p< 0.001$), but there was no difference in the repellent effect of OCW between the two assays Analysis of deviance, GLM; Chisq=3.70, df=2, p=0.06). The results with OCT (higher proportion of beetles repelled for the thigmotactic assay) suggest that the thigmotactic assay is more effective than the dual choice tube assay. OCW was less repellent, but that may because OCW was found to have a very much lower concentration of the most bioactive compounds than OCT (Table 8.2), it could be that a higher dose is required to increase the efficacy of the assay.

When the comparative 'efficiency' of the two assays is considered (i.e., assays with a high proportion of beetles found in either the treated or control bags are more efficient) a significantly higher proportion of beetles (Chis-sq=4.51, df=1, $p<0.05$) made a choice in the thigmotactic assay than in the dual-choice assay in the experiments with OCT or OCW. This indicates that the thigmotatic assay was more efficient than the dual-choice assay and highlights the importance of using the most appropriate bioassay in testing the behaviour of pest insects.

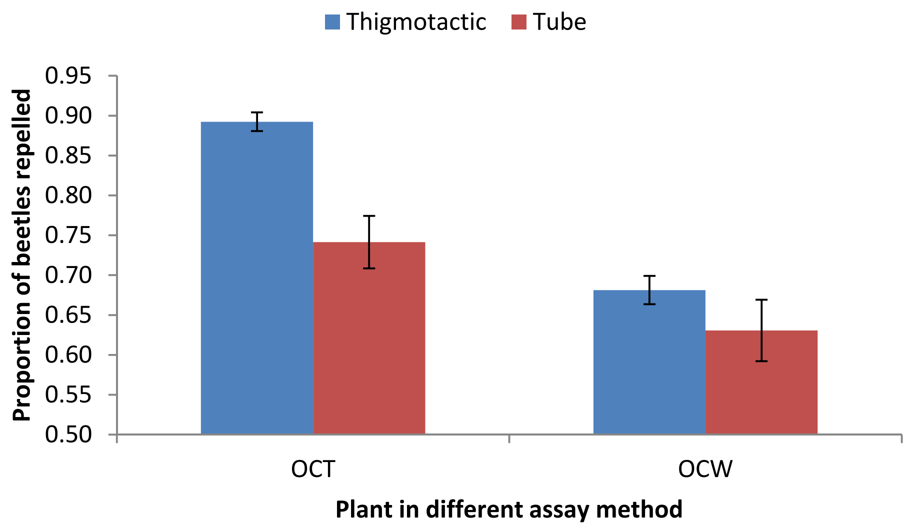


**Fig 8.9 Comparison of the mean proportion (±SE) of beetles repelled by treated grain by two different types of *Ocimum* species (OCT and OCW at 1% w/w) tested by two bioassay methods (Thigmotactic and Tube assay) to test their efficacy**. Standard error bars were calculated from the analysis of deviance residuals in a binomial test. N=30 beetles, the experiment was replicated eight times, and run for 4 h. There was a significant effect of bioassay type on response of beetles to the plant materials (Analysis of deviance, $p<0.05$).

## 8.4 Discussion

The work of this study indicates that the different samples of *Ocimum* collected from farmers have differing chemical compounds and ratios that can be used to distinguish most of them from one another. Similarly, the processing methods practiced by farmers resulted in samples of the same type of plant having different profiles of volatiles, with chemical components occurring at different ratios depending on whether they were sun or shade dried, and, consequently, differing in their behavioral effects on *T. castaneum*. Hence, the perception of farmers that the different plants commonly referred to by the same name as "*Dodoyya*" are the same species is not valid.

Dr Alan Paton a world specialist on *Ocimum* species at Kew Gardens, UK and Prof. Philip Stevenson (NRI and Kew Gardens) confirmed the identity of the *Ocimum* samples, by considering the morphological features of each sample. Paton confirmed that OCT and OCK are both *O. basilicum* (but collected from different locations), OCW is *O. africanum*, which is a closely related, wild version of *O. basilicum*, and OCZ is *O. gratissimum*, which is the least closely related species to the others (Paton *et al*., 1999). This was supported by the GC chemical analysis, which revealed that the profile of volatile chemical compounds in the four plant samples differed considerably.

The study demonstrated that OCT, which was the cultivated species, had the highest amounts of volatile chemical compounds. The profile of chemicals in OCK and OCW were similar to OCT, but differed in the ratios of the same chemical, whereas, for OCZ, an entirely different profile of volatile compound was present. The finding of Grayer *et al*. (1996), that the major components of *O. basilicum* are linalool, estragole, eugenol and methyl eugenol, is consistent with the findings of this study. However, in this study the cultivated varieties, OCT and OCK differed in the amount of 4-terpineol and the amount of caryophyllene. The 4-terpineol and caryophyllene found in this study was in addition to what Kasali *et al*. (2005) found in *O. basilicum* grown in southern Nigeria. However, the differences in the chemical compounds present and their relative amounts could be a result of environmental differences in the growing location (Pascual-Villalobos & Ballesta-Acosta, 2003; Boeke *et al*., 2004b) or genetic diversity of the plant species (Quereshi *et al*., 2011) and even plant morphological characters (Grayer *et al*., 1996; Vieira & Simon, 2006). For instance, Moudachirou & Yayi (1999) reported different cultivars of *O. basilicum* collected in different locations in Benin republic to show differences in composition and types of the major compounds such as linalool, eugenol and methyl chavicol. However, *O. basilicum* collected in different locations in India were found to have either estragole or estragole and linalool as their major component(s), but in differing ratios (Verma *et al*., 2012). The findings of this study, that OCZ (*O. gratissimum)* was high in thymol and o-cymene is consistent with the finding of Asawalam *et al*. (2008), that *O. gratissimum* grown from Nigeria was rich in thymol and para-cymene or eugenol and cis-ocimene (Saliu *et al.,* 2011). Similarly, *O. gratissimum* from Benin was found to be rich in p-cymene and thymol (Kpoviessi *et al*., 2012). OCW, which was grown wild and identified as *O. africanum* was found to contain estragole, 4-terpineol and linalool as the major compounds but at a reduced ratio to other compounds compared to OCT and OCK. This may indicate that cultivated *Ocimum* has more active chemical compounds in terms of both types of chemicals and relative ratios than wild types. *Ocimum africanum* is one of the *Ocimum* species known to grow wild in Africa and to be closely related and often confused with *O. basilicum* (**http://www.kew.org/plants-fungi/Ocimum-basilicum.htm**). Recently, two distinct chemotypes of *O. africanum* (i.e geranial/neral and estragole) were identified (Carovic-Stanko *et al*., 2011). It could be argued that this species merits further selection and cultivation to improve promising characteristics that might increase its value in pest control locally.

The similarity of chemical types and composition demonstrated by the different samples of Lemongrass (LGY, LGZ and LGD) could indicate that the samples of the Lemongrass were the same species. Although there was a little difference in the ratio of the active ingredients between the samples, this may be because of differences in the growing location. The presence of caryophyllene, geranial and neral as the major compounds in all the Lemongrass samples helped to identify it as *Cymbopogon nardus*. This finding was supported by the work of Mahalwal & Ali (2003), Nakahara *et al*. (2003) & Silva *et al*. (2011), although they all found caryophyllene in negligible amounts, and it was the major compound found in the samples extracted with hexane in this study. This emphasizes the importance of carefully choosing the solvent used for extraction of volatile compounds. This was confirmed in this study where it was found that more types of active compounds and ratios were found in plant samples extracted using methanol compared to hexane. This suggests that some compounds dissolve better in methanol as a solvent than in hexane; e.g., o-cymene was found only in hexane for *Ocimum* species, and caryophyllene in methanol extracts of *Ocimum* and Lemongrass. Ahmad *et al*. (2009) and Mousavi *et al*. (2012) also observed variation in the type and amount of active compounds for different solvent extraction methods.

This study demonstrates that drying methods can have a significant effect on the types and amounts of active compounds found in plants. The plant samples dried in the sun lost more active compounds than the samples that were dried in the shade. It could be that when plants are sun dried their volatile compounds are evaporated by the sun more quickly than if they are in the shade. Barbieri *et al*. (2004) confirmed that a lot of physiochemical processes occur in plants during drying, which are also influenced by environmental factors, such as temperature, humidity and air velocity (Rocha *et al*., 2011), which may affect the type and amount of volatile compounds that remain in the plants (Díaz-Maroto *et al*., 2004). In the study conducted by Grayer *et al*. (1996) there was significant variation in the types and amount of active compounds found in plants subjected to different drying methods. An intensive review of the influences of drying processes on the quality of medicinal plants was conducted by Rocha *et al*. (2011), and indicated that most of the studies that have been conducted on the effect of drying on plant volatile compounds has been done without due consideration of the farmers' situation, which often requires them to use direct sun to dry their plant materials to be used for grain protection. Rocha *et al*. (2011) reported that intensive solar radiation has adverse effects on the quality of plant volatile compounds, hence causing losses in the active compounds in the plants. It was found in the study presented here that the effect of sun drying on the active compounds was greater for some plant species than others. The major compounds in OCT and OCZ were affected the most, and there was little effect for OCW and OCK. This may suggest that the effect of sun drying depends on particular plants and their active compounds. Kpoviessi *et al.* (2012) observed a significant reduction in the amount of thymol and *p*-cymene in *O. gratissimum* harvested at the apex of the sun than in the morning. Similarly, Díaz-Maroto *et al*. (2004) observed a considerable reduction in linalool and little in eugenol content of *O. basilicum* during oven and freeze-drying. Grayer *et al* (1996) reported that methyl chavicol and eugenol were susceptible to drying processes. It was observed that volatile compounds stored on the plant leaves are lost through expansion and relaxation of the cuticle layer of the plant leaves during drying process; however, the rate of expansion was very negligible in some plants (Díaz-Maroto *et al*., (2004). Hence, this could explain the little effect of sun drying on the major compounds of OCW and OCK.

The results of the bioassay conducted to determine if differences in the chemical compounds in the plants samples correlated with their biological activity against *T. castaneum* indicated that the variability in the type and amount of active ingredients in the different samples of *Ocimum* species had an effect on their repellency to *T. castaneum*. Furthermore, the results confirmed that changes in the active compounds of the different *Ocimum* species due to differences in the effects of sun vs shade drying may have affected the degree of repellency for *T. castaneum*. As expected the cultivated *Ocimum* (OCT), which contained higher amounts of active chemical compounds was found to be more repellent to *T. castaneum* than any of the other samples of *Ocimum*. Even though OCK and OCT were identified to be the same species (*O. basilicum*), their active compounds differed considerably, which was reflected in differences in their repellency. These finding are supported by Švecová *et al*. (2010), who reported that the same plant species grown in different locations could have different active compounds, which could affect their biological activity on insects (Isman, 2006). OCK and OCZ demonstrated a similar degree of repellency, even though the types of the active compounds in the two plants were entirely different. Interestingly, their efficacy was better than OCW (a wild species). These findings suggest the possibility that famers using different species (cultivated vs. wild) grown in different locations are likely to experience different effectiveness in protecting their grain, even though they may be unasure that they have used plants with very different chemical profiles. Hence, it is important that farmers learn about these potential problems with using locally grown plant material, and that using cultivated *Ocimum* is likely to have a greater efficacy.

This study demonstrates that the repellent efficacy of the samples of *Ocimum* on the beetles was affected by the dose of the plant used. For instance all the samples of *Ocimum* were shown to have a linear dose repellence effect on *T. castaneum,* with the greatest effect at 1-2% w/w. OCW was shown to have the least repellent effect at all doses compared to all other samples of *Ocimum,* even when used at the highest dose (2%). The finding that the efficacy of OCT, OCK and OCZ was still increasing with dose up to 2% could indicate that even higher doses might increase the amount the repellent effect, although the curves in Fig. 8.6 were beginning to level off at 2%. However, for OCW it is likely that doses much higher than 2% could increase the efficacy of this species of *Ocimum,* hence, there is some indication that the efficacy could be improved. However, if high doses of plant are required for some *Ocimum* species, it may be better to use the essential oils rather than harvest and process large quantities of plant material to treat the double bags The previous research of Asawalam *et al*. (2007), Mikhaiel (2011) and Mishra *et al*. (2012) has demonstrated the repellent efficacy of essential oils from *O. basilicum* (OCW and OCK) and *O. gratissimum* (OCZ) against stored product pests. No information was found on the efficacy of *O. africanum* (OCW) on stored product pests to compare the present results with.

It was found in this study that differences in the active compounds between sun and shade-dried *Ocimum* had a significant effect on the repellency of *T. castaneum*. A greater repellency was observed with shade-dried than sun-dried OCT and OCZ, which is consistent with the finding that shade-dried plants had higher levels of active compounds. This was confirmed in the dose experiment of this research and in the study conducted by Phillips *et al*. (1995), Kim *et al*. (2010) and Suthisut *et al*. (2011), where the response of *T. castaneum* and other stored product insects to active compounds such as linalool, 4-terpineol, caryophyllene and thymol was dose dependent. However, no difference in repellency was observed between shade-dried and sun-dried OCW. This may be because of the fewer types and amount of the active compounds observed in both the shade- and sun-dried OCW, which may be too low to elicit a difference in response between the shade- and sun-dried OCW.

The results of the cluster analysis indicate that the chemical characteristics and biological activity of some of the *Ocimum* samples were similar, and hence grouped in the same cluster. The cluster analysis based on chemical characteristics put shade-dried OCT into a different cluster to all the others, which distinguished it from other samples of *Ocimum*, which was reflected in its performance in the bioassay, with the highest repellency effect of all the *Ocimum* samples. The similar repellent efficacies of sun-dried OCT, sun-dried OCK and shade-dried OCK supports their grouping in the same cluster. The number and type of active compounds were highest in the shade-dried OCT. The effect of sun drying on OCT reduced the type and amount of active compounds to be more similar to that found in OCK; hence these were in the same cluster. Moreover, sun-dried and shade-dried OCW and OCZ were placed in the same cluster, but only shade-dried OCZ appeared to have a significant difference in repellency, which may be because it contained high amounts of thymol. This pattern of repellency demonstrates the variable efficacy of different active compounds.

The opportunity was taken in this study to compare the efficiency and efficacy of the thigmotactic assay compared to the standard dual-choice tube assay to assess the relative repellency of the two samples of *Ocimum* (OCT and OCW) on *T. castaneum*. The findings indicate a greater level of repellency of beetles to OCT in the thigmotactic assay than in the dual-choice tube assay. The lower efficacy demonstrated by the tube assay could be due to the effect of the tube design which may have affected the beetles' behaviour (Morgan *et al*., 1998). By direct observations during the experiment, it was clear that the long narrow tube restricted the movement of the beetles along the sides of the tube. This effect was contrary to the natural behaviour of the beetles, which is to prefer to move around their environment until they get to edges they can follow (Semeao, *et al*., 2012). It was observed that when the beetles attempted moving up the sides of the tube they often fell on their backs and the smooth surface of the tube made it difficult for them to turn over and continue to move toward their choice of grain bags. Hence, the dual- choice assay had a lower proportion of beetles responding to the test stimuli, which resulted in a greater proportion of the beetles not making a choice of either the treated or untreated bags of grain. However, the thigmotactic assay provided the beetles with more of their natural environment, with stones throughout the tray floor providing beetles with more chances of movement around the tray area so they could make a choice. It should be noted that the test for the efficacy of the assay methods was done only with two types of plants; a more accurate comparison of the two assays could be obtained by testing more plants.

The findings of this research have helped in increasing our understanding that the repellency of *T. castaneum* by *Ocimum* species depends on the active compounds and their relative concentrations in test plants. It was also shown that a weak repellent efficacy of plants is correlated with low levels of active compounds due to either the habitat they were grown in, or loss of active compounds as a result of being sun-dried. These findings also help to understand the reasons for a wide range of efficacy of plant materials experienced by famers in their attempts at pest control. For instance, it is clear from these experiments that farmers using cultivated shade-dried *Ocimum*, especially shade-dried cultivated OCT, are likely to have better results than famers using sun-dried *Ocimum* of any of the other samples. It was also found that the best results were obtained when grain was treated with shade-dried *Ocimum* at 2% w/w, although 1% w/w could equally give at least 50% repellency. However, for OCW, which demonstrated low efficacy, better results could have been achieved with shade drying and increasing the dose to > 2% w/w. Finally, a further comparison of two bioassay methods confirmed the findings in Chapter 5 that the response of beetles to test plants/compounds in a bioassay are better understood when the test environment includes features that are similar to their natural environment.

# CHAPTER 9

## General summary, discussion, conclusions and recommendations

### 9.1 Introduction

The surveys of Chapters 3 and 7 highlight that sorghum is the most important food for small-scale farmers in Kebbi state; after harvest a large proportion of the grain is stored in various traditional storage structures. This grain is used mainly to satisfy immediate food requirements and the surplus is sold to pay for other socio-economic needs. However, post-harvest losses, mainly due to storage pests, have been an obstacle to realizing the full benefits of grain storage. Information about the most important pests that attack grain is incomplete and the underlying factors that facilitate the problems caused by the pests are not well understood. Although, the reports of KARDA (2004) and COA (2009) highlight *Tribolium castaneum*, *Rhyzopertha dominica* and *Sitophilus zeamais* as among the most common pests of stored grain in the state, there seems to be no scientific evidence to prove this. However, the evidence from Lale & Yusuf (2000) and Chimoya & Abdullahi (2011) indicate that *T. castaneum* and *Sitophilus* species are major pests of stored sorghum and millet in northern Nigeria. The proliferation of pest infestations in farmers' grain stores are probably due mainly to inappropriate storage methods, ineffective grain protectants and inefficient methods of processing and application of grain protectants.

The uses of synthetic insecticides, application of local plant materials or leaving grain untreated have been the main approaches to storing grain. Leaving grain untreated due to unavailability of chemicals or the lack of financial resources to buy chemicals compounds the problems of pest infestation. Improper use of pesticides, inadequate knowledge of the correct pesticides to use or the correct doses can result in more problems. Insecticides are also hazardous for small-scale farmers due to toxic effects of the chemicals for humans and non-target organisms in the environment, and the risk that insecticide resistance will develop, leaving farmers with even fewer solutions to the problem of grain loss (Arthur *et al*., 1988; Arthur et *al*., 1990; Xue *et al.* 2006; Assie *et al*., 2007; Snelder *et al*., 2007).

Farmers already use a number of different locally available plant materials as grain protectants, which they consider to be cheap sources of pest control. It appears that small-scale farmers of Kebbi have no standard methods of preparing plants for use as grain protectants. Belmain & Stevenson (2001) reported that variations in the efficacy of local varieties of plant materials and ineffective methods of preparation and application can result in poor grain protection. Evidence from Belmain & Stevenson (2001) and Isman (2006 & 2008) indicate that effective preparation and standardization of materials and methods used by farmers to protect their grain can help improve the efficacy of botanical protectants.

Therefore, the overall objective of this research work has been to help farmers by developing a promising new approach to grain protection that involves using cheap, locally available material to protect stored grain from insect infestation. An important part of my approach was to learn as much as I could in the time allowed about the farmers' perceptions of their pest problems with grain storage in their particular socio-economic and environmental context and to identify locally available and affordable materials that could be used to mitigate their problems. My aim is to use this information to develop an effective method of using these materials to help farmers in Kebbi state to realize the full benefits of good grain storage. However, this could not be achieved without prior knowledge of farmers' existing methods of grain storage and protection. Therefore, the first step was to undertake an interactive survey of famers, to gain a greater understanding of their perceptions of their existing methods, and use the information obtained as the basis for developing improved control strategies (Chapter 5).

The surveys in Chapters 3 & 7 found that sorghum was the main staple food for the small-scale farmers of Kebbi. The methods of its preparation and storage have a great effect on the types of insects that attack the grain, with *T. castaneum* being the most important pest. The grain suffers most infestation by insect pests when stored in the threshed form and stored in store-rooms. This evidence was obtained mainly from the southern region of Kebbi state. The magnitude of loss and infestation necessitates farmers to use different approaches to pest control, although many favour botanical pesticides/repellents (e.g., *Ocimum* species). However, even though repellent plant materials are a cheap and easily available source of pest control, efficacy is reported to be inconsistent, which may be due to inadequate knowledge of methods of pre-storage preparation and the use of ineffective plant materials, which necessitates a search for improved approaches to grain protection. Improvement in efficacy and availability could increase farmers' acceptance and uptake of botanical protectants.

Therefore, experimental work in the laboratory and in the field has led to the discovery that Lem-ocimum, a combination of two repellent plant species, *O. basilicum* (the most common plant species used by farmers to protect grain) and *C. nardus* (found growing locally and known to be repellent to storage insect pests) has the potential to protect stored sorghum from the most serious pest species, *T. castaneum*, when applied in between layers of double bagged grain. The experiments that led to this outcome were divided into three phases:

(A) Laboratory-based experiments to develop a new bioassay method that is more efficient and effective than standard bioassays in screening a range of plants used by farmers to identify plants with maximum repellency properties against *T. castaneum* and to identify optimal doses, as well as to develop new methods of incorporating plant materials into double bagging storage methods (Chapter 5).

(B) Field experiments in farmers' store-rooms on the efficacy of the new repellent-treated double bagging method (Chapter 6). Several surveys were also undertaken to identify differences in the perception of men and women regarding the factors that influence use of botanicals as grain protectants and how these could be improved to increase acceptance and uptake of the new method in local communities (Chapter 7).

(C) Analysis of the chemical profile of plants to identify the different species and varieties of *Ocimum* and *Cymbopogon* plants collected from different farmers in Kebbi, as well as to determine if chemical variation between the plant species and methods of processing botanicals affects their repellency against *T. castaneum* (Chapter 8).

The results of each experimental phase have been discussed in previous chapters, and, therefore, this chapter aims to review the importance and limitations associated with the development of a new bioassay method to screen for optimal plants and plant compounds to repel *T. castaneum,* and the significance and implications of using Lem-ocimum in double bags and the preparation and application of varieties of *Ocimum* species local to Kebbi to improve small-scale stored grain protection systems. This chapter will conclude with suggestions for implementation of the present findings and recommendations for further studies.

## 9.2 Summary of the findings and discussion

### 9.2.1 The significance of using a thigmotactic bioassay (tray with pits and stones) to obtain more meaningful responses from beetles

Bioassays have proved to be an effective technique for measuring the behavioural responses of insects to test stimuli (Robertson e*t al*., 2007; Stefanazzi *et al*., 2011). However, the major challenge in using bioassay arenas to study natural responses of insects such as *T. castaneum* to test stimuli is that the strength of response of the beetle to test materials tends be low for some of the standard bioassay designs (Olsson *et al*., 2006; Duehl *et al*., 2011; Campbell, 2012), which affects the efficiency of particula bioassay designs in assessing the effectiveness of the test material (Arthur *et al*., 2011). For example, beetles are often found in the corners or edges of a bioassay arena and, therefore, may never get close to the source test or control stimuli. This behaviour is probably due to the lack of some physical features that insects normally use to explore their natural environment and consequently affect the ability of that particular bioassay design to measure effectively the response of the insects to the test stimuli (Campbell, 2012).

The experiments conducted in this study (Chapter 5) indicate that the response of *T. castaneum* to test stimuli in an arena can be increased and better understood if their natural behavioural responses to their natural environment are taken into consideration in bioassays. As a result, the efficiency and effectiveness of bioassays can be improved. This point is supported by the findings of Hussain *et al*. (1994). Duehl *et al*. (2011) showed that modifications to an assay arena that support the beetle's natural behaviour can improve their response to the test stimuli. However, the Duehl *et al.* (2011) study did not address the problem that beetles frequently do not respond to treatments due to excessive time spent moving along the edges of the arena or becoming immobilised in the corners of the arena (Hagstrum and Campbell, 2002). The study presented in Chapter 5 has shown that the addition of features such as pits to hold mounds of test grain (to allow beetles to respond with positive geotaxis and negative phototaxis) and more importantly stones (to enable thigmotaxis) resulted in a significant increase in the efficiency and effectiveness of the bioassay, which was threefold that of standard assays (pitfall and open arena).

In addition to this, the comparison of a dual choice tube assay (standard) with the thigmotactic assay on the response of *T. castaneum* to two different *Ocimum* species (Chapter 8) further indicates how much better the thigmotactic assay is. The bioassays in Chapter 8 compared the repellency of two closely related *Ocimum* species (OCT, *O. basilicum* and OCW, *O. africanum*, the wild variety of *O. basilicum* in Africa), and found a significant difference in the proportion repelled by the two species in the thigmotactic bioassay, suggesting that OCT contained significantly higher levels of repellent compounds than OCW, which was verified by the chemical analysis of the two plants. No significant differences were found between OCT and OCW in the dual choice tube bioassay, thus demonstrating the value and importance of using sensitive bioassay methods to screen candidate repellent plants or compounds. This also suggests that the standard bioassays are lacking some physical features that can allow beetles to explore better the bioassay environment and make a more natural choice of the test materials.

Since *T. castaneum* are naturally thigmotatic, their response to a test compound in an environment lacking this stimulus could be difficult to understand, because of interference of the source of touch stimuli. However, the new thigmotactic bioassay has improved the response of beetles to test stimuli by solving the problem of insects being trapped into moving along the edges of a test arena. The addition of stones in the tray arena provided the beetles with thigmotatic touch, increasing their response to the test stimuli and efficacy of the method. Semeao *et al.* (2011) reported that the response of *T. castaneum* was enhanced when test materials were close to dark shapes, and this may be due to a negative phototaxis response to the shapes. Moreover, the addition of characteristics of the thigmotactic bioassay resulted in rapid screening (within 4 hrs) of the most promising repellent plants; *O. basilicum* and *C. nardus,* against *T. castaneum*; longer time were required to obtain a good result with the other assay methods (Loschiavo, 1952; Jang *et al.*, 1982; Fields *et al*., 2001; Othira *et al*., 2009).

In conclusion, the use of bioassays that use more of the beetle's natural environmental conditions is essential in obtaining a better response and thus a better understanding of the true response of beetles to test stimuli. Although the higher levels of response were in the range of 70-82%, further improvments to this level of response may be possible by designing new biossays that take into account additional biotic and abiotic factors.

### 9.2.2 The potentials and limitations of using the plant preparation Lem-ocimum incorporated in layers of double bags as a grain protectant

Pesticidal/repellent plant materials have been an available and affordable alternative means of pest control used by small-scale farmers in Nigeria (Poswell and Akpa, 1991; Salako *et al*., 2008). However, the major challenge faced by small-scale farmers in the use of this material is their slow action and/or inconsistency in their efficacy (Isman, 2006; Rajashekar *et al*., 2012). This problem has been linked to improper use of grain storage materials and protectant materials (Belmain, 2002). The results of farmer surveys in this study indicate that the amount of insect infestation experienced by small-scale farmers of Kebbi in their stored sorghum protected with plant materials, notably *O. basilicum* in polypropylene bags, has been inconsistent. Previous studies have shown that both *O. basilicum* and *C. nardus* are effective against *T. castaneum* (Mikhaiel, 2011 and Mishra *et al*., 2012), however this was based on essential oils in laboratory bioassays, and there was little effort to determine the efficacy of this material as grain protectants as applied in the typical ways in which farmers protect their grain.

Evidence from the study presented here indicates that treating a double bag with a combination of ground plant materials (Lem-ocimum) (0.5-1% w/w) has the potential to reduce infestations of the most important pest of stored grain, *T. castaneum*. This is an improvement to the current method of farmers mixing grain with dried plant materials, which was found to have the limitations of higher pest infestations and unwanted plant residues in the treated grain.

Although the new Lem-ocimum treated bags involve use of extra bags, this study takes into account the perception of small-scale farmers on the use of extra bags and plant materials. If farmers are to use the new method they may need extra bags, plant materials and time required to prepare the Lem-ocimum material and treat the double bag. In this study the main conclusion, that it might be worthwhile for farmers to store only a proportion of their grain in treated double bags, was tested in field experiments (Chapter 6), and it was found that the more treated bags (at least from 9 x 5kg treated bags) placed near each other in store-rooms containing ~20 x 60kg untreated bags, the better protection was obtained for the bags of treated grain (~0.6%±0.08% weight loss). This was significantly better than 0.82%±0.16% of monthly grain weight loss obtained in store-rooms with small numbers of 5kg treated double bags and in stores with untreated 5kg single bags (2.36%±0.85% per month). Although the effect of damage was not determined over longer storage times, i.e., for 1 year or more, this study shows that the low level of beetle infestation and grain damage in stores that have a high number of treated double bags may still be good enough to keep the infestation level within accepted market ranges, even after 1 year of storage.

Although the bag sizes of the treated bags used for this experiment were small; 5kg against 60kg of farmers' untreated bags, this study demonstrated a proof-of-principle that this method of grain protection has the potential to reduce beetle infestation, using relatively small amounts of plant materials, keeping the clean grain separate from repellent plant residues by double bagging, thereby reducing the labour-efforts of women to winnow stored grain.

The results in Chapters 4 and 6 of this study indicate that famers tend to use their extra stored grain for food over the extended farming season or for cash if there is surplus grain. However, the market value of damaged grain is low or worthless, which signifies a need for improvements to grain protection. This study has shown that 5kg treated bags can reduce grain damage, and therefore it remias to be seen if larger, standard 60 kg bags can also be protected well enough to provide high quality food and to sell in the market.

It is not always easy to convince farmers to change from their existing method to a new method until they are convinced that the new method is cheaper and can provide a better benefit than their own methods. The follow-up survey conducted with farmers that had participated in Experiment 2 testing different levels (small, medium or high) of treated bags in their stores (Chapter 6), indicates that farmers approved of the new method, however the acceptance and uptake of the new method may be affected by the extra work and additional costs. The farmers' views on this were dependent on gender to some extent, because of differences in their access to resources such as time, cash and the plant materials. The impression of respondents who tested high levels of treated bags in their store-rooms was positive, in that the new method can give them more food to eat or sell, irrespective of the extra work. This view may have been affected by how effective the method was in their group of high level treated bags; farmers that used a lower level of treated bags were, understandably, not as convinced that the new method works because levels of protection were not as high. Generally, however, famers may be easily convinced to use the new method because plant materials and polypropylene bags are locally available and the efficacy of the new method results in low grain loss if a sufficient number of bags are treated. Belmain and Steveson, (2001) indicated that farmers are more easily convince to use a new method if the materials used are cost effective and can be sourced locally. Women may be more easily convinced than men to use the new method because they have more access to the plant materials than men have, and a majority of them stated that the new method was easier to practice than their own methods, which were not the same perspective as the men. However, the cost of extra bags is a problem, as mentioned by some women. Men were more concerned with the efficacy and positive financial prospects; they were more willing to accept the new method if they were convinced they could get a better price for the grain they could sell.

It should be noted that collection of large quantities of plant material, changes in flavor of the treated grain, time taken to prepare treated bags and the need for winnowing the grain were reported as some of the disadvantages of using botanical grain protectants in the traditional way of mixing them with (Chapter 7). However, the new method of treating double bags is more cost effective, as it requires smaller amounts of plant materials to treat double bags compared to the amount required to mix with grain (i.e., the farmers' existing method). The new idea of separating grain from direct contact with repellent plant materials may reduce the effort women go through to winnow grain before using it, and also increasing the market value since the grain has less plant residues.

Due to limited time and resources, the study presented here was based on treating bags of grain that were only 5kg; less than 10% the normal size of a bag of grain (60kg). Therefore, further research is needed to establish whether this method would work with the standard famers' bag size, what proportion of bags would need to be treated to improve grain protection and to establish what level of benefits would be obtained in relation to the added cost of using extra bags and treating them with Lem-ocimum before any recommendation could be made to the farmers.

### 9.2.3 The potential and limitations of using different species of *Ocimum* processed in different ways as grain protectants

One of the problems with the use of botanical pesticides in grain protection is variability in the performance of plant products (Isman, 2006), which is linked to variability in the type and amount of active ingredients, mostly affected by type of species, seasonal variation, method of plants preparation and soil type between regions (Raseetha Vani et *al*., 2009). Information has been recorded on how farmers prepare and use botanical pesticides as grain protectants, and it has been show that this varies with locality and farmers (Obeng-Ofori, 2010).

To promote effective use of pesticidal/repellent plants as grain protectants the correct species, methods of preparation and application needs to be determined (Obeng-Ofori, 2010). This could be achieved by recording the different species use locally by farmers, how these are being used and the implication for pest management. In Kebbi state small-scale farmers currently use a range of *Ocimum* species collected from different locations and dried by a range of methods before use as grain protectants. Although samples of plants may look different, farmers often referred to them by the same name, even though Kew verified that they were separate species. The findings of this study (Chapter 8) indicate that the different samples of *Ocimum* species used by famers as grain protectants were chemically not the same. Similarly, it was found that, the different methods used to dry the different species (sun or shade dried) affected their chemical constituents, which consequently affected their bioactivity. This indicates the importance of famers to know that the different species they are using are chemically not the same, which may be one of the reasons they have had inconsistent results with botanical grain protectants, as described in Chapters 3 and 4 of this study, as highlighted by the findings of Belmain and Steveson (2001) that methods of drying and place of plant collection can affect the composition of active ingredients in plant materials. Cultivated *Ocimum*, confirmed to be *O. basilicum,* was found to have more active chemical compounds and provided better repellency than all the other *Ocimum* samples, particularly the wild species, confirmed to be *O. africanum*. Moreover, shade dried *Ocimum* plants were found to contain a better range of active compounds and greater efficacy in bioassays than sun dried *Ocimum*.

This study highlights the possible implication of using different *Ocimum* species among farmers, particularly for men who mostly use sun-dried wild species. It is important to note that the findings of the survey in Chapter 7 indicated that women mainly grow cultivated *Ocimum* and shade dried the plants, whereas men did not cultivate *Ocimum,* preferring to collect wild plants, which turned out to be the least bioactive samples, and they preferred to sun dry their plants, which also reduced the bioactivity of their plants. The findings of this study indicate that greater than 50% repellency can be achieved with cultivated *Ocimum* even at a low dose of 0.5% w/w. The equivalent effect could be found with 4 times as much (2% w/w) wild *Ocimum*, which suggests that for men to get a reasonable result with the wild species, they would need to treat their grain with at least 2% w/w of the wild species. Conversely, they may ask their wives to grow more cultivated *Ocimum* for their own use.

Since the women already use whole shade dried cultivated *Ocimum* to protect their grain, they are likely to benefit more from this new method that keeps the grain uncontaminated with plant residues. Since men use sun-dried wild *Ocimum*, they are likely to have less success than the women with their current methods, but dissemination of the findings reported here may help them to adjust their approach. However, the ability of women to grow cultivated *Ocimum* is a valuable starting point, and could help make the new method more widely available and acceptable. It is important to determine if men could be convinced to use a similar method use by women.

## 9.3 Conclusions

The results of this study support the following conclusions:

- Modifications to bioassay arenas to take into account the natural behaviour of beetles can improve the efficiency of the assay and consequently increase improve the outcomes.
- Addition of plant materials in between layer of 5kg double bags can effectively deter beetles, thereby disproving the first null hypothesis.
- Addition of extra treated double bags in store-rooms can reduce beetles infestation and increase the amount of clean grain.
- There are gender differences in the perception and use of pesticidal/repellent plants as grain protectants, thereby disproving the second null hypothesis.
- The different *Ocimum* species used by farmers differ both in active compounds and in efficacy against *T. castaneum,* thereby disproving the third null hypothesis.

However, the following future work is suggested for improvement to the use of pesticidal/repellent plant materials with the new method as grain protectants.

## 9.4 Suggestions for future work

It is recommended that future research should be carried out with the new bioassay, comparing its efficacy with more standard bioassay methods, using different plant species. The thigmotatic behaviour of *T. castaneum* may be different for other species, strains, or even sexes. It is important to determine if this differs with particular species, strains or sexes of *Tribolium* species.

Considering the time and resources at hand, the field research was conducted with improvised 5kg double bags instead of 60kg farmers' bags; meaning that very small amounts of grain were protected compared to the amount of farmers' untreated grain, and only one dose of Lem-ocimum was tested. A bigger field experiment could be carried out to assess the effect of variables, such as dose of Lemocimum applied to double bags under field conditions, effect of the size of treated bags (where the ideal size would be 60kg bags) and effect of the proportion of treated to untreated bags. For example, future field studies could test:

1) Dose-response of Lem-ocimim added to 60kg double bags to determine which dose is most cost-effective for protecting grain for selling in the market,

2) Optimal size of treated bags; does the size of bag affect how well Lemocimu-treated double bags protect grain?

3) Optimal proportion of treated bags; what is the cost/benefit of treating 10%, 33% or 50% of bags. This would be determined partly by what proportion of grain bags a farmer could afford to rotect as high quality grain.

Due to limits of time and resources, it was not possible to monitor the infestations in the farmers' untreated bags beyond the initial sampling at the beginning of the field experiments and the farmers' qualtitative assessment of in the same store-rooms in the last month of the experiments. It is recommended that a similar future study should be carried out with the farmers' untreated bags; collecting insect samples from the farmers' untreated single bags each month to see if the treated bags have any effect in reducing beetle numbers in adjacent untreated bags, and if the way treated bags are distributed in the farmers' store-rooms matters, e.g., treated bags kept close together or placed randomly amongst the untreated bags. The use of many of the 5kg treated bags placed close to each other in a storeroom was shown to be the best method of using this technology. Although in this study the 5kg threated bags was placed on top of the farmers untreated bags, a different position might have had different effects. The effects of treated bag placement in store-rooms should be investigated further.

Similarly, the status of untreated bags stored with treated bags in the same room was not determined. There was no significant effect of number of farmers' untreated bags on the number of beetles in the treated double bags, which may be related to the relative sample sizes. This could be taken into consideration in a future study by having a large and equal number of sample sizes between farmers' stores sizes. The field experiment was started at the middle of the storage season and was monitored for only 5 months of storage. It is recommended that future experiments should be conducted at the beginning of the storage season and monitored for more than 5 months and at least 9-12 months. This will allow a fuller understanding of the trend under different weather condition, as well as the effect of long-term storage on the plant materials. *Tribolium castaneum* are known to be attracted to food infested by other beetles (Dobie *et al*., 1991), which means that the efficacy of treating infested grain with repellent plants is questionable, especially if there are other species present that are not repelled by the particular botanical used. Hence, more research is required on the efficacy of infested grain treated with the new method presented here.

Although this work was focused on repelling adult *T. castaneum*, more research on the toxicity and fumigation effects of plants on all the developmental stages of the beetle may increase our understanding of the mode of action of these plants. This research shows that a combination of plant materials provide better repellent efficacy. It is recommended that in future, the inclusion of plant materials such as Neem products, which are available in the study area and which are known to have a wider spectrum of effect on different storage pest (Isman, 2006 & 2008) should be included as a possible synergist. This may enhance the longevity of the efficacy through different modes of action.

This study demonstrates that the other two less important beetles were less affected by Lem-ocimum treated bags. A further laboratory research should be carried out with these two beetles to confirm whether this was due to the plant products or other factors from the field.

The cost of using extra bags is a major concern of some women, especially if instead of selling it they prefer to use the high quality grain in their treated bags for home use, as seed grain, and for other uses. The implications this may have on the willingness of women to use the new method may need to be considered.

Since both plant materials in Lem-ocimum are used for traditional medicinal purpose and in food (James *et al*., 2008; Awoyemi, *et al*, 2012) they are expected to be safe to use as grain protectant. However, future work may be conducted on the potential of residues and possible effects in grain stored with these plant materials.

A majority of men use the weakest variety of *Ocimum,* which they collected from the wild. However, for them to have similar grain storage improvements as their wives they would have to increase the dose of their wild plants to > 2% w/w. alternatively, they could motivate their wives to grow more of the cultivated variety. Since shade drying was found to be the best method of drying plants, men need to be convinced to change their method of drying. They could be encouraged to dry it with seeds used to hang on trees or roof of their kitchen. Training women to use the improved method of grain protection may help them successfully store grain for longer periods and hence encourage them to plant more of their pesticidal plants, which would increase their availability.

The research presented here did not establish which of the active plant compounds was responsible for the bioactivity of *T. castaneum*. Future work could be carried out to consider this. Knowing the most active compounds of the repellent plants could help in developing optimum combinations of plants.

Farmers appreciate the possibility of obtaining a better price for their grain sold to traders. Including traders in future research may help to identify desirable grain qualities required by traders to agree higher prices. This may help in improving the farmer-trader marketing relationship, as well as increase use of botanical pesticides.

Akinnagbe & Ajayi (2010) reported that a new technology is easily promoted among rural farmers through national research institutes, government departments in collaboration with Agricultural extension workers, or non-governmental organization (IFAD, 2012). However, this study found that farmer - extension agent interaction is low regarding the use of pesticidal/repellent plants. It is important to disseminate new knowledge and developments in pest control to extension services so that well-organised extension services can provide help and updated information to farmers on new ways of using botanicals as grain protectants.

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# Appendices

## Appendix 1: Sample of Questionnaires

### 1.1 Chapter 3 survey Questionnaire

**SECTION A: Personal data**

Name…………………………………………..
Educational status..(i) Educated (level)………… (ii) Uneducated
Sex…………Male……………….Female……….
Age…………………………………………
Occupation…………………………………….
Village name…………………………………..
District…………………………………………
Family size……………………………………

**SECTION B: Grain storage**

1. Do you own farmland?    Yes    No
2. If yes what is the size of your farm land?..........
3. What type of cereal grain crop(s) do you grow?
   ………………………………………………….
4. How do you source your seed?
   a) purchase from open market    b) from friend
   c) government (KARDA)    d) own seed
5. When do you normally harvest your grain crops?
   ……………………………………………….
6. Do you dry your grain crops before storage?    Yes    No
7. If yes, how do you dry your grain crops?
   ……………………………………………………..
8. For how long do you dry your grain crop before storage?
   ………………………………………………………
9. What problems do you encounter during drying grain?
   ……………………………………………………
   ……………………………………………………
10. Do you store grain crops after harvest?    Yes    No
11. If yes, what type of grain crops do you store?
   …………………………………………………….
12. For what purpose do you store your grain crop?
   a) domestic    b) commercial    c) both    d) other……………….
13. From what time of the year do you begin to use the store for storing of the grain crops mentioned above?    ………………………..
14. What time of the year do you empty your store?
   ………………………………………………………
15. What quantity of the grain crops mentioned above do you store?
   …………………………………

**SECTION C: Grain Storage System**

1. What types of storage structure do you use for your grain storage?
   …………………………………………………….
2. Why do you use such a storage structure?
   …………………………………………………….
3. In What form do you store your grain?
   …………………………………………………….
4. Why do you use such methods?
   ……………………………………………………

**SECTION D: Storage Insect Infestation**

1. Do you have a problem of insect pest infestation in your grain store?
   Yes No
2. If yes, what type of insect pests attack your stored grains?
   ………………………………………………….
3. What is the population of the insect pest mentioned above in your store?
   High………..Moderate………Low………..None
4. Which period of the following storage year insect infestation is high in your Store?
   a) Rainy season b) Dry season c) Harmattan
   ………………………………………………….
5. What type of insect pests commonly found during such periods?
   a)…………………………………………………
   b)…………………………………………………
   c)…………………………………………………
6. What other pests attack your stored product?
   …………………………………………………….

**SECTION E: Stored grain insect Pest Control**

1. Do you apply any chemical for control of stored insect pests? Yes No
2. If No why?...................................................................
3. If Yes, what type of chemicals do you apply?
   ……………………………………………………
4. Do you use botanicals methods of insects' control? Yes No
5. If No, why?.............................................................................
6. If Yes, what are they?………………………………………………………
7. What part of them do you use? ………............................................................
8. How do you apply them?.......................................................................
9. Do you think the method is effective? Yes No

**1.2 A sample of a follow-up questionnaire on the view of farmers on the efficacy and use of treated double bag method as a new grain protection method tested in their grain store rooms**

1) Name of respondent..............................................................
2) Village.......................................................................
3) Gender.......................................................................
4) Type of experiment tested........................................................

4) How can you compare the effectiveness of this new method of grain treatment with your existing method in terms of grain protection?
a) Very effective
b) A bit effective
c) Similar
d) A bit ineffective
e) Not effective
f) Cannot say

5) Do you think the treatment you tested has reduced damage to the extent of giving you more food to eat or sell?
a) Yes
b) No
c) Not sure

6) How easy was it to make and use the improved botanicals compared to your old/existing method
a) Much easier
b) A bit easier
c) Similar
d) A bit harder
e) Much harder
f) Don't know

7) Is the extra work likely to affect uptake of the new method because?
a) People won't want to do all that work even if it means they have more food or money as a result
b) People will do the new method even if they are not sure they will have more food or money as a result
c) People will do the new method if they are sure of having more food or money as a result
d) People will wait and see if the effort is worth it for other people before they try
e) The people who do the work don't benefit from extra cash
f) Don't know

8) How can you compare the residues of plant material in the treatment you tested to your old method?
a) No residues
b) Few residues
c) The same

d) A few more residues
e) Much more residues

f) Don't know

9) What would persuade you to change how you store your grain?
a) Effectiveness of current method
b) Current method found easy to practice
c) New method is more cost effective
d) What other farmers say
e) What other 'experts' say
f) Receive higher price for better quality
g) Other reason, specify.................................

10) Who in the household prepared the grain for storage before (using existing method)?
a) Head of the household
b) Other Men of the household
c) Other Women of the household
d) Men and women
e) Other.....................................................................................

11) Who in the household would prepare the grain if it was done the way of the experiment?
a) Head of the household
b) Other men of the household
c) Other women of the household
d) Men and women
e) Other....................................

12) Would you keep using the same treatment that you tested?
a) Yes the same
b) No, lower number of treated bags
c) No, higher number of treated bags
d) No, go back to what we did before (How did used to protect your grain?),
e) No, change to a different method which is..................................................
f) Not sure

Any other comments...............................................................................................

……………………………………………………………………………………..

**1.3 Chapter 7 (second) survey Questionnaire**

**Questions for Individual Farmer on grain storage and protection**

This survey is being undertaken on behalf of a researcher from Kebbi State University and seeks to gain detailed knowledge about how farmers control grain pests. Any information provided will be kept in confidence and participation is entirely voluntary. The respondent can opt out of the interview at any time and does not have to give a reason.

**1.0: GENERAL INFORMATION**

1.1 Date of Interview (dd/mm/yyyy) ……/…../ ……/……..

1.2 Name of Interviewer ………………………

1.3 Name of village...........................................

1.4 Name of Respondent …………….............

1.5 Ethnic group..................................................

1.6 Major occupation...... (1-Farming; 2-Trading; 3-Fishing; 4-Civil servant)

1.7 Gender of Respondent………… (1-Male; 2-Female)

1.8 Age……………….

1.9 Education level completed… (1-No education; 2-Primary; 3-Secondary; 4-Tertiary)

1.10 Household size............

| | Males | Females |
|---|---|---|
| Less than18 years | | |
| 19-40 years | | |
| >41 | | |

## 2.0 Grain storage and uses

2.1 How many sacs of the following grains do you produced?

☐ Sorghum..... ☐ Millet..... ☐ Rice..... ☐ Maize.....

2.2 What is the reason that you produce the mentioned grain type the most?

☐ It is our staple food ☐ There is good market for it ☐ other specify...................

2.3 Do you store Sorghum grain? ☐ Yes ☐ No

If sorghum not stored, thank the respondent and stop the interview.

2.4 How do you process your sorghum before storage?

☐Thresh it (who does the threshing and how?.......................................

☐ Unthreshed

☐ On stick

☐ Off stick

2.5 Where do you store your grain?

☐ in a Room

☐ Granary

☐ Shade

2.6 Why do you use this location to store your grain?..................................................

2.7 What container do you use for grain storage?

☐ Sack ☐ Plastic container ☐ Other, specify....................................

2.8 For how long do you store your grai**n?**

☐ Up to 2 month

☐ 2- 6 months

☐ 6 months to 1 year

☐ More than 1 yr

2.9 How grain stock is being used within your family?

☐ Mainly for household feeding ☐ Mainly selling ☐ Feeding & selling

## 3.0 Pest infestation in sorghum

3.1 Do you have a problem with pest infestation in your stored sorghum?

☐ Every year ☐ Sometimes □ Occasionally □ Never

3.2 If yes, what type of pest do you have most in your store?

☐ *Tribolium castaneum* ☐ *Rhyzopertha dominica* ☐ *Sitophilus zeamais*

☐ *Plodia interpunctella* ☐ Other, specify...................................................

3.3 Does pest damage affects the amount of food you have to eat?

☐ Yes ☐ No

3.4 Does pest damage affects the amount of grain you have to sale?

☐ Yes ☐ No

3.3 Do you clear your store (granary/store room) of grain every year before loading the new crop?

☐ Yes ☐ No

3.4 What is the level of the pest infestation right now in your store?

☐No pests ☐ Low ☐ Medium ☐ High

## 4.0 Grain protection

4.1 Do you use plant materials to protect grains against pest infestation?

☐ Yes ☐ No

4.2 What plant materials do you normally used to protect grain?

☐*Ocimum basilicum* (sweat basil ) ☐ Wood ash ☐ground pepper

☐ *Erythropleum guineeses* ☐ *Nauclea diderrichii*

☐*Vernonia amygdalina* ☐ Others? Names: .................................................

4.4 Where do you source information about plant materials for use as a grain protectant?

☐ Extension agent ☐ Radio ☐ Friends ☐ Parent ☐ Grant parent

☐ Other source specify.....................

4.5 Where do you collect/obtain this plant material?

☐ Backyard garden ☐ Forest around your village ☐ Forest far from village

☐Cultivate it ☐ Other – specify.............................

4.6 Which part of the plant do you collect?

☐ Whole plant ☐ Leaves ☐ Stem bark ☐ Root ☐ Flower

4.7 How do you prepare your plant material?

☐ Dry in the sun and ground to powder ☐ dry in the shade and ground to powder

☐ Dry in the sun as whole plant ☐ dry in the shade as whole plant

☐ prepare a plant water extract ☐ other, specify................

Additional comment..........................................................................................................

4.8 How do you treat your grain with plant material?

☐ Mixed ground (powder) plant material with grain ☐ mixed grain with plant water extract ☐ used as a layer in between grain ☐ treat the container

4.9 Is there any factor you consider when choosing a plant for use as a grain protectant?

☐ Yes ☐ No

4.10 If yes, what are they?...........................................................................................

☐ Smell ☐ Availability ☐ Effectiveness ☐ No toxicity/harmfulness

☐ other, specify...........................

4.11 Does plant material meet your needs as grain protectant?

☐ Yes ☐ No [ ] Don't know

4.12 If yes how does it work?

☐ It kills insects ☐ Send insects away ☐ Insects stay but cause no damage

☐ Don't know

4.13 What is the advantage/disadvantage of grain treated with plant materials?

..........................................................................................................................

4.14 To whom do you sale your grain?

☐ Traders ☐ Other farmer ☐ Consumer ☐ Relation

4.15 Do buyers offer a higher price for grain treated with a botanical protectant?

☐ Yes ☐ No ☐ Don't know

4.16 Why do you use plant pesticides rather than synthetic chemicals?

☐ cost effective ☐ More available ☐ Less toxic/harmful ☐ other, specify............................

4.17 What other things apart from grain protection plant pesticide is used for?

..............................................................................................................................

End – Thank the participant

# Appendix 2: Data Analysis

## 2.1 Analysis for Chapter 3

**ANOVA for the quantity of the main three grain stored per household by region**

| | Df | Sum Sq | Mean Sq | F value | Pr(>F) |
|---|---|---|---|---|---|
| Grain | 2 | 8239 | 4119.3 | 22.6194 | 2.995e-10 *** |
| Region | 2 | 168 | 83.9 | 0.4605 | 0.6312 |
| grain:Region | 4 | 11228 | 2807.0 | 15.4135 | 4.254e-12 *** |
| Residuals | 711 | 129481 | 182.1 | | |

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

## 2.2 Analysis for Chapter 4

**Analysed data obtained from different types of grain stored in different storage structures using two ways ANOVA**

| | Df | Sum Sq | Mean Sq | F value | Pr(>F) |
|---|---|---|---|---|---|
| Grain | 3 | 437.92 | 145.975 | 18.7144 | 4.206e-10 *** |
| Storetype | 1 | 126.97 | 126.971 | 16.2781 | 9.422e-05 *** |
| Region | 2 | 116.78 | 58.392 | 7.4861 | 0.0008471 *** |
| Grain:Storetype | 3 | 21.72 | 7.240 | 0.9281 | 0.4293027 |
| Grain:Region | 6 | 183.57 | 30.595 | 3.9224 | 0.0012559 ** |
| Storetype:Region | 2 | 5.16 | 2.579 | 0.3307 | 0.7190557 |
| Grain:Storetype:Region | 5 | 75.83 | 15.165 | 1.9442 | 0.0915691 . |
| Residuals | 126 | 982.82 | 7.800 | | |

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

**Analysed grain layer data from grain storage sampling using two ways ANOVA**

| | Df | Sum Sq | Mean Sq | F value | Pr(>F) |
|---|---|---|---|---|---|
| grain | 1 | 192.39 | 192.391 | 35.4769 | 2.405e-08 *** |
| layers | 2 | 226.08 | 113.042 | 20.8449 | 1.512e-08 *** |
| grain:layers | 2 | 3.73 | 1.866 | 0.3441 | 0.7095 |
| Residuals | 126 | 683.30 | 5.423 | | |

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

### 2.3 Analysis for Chapter 5

**Analysis of deviance table for comparing the efficiency of four types of bioassay**

| | Df | Deviance | Resid. Df | Resid. Dev | Pr(>Chi) |
|---|---|---|---|---|---|
| NULL | 27 | 120.053 | | | |
| Types of bioassay | 3 | 82.42 | 24 | 37.633 | < 2.2e-16 *** |

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

**Analysis of deviance table for comparing the effectiveness of four types of bioassay**

| | Df | Deviance | Resid. Df | Resid. Dev | Pr(>Chi) |
|---|---|---|---|---|---|
| NULL | | | 27 | 44.773 | |
| Types of bioassay | 3 | 14.169 | 24 | 30.605 | 0.002684 ** |

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

**Analysis of deviance table for beetles responded to different concentration of chemical compounds and period of exposure to the compounds**

| | Df | Deviance | Resid. Df | Resid. Dev | P(>\|Chi\|) |
|---|---|---|---|---|---|
| NULL | | | 41 | 72.215 | |
| conc | 1 | 26.5480 | 40 | 45.667 | 2.571e-07 *** |
| time | 1 | 21.0742 | 39 | 24.593 | 4.418e-06 *** |
| conc:time | 1 | 1.1548 | 38 | 23.438 | 0.2825 |

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

**Analysis of deviance table for the beetles responded to different concentration of citronella (1, 2 and 10mg)**

| | Df | Deviance | Resid.Df | Resid. Dev | Pr(>Chi) |
|---|---|---|---|---|---|
| NULL | | | 27 | 68.072 | |
| conc | 1 | 44.865 | 26 | 23.207 | 2.111e-11 *** |

---

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

**Analysis of deviance table for the beetles' responded to different concentration of Methyl salicylate (1, 2 and 10mg)**

| | Df | Deviance | Resid. Df | Resid. Dev | Pr(>Chi) |
|---|---|---|---|---|---|
| NULL | | | 27 | 79.018 | |
| conc | 1 | 57.901 | 26 | 21.117 | 2.757e-14 *** |

---

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 '' 1

**Analysis of deviance table for the beetles' responded to different concentration of ground powder of *Cymbopogon citratus* (0.25, 0.5 and 1% w/w)**

| | Df | Deviance | Resid. Df | Resid. Dev | Pr(>Chi) |
|---|---|---|---|---|---|
| NULL | | | 27 | 237.631 | |
| conc | 1 | 207.41 | 26 | 30.218 | < 2.2e-16 *** |

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

**Analysis of deviance table for the beetles' responded to different concentration of ground powder of *Cymbopogon nardus* (0.25, 0.5 and 1% w/w)**

| | Df | Deviance | Resid. Df | Resid. Dev | Pr(>Chi) |
|---|---|---|---|---|---|
| NULL | | | 27 | 251.809 | |
| conc | 1 | 227.02 | 26 | 24.791 | < 2.2e-16 *** |

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

**2.4 Analysis for Chapter 6**

**Experiment 1**

**ANOVA and Tukey test for significant on a) total b) live and c) dead adult number of *T. castaneum* found between grain stored with single untreated, untreated double and treated double bags**

| a) | Df | Sum Sq | Mean Sq | F value | Pr(>F) |
|---|---|---|---|---|---|
| treat | 2 | 410.8 | 205.41 | 101.5 | <2e-16 *** |
| Residuals | 87 | 176.0 | 2.02 | | |

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

TukeyHSD

Tukey multiple comparisons of means

95% family-wise confidence level

| | diff | lwr | upr | p adj |
|---|---|---|---|---|
| untreatdoublebags-treatbags | 1.590476 | 0.7148243 | 2.466128 | 0.0001165 |
| untreatsinglebags-treatbags | 5.113050 | 4.2373983 | 5.988702 | 0.0000000 |
| untreatedsinglebags-untreatdoublebags | 3.522574 | 2.6469222 | 4.398226 | 0.0000000 |

| **b)** | Df | Sum Sq | Mean Sq | F value | Pr(>F) |
|---|---|---|---|---|---|
| treat | 2 | 171.26 | 85.63 | 105.9 | <2e-16 *** |
| Residuals | 87 | 70.35 | 0.81 | | |

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 '' 1

TukeyHSD

Tukey multiple comparisons of means

95% family-wise confidence level

| | diff | lwr | upr | p adj |
|---|---|---|---|---|
| untdbl-ttdbl | 0.640000 | 0.08635063 | 1.193649 | 0.0193092 |
| untsgl-ttdbl | 3.193333 | 2.63968397 | 3.746983 | 0.0000000 |
| untsgl-untdbl | 2.553333 | 1.99968397 | 3.106983 | 0.0000000 |

| c) | Df | Sum Sq | Mean Sq | F value | Pr(>F) |
|---|---|---|---|---|---|
| treat | 2 | 346.0 | 173.02 | 63.27 | <2e-16 *** |
| Residuals | 87 | 237.9 | 2.73 | | |

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

TukeyHSD
Tukey multiple comparisons of means
95% family-wise confidence level

| | diff | lwr | upr | p adj |
|---|---|---|---|---|
| untdbl-ttdbl | 0.8433333 | -0.1748309 | 1.861498 | 0.1244975 |
| untsgl-ttdbl | 4.5166667 | 3.4985025 | 5.534831 | 0.0000000 |
| untsgl-untdbl | 3.6733333 | 2.6551691 | 4.691498 | 0.0000000 |

**ANOVA and Tukey test for significant on a) total b) live and c) dead adult number of *R. dominica* found between grain stored with single untreated, untreated double and treated double bags**

| a) | Df | Sum Sq | Mean Sq | F value | Pr(>F) |
|---|---|---|---|---|---|
| treat | 2 | 83.8 | 41.89 | 10.37 | 9.13e-05 *** |
| Residuals | 87 | 351.5 | 4.04 | | |

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

TukeyHSD
Tukey multiple comparisons of means
95% family-wise confidence level

| | diff | lwr | upr | p adj |
|---|---|---|---|---|
| untdbl-ttdbl | 0.7314286 | -0.5060344 | 1.968892 | 0.3405345 |
| untsgl-ttdbl | 2.3120592 | 1.0745962 | 3.549522 | 0.0000732 |
| untsgl-untdbl | 1.5806306 | 0.3431676 | 2.818094 | 0.0085462 |

b)

| | Df | Sum Sq | Mean Sq | F value | Pr(>F) |
|---|---|---|---|---|---|
| treat | 2 | 20.44 | 10.218 | 13.96 | 5.53e-06 *** |
| Residuals | 87 | 63.70 | 0.732 | | |

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

TukeyHSD(m1)

Tukey multiple comparisons of means

95% family-wise confidence level

| | diff | lwr | upr | p adj |
|---|---|---|---|---|
| untdbl-ttdbl | 0.2657143 | -0.2610912 | 0.7925198 | 0.4547939 |
| untsgl-ttdbl | 1.1171686 | 0.5903631 | 1.6439741 | 0.0000070 |
| untsgl-untdbl | 0.8514543 | 0.3246488 | 1.3782598 | 0.0006445 |

c)

| | Df | Sum Sq | Mean Sq | F value | Pr(>F) |
|---|---|---|---|---|---|
| treat | 2 | 22.02 | 11.010 | 7.072 | 0.00143 ** |
| Residuals | 87 | 135.45 | 1.557 | | |

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

TukeyHSD

Tukey multiple comparisons of means

95% family-wise confidence level

| | diff | lwr | upr | p adj |
|---|---|---|---|---|
| untdbl-ttdbl | 0.4695238 | -0.29867240 | 1.237720 | 0.3164512 |
| untsgl-ttdbl | 1.2020592 | 0.43386300 | 1.970255 | 0.0009803 |
| untsgl-untdbl | 0.7325354 | -0.03566081 | 1.500732 | 0.0649159 |

**ANOVA and Tukey test for significant difference in total number of adult *L. serricorne* found between grain stored with single untreated, untreated double and treated double bags**

| | Df | Sum Sq | Mean Sq | F value | Pr(>F) |
|---|---|---|---|---|---|
| treat | 2 | 3.72 | 1.862 | 1.704 | 0.188 |
| Residuals | 87 | 95.05 | 1.093 | | |

TukeyHSD(m1)
Tukey multiple comparisons of means
95% family-wise confidence level

| | diff | lwr | upr | p adj |
|---|---|---|---|---|
| untdbl-ttdbl | 0.0228570 | -0.6206704 | 0.6663844 | 0.9960535 |
| untsgl-ttdbl | 0.4424839 | -0.2010435 | 1.0860112 | 0.2346715 |
| untsgl-untdbl | 0.4196269 | -0.2239005 | 1.0631542 | 0.2707637 |

**ANOVA and Tukey test for significant difference in a) total b) live c) dead number of adult *T. castaneum* found between stores with small, medium and high number of treated double bags**

| **a**) | Df | Sum Sq | Mean Sq | F value | Pr(>F) |
|---|---|---|---|---|---|
| Storesize | 2 | 40.74 | 20.372 | 16.13 | 6.52e-07 *** |
| Residuals | 117 | 147.81 | 1.263 | | |

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘’ 1

TukeyHSD
Tukey multiple comparisons of means
95% family-wise confidence level

| | diff | lwr | upr | p adj |
|---|---|---|---|---|
| medium-small | -0.6700000 | -1.266627 | -0.0733732 | 0.0236462 |
| high-small | -1.4264286 | -2.023055 | -0.8298018 | 0.0000003 |
| high-medium | -0.7564286 | -1.353055 | -0.1598018 | 0.0089245 |

| b) | Df | Sum Sq | Mean Sq | F value | Pr(>F) |
|---|---|---|---|---|---|
| Stosize | 2 | 6.429 | 3.215 | 14.36 | 2.66e-06 *** |
| Residuals | 117 | 26.198 | 0.224 | | |

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘’ 1

TukeyHSD

Tukey multiple comparisons of means

95% family-wise confidence level

| | diff | lwr | upr | p adj |
|---|---|---|---|---|
| medium-small | -0.2171429 | -0.4683269 | 0.03404116 | 0.1044961 |
| high-small | -0.5621429 | -0.8133269 | -0.31095884 | 0.0000016 |
| high-medium | -0.3450000 | -0.5961840 | -0.09381599 | 0.0041309 |

| c) | Df | Sum Sq | Mean Sq | F value | Pr(>F) |
|---|---|---|---|---|---|
| Storesize | 2 | 16.33 | 8.166 | 15.92 | 7.69e-07 *** |
| Residuals | 117 | 60.02 | 0.513 | | |

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

TukeyHSD

Tukey multiple comparisons of means

95% family-wise confidence level

| | diff | lwr | upr | p adj |
|---|---|---|---|---|
| medium-small | -0.4614286 | -0.8416275 | -0.08122962 | 0.0129962 |
| high-small | -0.9035714 | -1.2837704 | -0.52337247 | 0.0000004 |
| high-medium | -0.4421429 | -0.8223418 | -0.06194390 | 0.0182517 |

## 2.5 Analysis for Chapter 8

**Analysis of deviance table for the effect of plant types, assay method and methods of drying plants**

| | Df | Deviance | Resid. Df | Resid. Dev | p-value |
|---|---|---|---|---|---|
| NULL | | | 183 | 514.78 | |
| plant | 4 | 127.278 | 179 | 387.50 | < 2.2e-16 *** |
| method | 1 | 34.329 | 178 | 353.17 | 4.654e-09 *** |
| assay | 1 | 4.022 | 177 | 349.15 | 0.04491 * |
| factor(dose) | 2 | 78.409 | 175 | 270.74 | < 2.2e-16 *** |
| plant:method | 2 | 6.980 | 173 | 263.76 | 0.03050 * |
| plant:assay | 1 | 3.671 | 172 | 260.09 | 0.05537 . |

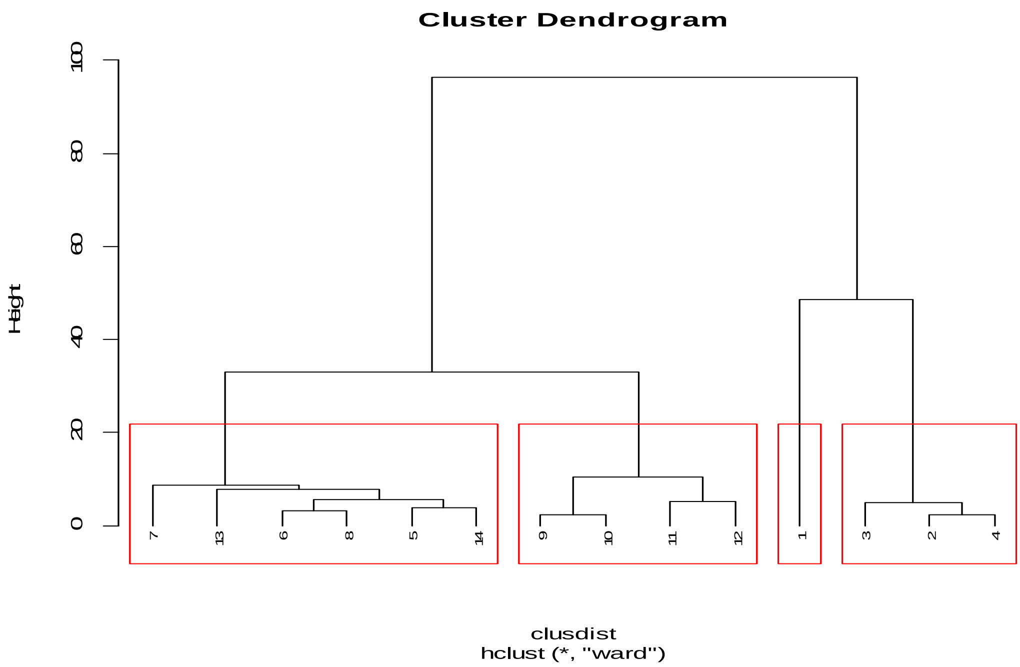
---

**Multiple comparison of plant types with t-test and corrected using Bonferroni correction method**

| | t-test | df | p-value | B-correction | Sign |
|---|---|---|---|---|---|
| OCT X OCK | 3.294704 | 14 | 0.01 | 0.09 | NS |
| OCT X OCW | 8.980479 | 14 | 0.0005 | 0.0045 | * |
| OCT X OCZ | 4.276842 | 14 | 0.0005 | 0.0045 | * |
| OCT X CNT | 7.748865 | 14 | 0.0005 | 0.0045 | * |
| OCK X OCW | 4.205094 | 14 | 0.0005 | 0.0045 | * |
| OCK X OCZ | 0.446546 | 14 | 0.25 | 2.25 | NS |
| OCK X CON | 5.335483 | 14 | 0.0005 | 0.0045 | * |
| OCW X OCZ | -4.10413 | 14 | 0.0005 | 0.0045 | * |
| OCW X CON | 2.76473 | 14 | 0.005 | 0.045 | NS |
| | | | | | |

**Appendix 3: Chapter 8 cluster dendrogram to identify plant samples with similar chemical characteristics**

## 3.1 Cluster Dendrogram



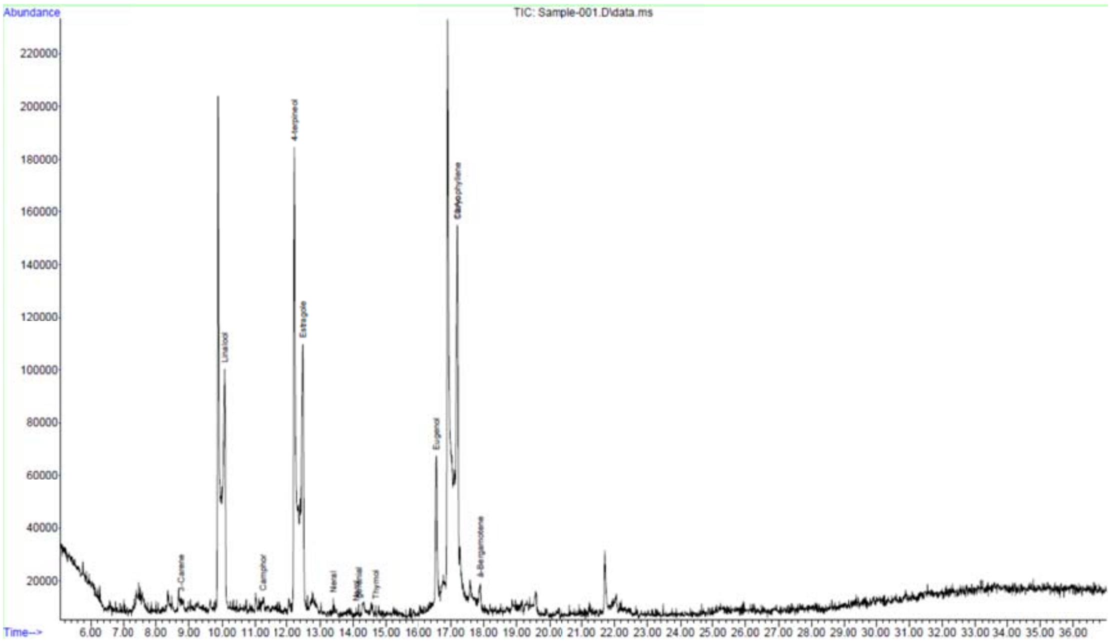

## 3.1 Cluster code

| | |
|---|---|
| 1 | OCT shade |
| 2 | OCT sun |
| 3 | OCK shade |
| 4 | OCK sun |
| 5 | LGY shade |
| 6 | LGY sun |
| 7 | LGZ shade |
| 8 | LGZ sun |
| 9 | OCW shade |
| 10 | OCW sun |
| 11 | OCZ shade |
| 12 | OCZ sun |
| 13 | LGD shade |
| 14 | LGD sun |

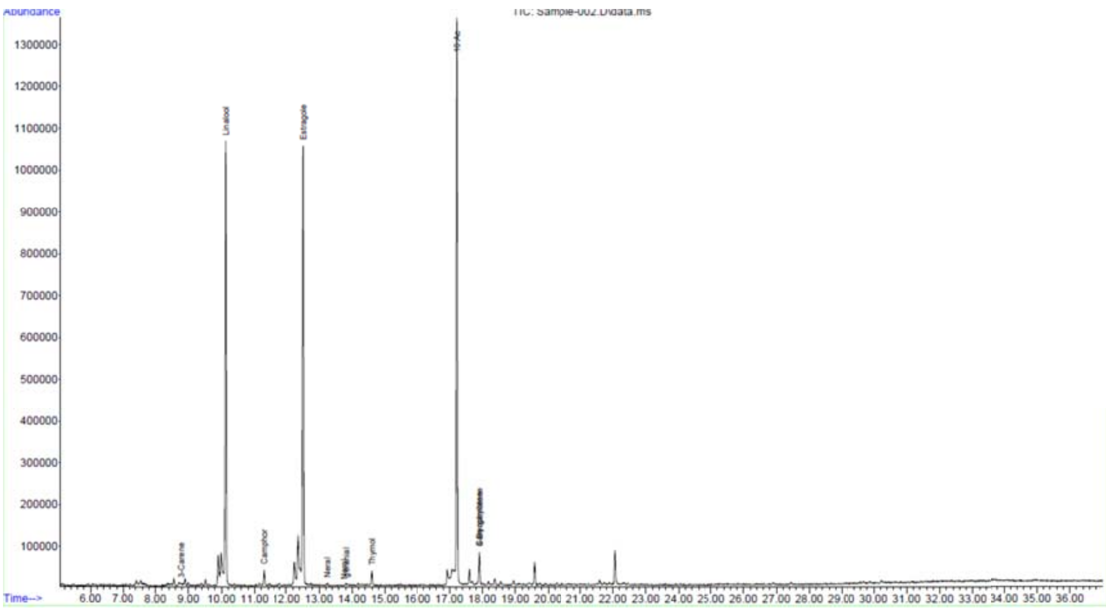
**Appendix 4: Chapter 8 chromatograms peaks for the different compounds found in different samples of Ocimum and Cymbopogon species extracted with hexane and methanol and analysed using GC-SM**

**4.1 a) shade dried OCT extracted with methanol b) shade dried OCT extracted with hexane**

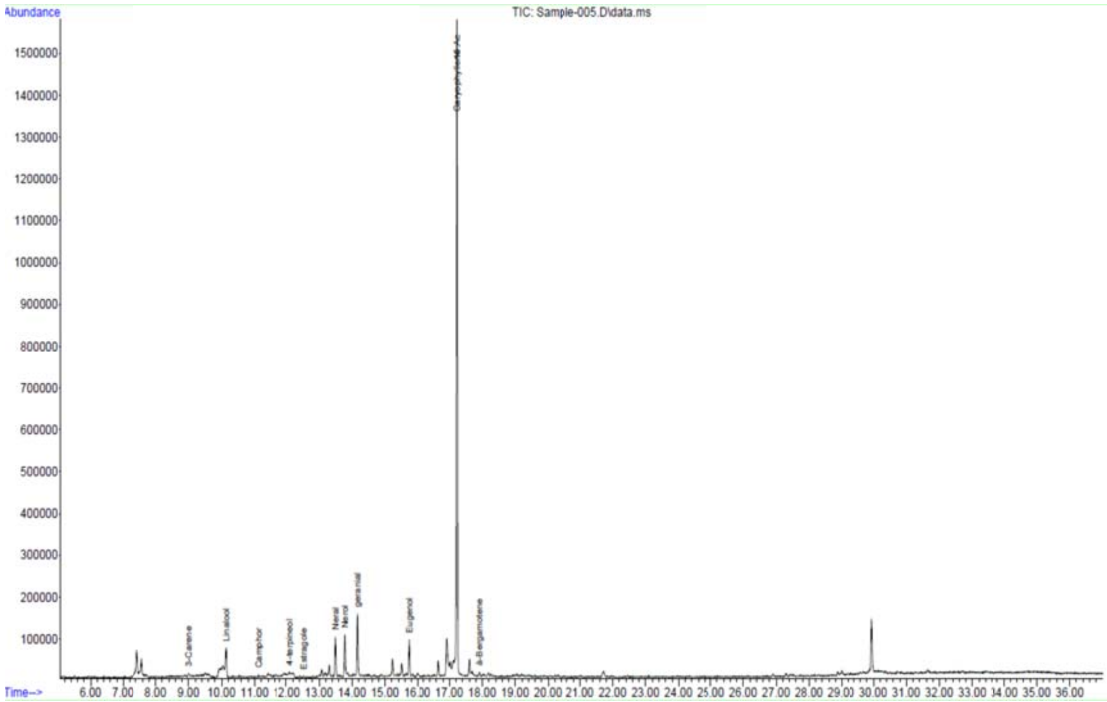
**a)**



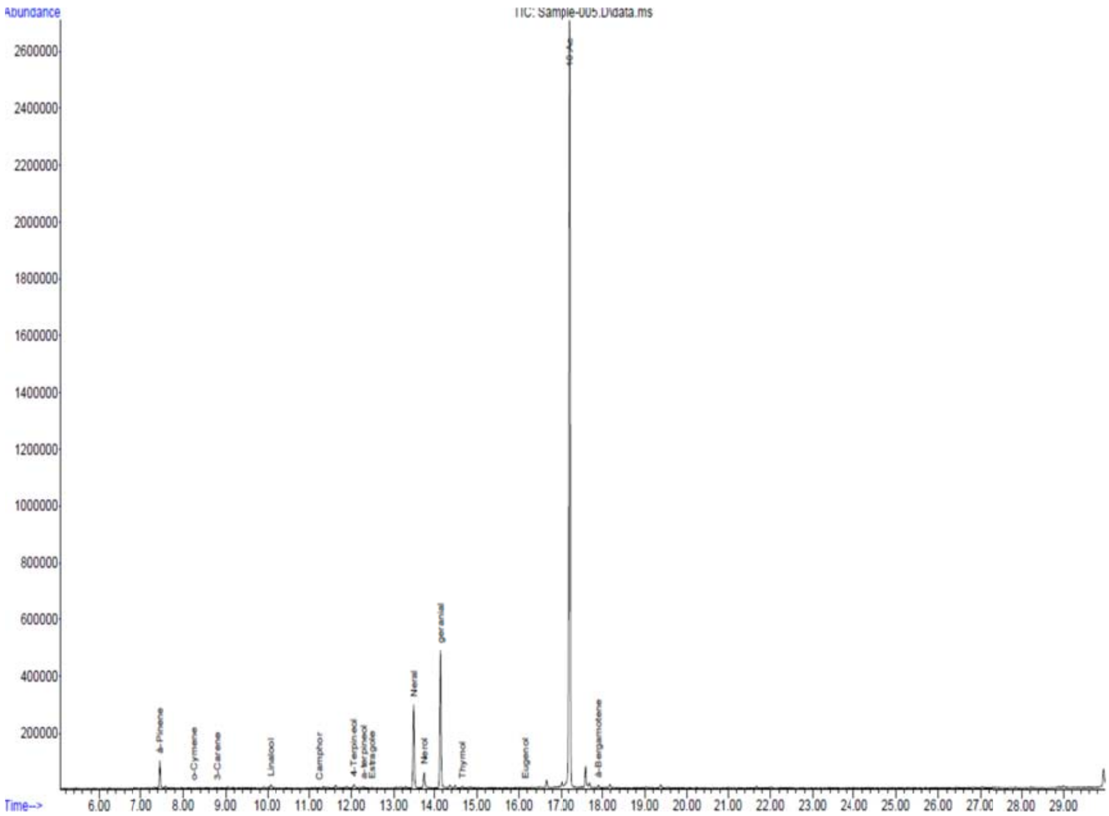

**b)**



**4.2 a) shade dried LGY extracted with methanol b) shade dried LGY extracted with hexane a)**

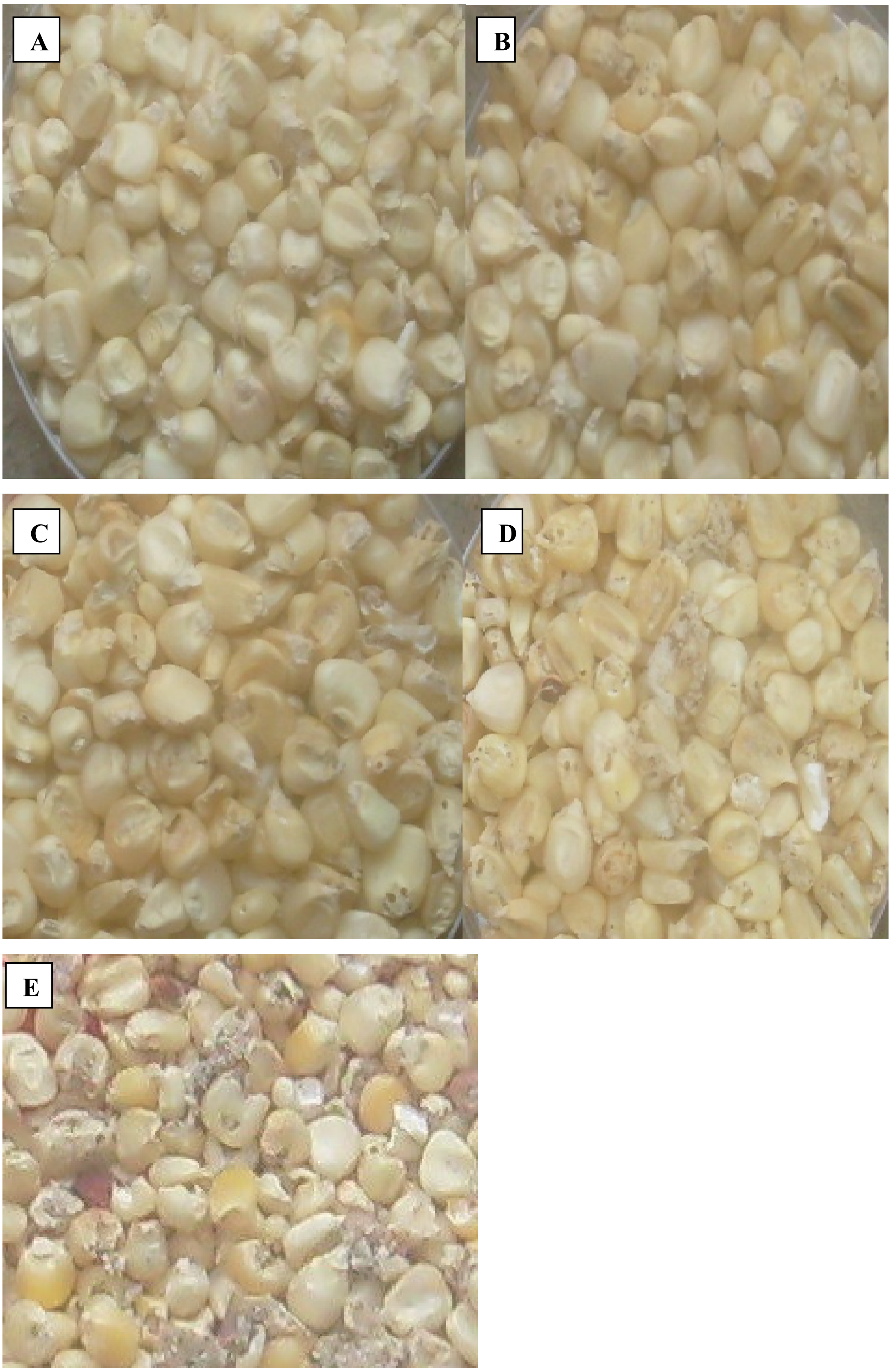



b)



**Appendix 5: Picture of different grains calibrated into different damage grades**

**5.1 Threshed maize calibrated into different damage grades**



## 5.2 Threshed sorghum calibrated into different damage grades

## 5.3 Threshed millet categorized into different damage grades

## 5.4 Threshed millet categorized into different damage grade