THE INSECTICIDAL POTENTIAL OF *NEORAUTANENIA MITIS* (A.RITCH) AGAINST *PROSTEPHANUS TRUNCATUS* (HORN) IN STORED MAIZE

BY

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ABSTRACT

This study was carried out to evaluate the insecticidal potential of *Neorautanenia mitis* to the Larger Grain Borers *Prostephanus truncatus* (Horn) (Coleoptera: Bostrychidae) and its mammalian toxicity. Laboratory studies were conducted to asses the efficacy and the basic mode of action of *N. mitis* on *P. truncatus*. Further studies were conducted to asses the toxicity of *N. mitis* on mammals using rats (*Mastomys natalensis*). The efficacy was assessed at six rates (0, 1, 2, 5, 7 and 10% w/w *N. mitis* in 50g of maize grains) with 5 replications. The repellency was evaluated in the choice bioassay at five rates (1, 5, 10, 15 and 20% w/w *N. mitis* in 50g of maize grains) in 5 replications. The contact mode of action was evaluated using Student's t or t-test. Anti-oviposition was evaluated in non choice bioassay system at six rates (0, 1, 2, 5, 7 and 10% w/w *N. mitis* in 50g of maize grains) in 3 replications. Actelic super dust at a rate of 0.05g in 1kg of maize was also used for comparison. Mammalian toxicity was evaluated by exposing rats to six rates (0, 5, 12.5, 25, 50 and 75% w/w *N. mitis* in Broiler mash) in 6 replications. The lethal dose (LD_{50}) was determined by the graphical method of Miller and Tainter. Split-plot arrangement in a Complete Randomized Design (CRD) was used while Two-way ANOVA was used in the analysis of data. The results showed that *N. mitis* powder and liquid extracts were statistically significant ($p<0.05$) in reducing the number of damaged grains. Repellency and ant-oviposition are presumed to be the key mode of action of *N. mitis*. The study indicated that the plant tuber is toxic to rats, therefore care should be exercised during the preparation and handling of the food stuff treated with *N. mitis*.

DECLARATION

I, ZENO PETER ASSEY, do hereby declare to the Senate of Sokoine University of Agriculture that this Dissertation is my original work and has not been submitted for a degree award in any other University.

15/06/2012.

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DEDICATION

In loving memory of my late parents:

Peter M. Assey and Juliet P. Assey

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LIST OF ABBREVIATIONS AND SYMBOLS

ANOVA	Analysis of Variance
et al	and others
°C	Degree Celsius
CRD	Completely Randomized Design
C.V	Coefficiency of Variation
Conc	Concentration
DAT	Days after Treatment
DMRT	Duncan's Multiple Range Test
e.g.	For example
FAO	United Nations Food Agriculture Organization
Fig	Figure
F1	First filial generation
g	Gram
HAT	Hours After Treatment
RIR	Reproduction Inhibition Rates
kg	Kilogram
L	Liter
LSD	Least Significant Different
LD_{50}	Lethal dose-50
kg. b. w	Kilogram body weight
m	Metre
ml	Milliliter

MSTATC	Micro Statistical Program, written using C programming Language
MC	Moisture Content
PMC	Pest Management Centre
PR	Percentage Repellent
pp	Pages
P<0.05	Significance at less than 5% level
^{0}C	Degree Celsius
R.H	Relative Humidity
RCBD	Randomized Complete Block Design
SV	Source of Variation
SUA	Sokoine University of Agriculture
spp	Species
S.E.	Standard Error
TDP	total development period
%	Percentage
U.K.	United Kingdom
w/w	Weight in weight

CHAPTER ONE

1.0 INTRODUCTION

1.1 Historical back ground

Maize (*Zea mays* L.) is an important food crop widely grown in Tanzania. It serves as a source of dietary carbohydrate for humans (Onwueme and Sinha, 1999). It is a staple food in many countries in Africa, Latin America and Asia. In Tanzania maize is largely produced in four Regions popularly known as "The Big Four" namely Iringa, Mbeya, Rukwa, and Ruvuma (Nsemwa and Lyimo, 2005). These Regions contribute more than 60% of the total maize produced in Tanzania (Rwamugira, 1996).

In Tanzania maize production is severely constrained by pre harvest losses in the field and post harvest losses in storage. Such losses are considered to be a major cause of food insecurity in Tanzania. According to Makundi *et al.*, 2007; Key and Mungereza (1998), *Prostephanus truncatus* Horn. (Coleoptera: Bostrichidae) and the maize weevil *Sitophilus zeamais* Mostch, (Coleoptera: Curculionidae) are the major storage insects pests causing losses of maize in Tanzania. Other insects pests reported in stored grain in Tanzania include; granary weevil *S. granaries* (L), lesser grain bores *Rhizopertha dominica* (F), indian meal moth *Plodia interpunctella* (Hubner) and angoumois grain moth *Sitotroga cerealella* (Olivier). In addition there are storage insects that are often regarded as secondary pests because they feed on processed maize products or damaged grains. They include the tropical warehouse moth *Ephestia cautella* (Walker) flour moth *Ephestia kuchniella* (Zeller), confused flour beetle *Tribolium confusum* (Jacquelin du val), red flour beetle *Tribolium casteneum* (Herbst), and rust grain beetle *Cryptolestes ferrugineus* (Stephens).

Losses during storage are still high despite farmers efforts in controlling them using various methods like using hermetic storage, synthetic insecticides, resistant maize varieties and by using hygienic and sanitation methods. Losses of up to 34% of maize have been reported in Tanzania after three months of storage with an average loss of 11.33% per month (Hodges *et al.*, 1983). Global post harvest grain losses caused by the insects and other bio agents ranges from 10 to 40 % (Raja *et al.*, 2001). Throughout the whole of Sub Sahara African an estimated 25-40% of grain crop is lost in storage each year due to insect damages related to poor storage structures, expensive and scarcely available storage insecticides (Koona and Njoya, 2004). Research proposes that, high losses occur between harvesting and consumption (Nchimbi-Msolla and Misangu, 2002).

Synthetic insecticides have been for a long time effective and the most important in mechanism of controlling storage pests. However, there are several disadvantages accompanied by their use. These include insecticides residues which can hardly be removed during grain processing (Sighamony *et al.*, 1990). The residues may ultimately contaminate the environment, promote faster development of resistant forms of pests and destroy natural enemies, turns formally innocuous species into pests and harm non-target species (Peddy and Peddy, 1987., Zettle and Cuperus, 1990).

These problems have necessitated the need for research on the use of alternative methods that can prevent cereal grains losses. Such methods include the use of botanicals pesticides. Botanical pesticides are seen as a promising alternative to the

use of synthetics and have recently received much attention (Golob *et al.*, 1999; Mohan and Fields, 2002; Facknath, 2006; Akob and Ewete, 2007).

1.2 Problem Statement and Justification of the Study

In Tanzania as in other developing Sub-Saharan Africa countries, continuous use of synthetic insecticides in the control of insect pests although effective, is expensive and has raised health and environmental concerns (Talukder, 2006; Isman, 2007). Surveys conducted in the Southern Highlands of Tanzania indicate that between 42-60% of the farmers use chemicals insecticides against grain storage pests particularly *P. truncatus* (Ashimogo *et al.*, 1995). Resource poor farmers fail to utilize insecticides at recommended dosages because they are expensive and are not readily available. There is also the problem of substandard and ineffective insecticides in the market (Mkoga *et al,* 1999). Consequently, some farmers have resorted to the use of natural plant materials which are less expensive, locally available, ecologically safe and socio-friendly in protecting their crops (Banwo and Adamu, 2003; Ogendo *et al.,* 2006; Talukder, 2006; Isman, 2007). Various products of plants have been tested recently with varying degree of success as protectants against a number of stored grain insect pests (Verma and Dubey, 1999). Pesticides of plant origin such as *Neorautanenia mitis* (A. Ritch) Verdcourt is one of the potential botanical pesticides that have not yet been fully exploited. This plant is available in many parts of Tanzania particularly in Southern highland Regions and is locally used by the farmers to control insect pests (Kabungo, 2004).

According to Bus and Park-Brown (2002) there is need for research on the active ingredients, pesticide preparations, application rates and environmental impact of botanical pesticides. It is important to note that botanical pesticides much as they are derived from plants, do not guarantee safety to humans and to the environment. Some may be quite toxic such as the rotenoids, which necessitates toxicological studies aimed at assessing the safety of these botanical pesticides should be done before they are used to avoid possible dangers (Belmain *et al.,* 2001).

The effectiveness of *N. mitis* in controlling pests has been reported by a number of workers. For examples Chimbe and Galley, (1999) reported its use against *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae) and *P. truncatus* in wheat and maize. Joseph *et al.* (2003) reported the larvicidal and mosquitocidal effects of crude extracts of *N. mitis* in Tanzania. The roots of *N. mitis* have also been reported to have pharmacological effects in rats and mice (Vongtau *et al.*, 2000; 2005). Van Puyvelde *et al.* (1987) isolated various compounds from the roots of *N. mitis* including hydroxyrotenone, a compound known to have insecticidal properties. Rahman and Talukder (2006) studied the bioefficacy of seven plant derivatives on *Callosobruchus maculates* (Fab) in Bangladesh. However, to date little is known on the standard application rate for use by farmers in Tanzania, mode of action of *N.mitis* (e.g. repellency, contact, and anti-oviposition / eggs laying inhibition) and safety of crude extracts to consumers. Lack of this information makes recommendations of proper application method for this botanical insecticide difficult to make.

1.3 Objectives

1.3.1 Overall Objective

To investigate the efficacy of *N. mitis* against *P. truncatus* attacking maize and assessing its mammalian toxicity.

1.3.2 Specific Objectives

i. To compare the effectiveness of two different formulations of *N. mitis* in controlling *Prostephanus truncatus.*

ii. To determine mode of action of *N. mitis* against *P. truncatus.*

iii. To determine mammalian toxicity of *N. mitis.*

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Maize, *Zea mays* L (Cyperales: Poaceae)

Maize is a member of the grass family Poaceae (Purseglove, 1990). Maize was first cultivated in Central America, notably in Mexico. The crop evolved through domestication of wild grass teosinte (*Zea Mexican*) which is native to Mexico. . In Tanzania maize production is mainly to meet house hold consumption and cash needs. Maize remains a major food crop and ranks high as compared to other food crops at 67% of the total cereal crops production (Makundi *et al*, 2007)

2.1.1 Economic importance of maize

Maize contains 80% starch, 13%water, 10% protein, 4% oil, 2% sugar and 3% fibers Maize is used as feed for livestock and poultry, particularly in the industrialized countries and material for many industrial products. Residues such as the stalks are used for fuel or compost (Oparaeke and Kuhiep, 2006).

2.1.2 Constraints to maize production

According to researchers, the most serious abiotic and biotic constraints for each of the maize production ecologies and geographic regions, include drought and poor soil fertility were the major ones. Drought is the most serious abiotic constrain in tropical maize produced under rain fed condition (Pingali, 2001).

Tropical soils are renowned for their low soil fertility, particularly low nitrogen, and consequently tropical soils ranks as the second most important abiotic constraint to maize production in tropical ecologies. Intensified land use and the rapid decline in

fallow periods, coupled with the extension of agriculture into marginal lands, have contributed to a rapid decline in soil fertility, particularly in sub- Saharan Africa. Nitrogen (N) and phosphorus (P) deficits are severe and widespread biophysical constraint to smallholder maize productivity, and in turn to the long-term food security

Inappropriate intensification of maize production systems, particularly in the hillsides of the Tropical lowlands and the mid altitude environments, has resulted in to high rates of soil erosion in many areas. Soil erosion and degradation are most often observed in areas where population growth is rapid, where land ownership right and use are not well defined, and where farmers face poor policy environment (Pingali, 2001).

Among the most important biological constraints at field and post harvest stages of maize production include insect pests. It is estimated that field and storage pests destroy approximately 43% of potential crop production in developing ,Asian and African countries (Jackobson, 1982; Ahmed and Grange, 1986; Ogendo *et al.*, 2004). In Tanzania insects pests have been the cause of reduction in maize production level (Temu *et al.*, 1995; Rugumamu, 2005).

Table 1: The common insect pests in developing world that affect maize production

Part of plant affected	Insect pest
Roots	Rootworms, wireworms, white grubs, and seed-corn maggots
Leaves	Aphids, armyworm, stemborers, thrips, spidermites, and grasshoppers.
Stalks	Stem borers, termites.
Ears and tassels	Stem borers, earworms, adult rootworms, and Armyworm.
Grain during storage	Grain weevils, grain borers, indian meal moth, and the angoumois grain moth.

Source; (FAO, 1983)

Insect damage can occur at any stage of maize production and storage. Its severity depends on the variety used, cultivation practices, levels of pest infestation, control strategies used and climate. There are two main classes of maize pests that are primary pests and secondary pests. The primary pest is the one which borers the kenel and develops inside it, by hiding their infestations in the grain mass (Cotton and Wilbur, 1982). The Larger Grain Borer *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae) is classified as a primary pest. In contrast a secondary pest is one that does not have the kernel boring capability and can only develop outside the kernels or feeding on broken kernel or on the grain dust. A good example is the Red flour beetle *Tribolium casteneum* (Herbst) and Confused flour beetle *T. confusum* (Jacquelin).

Table 2: Primary and secondary insect pests of stored maize

Scientific name	Common name	Family name
Primary pests		
Prostephanus truncatus (Horn)	Larger Grain Borer	Bostrichidae
Sitophilus oryzae (L.)	Rice weevil	Curculionidae
S. zeamais (Motschulsky)	Maize weevil	Curculionidae
S. granarius (L.)	Granary weevil	Curculionidae
Rhizopertha dominica (F.)	Lesser grain borer	Bostrichidae
Sitotroga cerealella (Olivier)	Angoumois grain moth	Gelechlidae
Secodary pests		
Oryzaephilus surinamensis (L.)	Saw toothed grain beetle	Silvanidae
PlodiaInterpunctella (Hubner)	Indian meal beetle	Pyralidae
Ephestia kuchniella (Zeller)	Mediterranean flour beetle	Pyralidae
Lasioderma serricome (F)	Cigarette beetle	Anobidae
Tribolium casteneum (Herbst)	Red flour beetle	Tenebrionidae
T. confusam (Jacquelin)	Confused flour beetle	Tenebrionidae

Source; (FAO, 1983)

2.2 *Prostephanus truncatus*

The larger grain borer, *P. truncatus* (Horn) is an important pest of maize *Zea mays*, and can infest the standing crop as well as maize in storage. It can also infest and

cassava, *Manihot esculenta* in Africa. The ability of the beetle to establish itself as a serious pest in both the hot, dry conditions of western Tanzania, the hot, humid conditions of Togo and up to an altitude of 2200 m in Mexico suggests that it has the potential to spread to all areas where maize is grown, and to other tropical and subtropical regions.

2.2.1 Description life history and type of damage

Prostephanus truncatus originates from meso-America where it infests maize stores and dead wood (Ramirez-Martinez *et al.*, 1994; Espinal *et al.*, 1996). It takes about 24-25 days to develop from an egg to adult under optimal conditions (32°C and 80% R.H). Eggs are laid in batches of 20 and each egg clutch is usually protected by tightly packed frass. Females can live for several months, laying an average of 430 eggs. There are three larval instars that develop within the food substrate, feeding on dust produced by adult boring (Borgemeister *et al.*, 2003). Males alone produce an aggregation pheromone when they have located a suitable breeding site, which attracts female flying adults. Oviposition begins 5-10 days after adult emergence, reaching a peak at 15-20 days (Bell and Watters, 1982). The mean development period under optimum conditions for eggs is 3 days, for larvae (3 instars) 13days, prepupae 4 days, and pupae 2 days (Demianyk and Sinha, 1988). Females tend to outlive the males, with a mean survival time of 61 days for females and 45 days for males (Shires 1980; Bell and Watters 1982).

2.2.2 Morphology of *P. truncatus*

The larger grain borer belongs to Bostrichidae a family of most of wood boring beetles. It is 3-4mm long cylindrical and dark brown in color. The thorax bears raw

of teeth on its upper front edge and with the head turned down underneath the thorax so that it can not be seen from above. The cylindrical shape and teeth on the thorax are also characteristic of other grain feeding insects in this family. In *P. truncatus,* the end of the wing covers is flattened and this sloping region has two curved ridges at the tips. The effect of the flattened ends to the wings cover and the ridges is to gives the *P. truncatus* a very square cut end. This feature distinguishes *P. truncatus* from other Bostrichids in particular *Rhizopertha dominica* (Lesser Grain borer) which are also known to attack stored products.

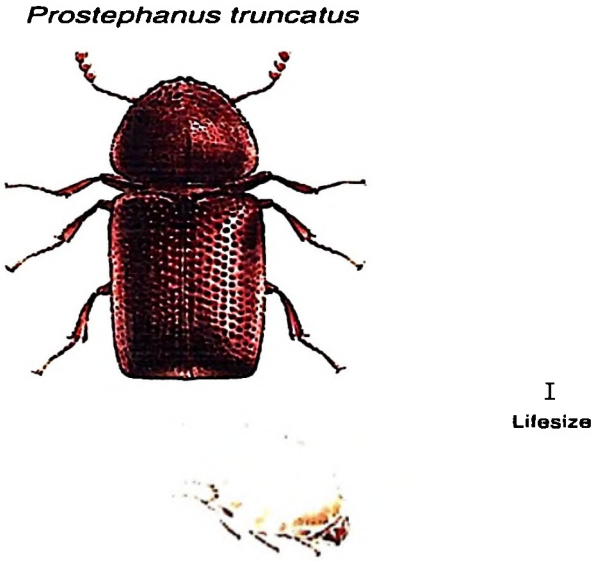

Figure 1 Adult beetle and larva (ICI copyright)

Figure 1: Adult beetle and larva (ICI copyright)

2.2.3 Geographical distribution of *Prostephanus truncatus*

Since the accidental introduction of the *P. truncatus* in East Africa in the late 1970s and West Africa in the mid-1980s from Central America and Mexico (Markham *et*

al., 1991) the pest has spread further to cover most other countries in Sub Sahara Africa (Hodges, 1994). The beetle has spread to 15 African countries (Hodges, 1994; Adda *et al.*, 1996; Sumani and Ngolwe, 1996; Roux, 1999, including Tanzania (1981), Kenya (1983), Burundi (1984), Togo (1984), Benin (1984), Guinea (1987), Ghana (1989), Burkina Faso (1991), Nigeria (1992), Malawi (1992), Rwanda (1993), Niger (1996), Zambia (1996), Namibia (1998) and South Africa (1999). It is a serious pest of stored maize and dried cassava roots, it attacks maize in the field just before harvest (Shires, 1977).

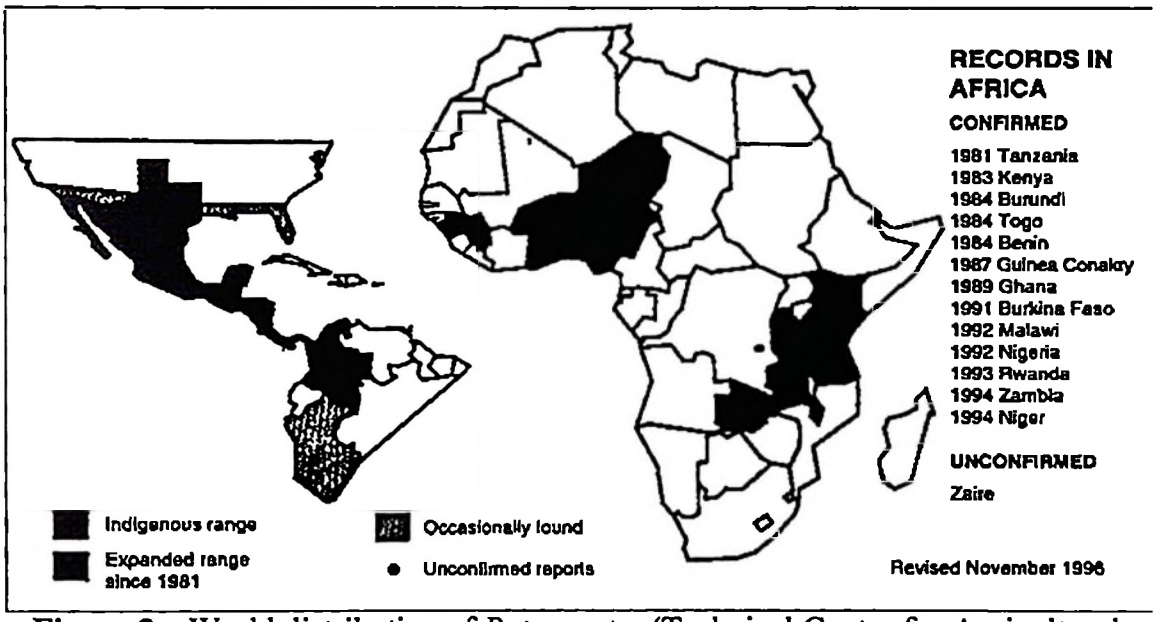

Figure 2: World distribution of *P. truncates* (Technical Centre for Agricultural and Rural Cooperation -CPA-Netherlands)

2.2.4 Historical background of *P. truncatus* in Tanzania.

In 1980, farmers from Tabora District in Western Tanzania reported the presence of an unknown pest destroying stored maize and dried cassava. It was once assumed that the pest had been introduced into the country through maize imports. Locally it became known as "Scania", referring to the shape of the head, which resembled the

shape of vehicles popular called scania, but was very distinctive compared to other important, traditional storage pests. Another name under which it became known was "Dumuzi", which is the noun of the verb "dumula" in the local Kinyamwezi language, meaning to grind maize into flour, which is what *P. truncatus* does to stored maize.

2.2.5 Pest status of *P. truncatus*

Losses caused by *P. truncatus* in maize and cassava are much higher than those caused by indigenous pests, especially because the *P. truncatus* inflict the losses in a relatively short period of time. At household level, the losses may be as high as 35% in 5-6 months of storage, and losses of up to 60% or more may occur over a 9-month storage period (Golob and Hodges, 1982; Hodges *et al*, 1983). Weight losses of this magnitude render the maize unfit for human consumption and therefore can be considered as a total loss. Such losses are jeopardizing Tanzania's efforts in achieving self-sufficiency in food production. Weight losses of up to 40% have been recorded in Nicaragua from maize cobs stored on the farm for 6 months (Giles and Leon, 1975). Both larvae and adults feed on the grain in the store and reduce them to powdery form and spoil the grain with their frass. Infested grains are often susceptible to caking and mouldness, which reduce the market value of the maize (Ofuya and Lale, 2001).

2.2.6 Management of *P. truncatus*

2.2.6.1 Prevention of infestation.

According to Makundi *et al,* (2006), the prevention of infestation of grain by *P. truncatus* is more effective than the control of infestation. Various strategies can be

used to prevent infestation and these include botanical toxicants, timely harvesting, using store hygiene, using resistant varieties, and using air tight containers for storage.

2.2.6.2 Botanical toxicants

While the insecticidal activity of extracts of some plant species, such as the neem *Azadirachta indica* (A. Juss) and Castor oil *Ricinus communis* (L) are well known, much research has recently focused on the use of extracts of local plants that may be toxic to insects and cheap for post harvest storage purposes. The application of ashes of wood and other materials that are locally available is widespread (Golob and Kilminister, 1982)

2.2.6.3 Timely harvesting and selection of uninfected seeds

This involve the altering of the time of harvest to obtain optimum drying of the grain and lowering field infestation methods by appropriate handling and drying of the grain after harvest. The selection of the maize cobs after the harvest has been proposed to avoid insect infestation at the beginning of the storage time. Early harvesting of maize 2–3 weeks after physiological maturity is recommended (Borgemeister *et al.*, 1998).

2.2.6.4 Hygienic and sanitary measures

The proper cleaning of the store prior to the next harvest is of utmost importance. Many storage pests are able to survive on residuals and debris of stored grain or within the stored structure and act as sources of new infestation.

2.2.6.5 The use of chemicals

In Tanzania, the current recommended insecticide for control of *P. truncutus* is the use of Permethrin+Pirimphos-Methyl (Actelic super dust) (Key and Mungereza, 1998) at a rate of 100g per 100kg of maize grains. The insecticidal formulation consisting of 0.3% permethrin and 1.6% pirimiphosmethyl.

2.2.6.6 The use of resistant varieties

The use of varieties with good husk cover characteristics (strong and thick husks, and long tip extension) (Meikle *et al.*, 1998) and varieties with strong seed kernel are other means of lowering post harvest losses.

2.2.6.7 The use of airtight containers/drums

Air tighten storage such as the metal drums prevent the pests from entering and increase the mortality of stored grain insects, due to high concentration of carbon dioxide and a low concentration of Oxygen (Henckes, 1992).

2.2.6.8 Biological control

Population growth of *P. truncatus* is inhibited by a number of parasitoid, predators and pathogens found in association with *P. truncatus*. The predator is a Histerid beetle *Teratriosoma nigrescence* (Borgemeister *et al,* 1997) is one of the predators of *P. truncatus.* As Holst and Meikle (2003) reported *T. nigrescens* reduced significantly the population growth rate of both *P. truncatus* and non target pests the weevil (*S. zeamais*).

2.2.7 Constraints to management of *P. truncatus*

The current management of storage pests include the use of air tight containers, synthetic insecticides, inert material (wood ashes, sand), smoking and resistant varieties. However, the use of synthetic insecticides such as potassium phosphide for control of *P. truncatus* is expensive (Banwo and Adam, 2003). The use of synthetic insecticide to control *P. truncatus* has contributed to the outbreak of pests beyond normal population levels due to the killing of natural enemies. Health and environmental concerns have also been high as a result of using synthetic insecticide (Tulukder and Howse, 1994). Shortages of the insecticide, high costs to farmers and/or traders especially when large quantities of maize are involved, reluctance of farmers or traders to use right dosage and comply with the law when there is no obvious *P. truncatus* infestation, lack of manpower and facilities at checkpoints have all contributed to the outbreak of the pest.

2.2.8 Host range of *P. truncatus*

P. truncatus is a serious pest of stored maize and dried cassava roots, and attacks the maize in the field just before harvest (Shires, 1977). The pest behaves as a typical primary pest of farm-stored grains; whole grains are attacked on the cob, both before and after harvest. Extensive populations of *P. truncatus* occur in the natural environment, and it has been recorded from a number of tree species in Central America and Africa (Nang'ayo *et al.*, 1993; Ramirez-Martinez *et al.*, 1994). As a typical bostrichid, *P. truncatus* has demonstrated to have the capacity to breed in a wide range of woody hosts. Some of which are important agro forestry species. Tree species such as *Commiphora africana* (Arn.) Engl. and *Commiphora riparia* Engl.

were found to support breeding populations of *P. truncatus* emphasizing the need to appreciate the natural environment as a reservoir for *P. truncatus* (Nang'ayo *et al.*, 1993). The abundance of LGB in the natural environment is positively correlated with ambient relative humidity and temperature (Nang'ayo, 1996). With many woody species appearing to support breeding populations of *P. truncatus* farmers are advised to avoid potential hosts of *P. truncatus* when constructing maize stores. Other findings indicate that maize cobs and Stover also harbor sufficient numbers of *P. truncatus* and should be buried or destroyed to reduce potential sources of infestation (Wekesa, 1994). Heavily infested stores were also found to influence pre-harvest infestation suggesting that where the circumstances allow, stores should be sited as far as possible from the maize fields.

Table 3: Some of the tree species that support breeding of the *P. truncatus* laboratory conditions

Scientific name	Family
Anacardium occidentale	Anacardiaceae
Mangifera indica	Anacardiaceae
Manihot esculenta	Euphorbiaceae
Cajanus cajan	Papilionaceae
Commiphora campestris	Burseraceae
Commiphora africana	Burseraceae
Cassia abbreviata	Caesalpiniaceae
Cassia siamea	Caesalpiniaceae
Delonix regia	Caesalpiniaceae
Leucaena diversifolia	Mimosaceae
Acacia polyacantha	Mimosaceae
Prosopis pallida	Papilionaceae

Source (Nang'ayo, 1996).

2.3 Botanical insecticides

Botanical insecticides are the insecticides originate from the plant. These have generated extraordinary interest in recent years as potential sources of natural insect

control agents. Today over 2000 species of plants are known that possess some insecticidal activity (Jacobson, 1989)

2.3.1 Definition of Botanical Insecticides

Botanical pesticides are an important group of naturally occurring, often slow-acting crop protectants that are usually safer to humans and the environment than conventional pesticides (Pavela, 2009). Botanicals are useful against insect pests such as ants, aphids, beetles, caterpillars, cockroaches, fleas, flies, leafhoppers, mosquitoes in yards and gardens. Some of the currently recognized botanical insecticides are limonene, neem, nicotine, pyrethrum, rotenone, ryania and sabadilla. Some organic products contain combinations of the above. As Jacobson (1989) suggests the most promising botanicals are to be found in the families of Maliaceae, Rutaceae, Asteraceae, Annonaceae, Labiaceae and Canellaceae. The most numerous are found in the families of compositae, Fabaceae, Labiaceae, Leguminaceae, Solanaceae and Umbelliferae. Secondary compounds include alkaloids, terpanoids, phenolics, flavonoids, chrombenes and other minor chemicals. These can affect insects in several different ways which induce disrupting major metabolic path ways, causing rapid death; acting as attractants, deterrents, phagostimulant and antfeedants or modifying oviposition. Indeed, research in this area has led to the discovery of substances with interesting activities on insects. The substances include insect growth regulators/inhibitors and antifeedants (Saxena, 1987; Rembold, 1994). The insect development inhibitors specifically affect development of insects. They may retard or accelerate development or interfere with the life cycle of the insects in many other ways (Bell *et al.*, 1990).

2.3.2 Importance of botanical insecticides vis-à-vis synthetic insecticides

The botanical insecticides are generally pest-specific and are relatively harmless to non-target organisms including man. Most of them are also biodegradable and harmless to the environment (Rembold, 1994). Furthermore, unlike conventional insecticides which are based on a single active ingredient, plant derived insecticides comprise an array of chemical compounds which act concertedly on both behavioral and physiological processes. Thus the chances of pests developing resistance to such substances are less likely (Saxena, 1987). One plant species may possess substances with a wide range of activities. For example, extracts from the neem tree have antifeedant, antioviposition, repellent and growth-regulating effects (Schmutterer, 1990). In contrast, the toxicity of conventional synthetic insecticides is mainly restricted to neuro-muscular function (Ware, 1994). Conventional synthetic insecticides require special safety procedures and equipment during production and application (Ware, 1994). Despite precautions, exposure to humans, the environment and food are inevitable (Franzen, 1993). The synthetic insecticides are expensive and have in many cases only produced moderate results along with major ecological damage. In contrast, the low toxicity of botanical insecticides makes processing and application of the product inexpensive. In many cases, the materials are locally available and affordable (Childs, 2001). Plants with insecticidal properties are abundant and can be propagated by farmers under minimum costs. It is comparatively safe and less knowledge intensive to the users compared to the synthetic insecticides. Botanical insecticide plants are far less persistent in the environment (Gaskins *et al.*, 1972; Isman, 2000).

2.4.4 Impacts of botanical insecticides on Environment

Pesticidal plants are an appropriate technology for pest management on small holder farms; but strengthening and promoting their use needs consideration and care. Although pesticidal plants are a relevant pest management technology, their use is under threat due to stagnating knowledge base that cannot keep up with the contemporary health, safety and reliability directives. One key aspect of this is the determination of safety of the material currently being used or promoted. A study on plants used to protect stored grain from insect pests in West Africa has shown that some of the most commonly found plants used by farmers can adversely affect growth and development (when fed to rodents), with potentially long term effects (Stevenson, 2007). Greatest risks exist when pesticidal plants are used for post-harvest treatment especially when toxic species which were originally developed and promoted for use in field pest management such as *Tephrosia* spp and *Derris* spp. are promoted by poorly informed extension organizations for use on stored food stuffs. There is a myth that because they occur naturally pesticidal plants are safe. Plants produce some of the most toxic substances known and many plant materials may be more dangerous than commercial synthetic chemical insecticides (Stevenson, 2007). It is therefore essential to develop the capacity to evaluate their toxicity and ensure appropriate promotion to end users.

2.5.5 Overview on efficacy of botanical insecticides

Currently, there is considerable interest among biochemists and botanists to screen plants for secondary chemical compounds, which could be used for developing medicines and pesticides. In general, plants with pesticidal properties can be

exploited as a key to synthesize a chemical compound which then could be produced industrially (Berger, 1994). Literature on the biological properties of crude extracts and isolated secondary substances of plants against different insects and other organisms is abundant. Talukder and Howse (1994) mention the toxic and repellent properties of extracts of Aphanamixispolystachya (L) against *S. oryzae*. Furthermore Boeke *et al.* (2004) have evaluated the efficiency of 23 different plant extracts on *Callosobruchus maculatus* and found repellency of volatile oils. Prakash and Rao (1997) conducted an extensive revision of plants containing active secondary substances against insects. These authors provide information on 866 plant products with insecticidal activities. In Brazil, Tavares and Vendramim (2005) studied the bioactivity of *Chenopodium ambrosioides* on *Sitophilus zeamais* and in Argentina this subject has received less attention. Novo *et al.* (1997, 1998) observed the repellent activity of several crude extracts of four native plants against *T. castaneum* and the antifeedant effect on *Anticarsia gemmatalis*. The current study was aimed at exploring the effectiveness of *N. mitis* in controlling *P. truncatus* and determines its related toxicological properties with a view to use it as a possible agent for controlling *P. truncatus*. The results obtained would lead to adequate understanding of the insecticide and warrant its safe use by farmers.

2.4 *Neorautanenia mitis*

2.4.1 Description and habitat

The plant is a leguminous creeper shrub which can be 1-1.5m high, with trifoliate leaves and hairy big pods, and round seeds. The flowers are pinkish or white in color. The stem originates from a huge root tuber. Normally these plants are

distributed in Southern high lands of Tanzania especially in the miombo wood lands, open woodlands and open grass lands.

Figure 3: Young *Neorautanenia mitis*

Figure 4: Matured *Neorautanenia mitis*

Figure 5: ***Neorautanenia mitis*** **tuber**

Figure 6: ***Neorautanenia mitis*** **flower**

2.4.2 Biology

N. mitis is a small leguminous plant which can be as high as 1.5m. The plant has broad leaves some of which have lobed sides making the leaves appear with three 'tips'. *N .mitis* produces sizeable tubers which can reach a diameter of around 75cm with a weight of around 50kg. It is the tuber which contains the insecticidal properties. *N. mitis* showed a great potential in the control of maize weevils and possibly other storage pests and can protect grains up to six months with a minimum damage (Kabungo *et al.*, 1998)

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Experimental Site

Experiments were conducted in laboratories at Sokoine University of Agriculture (SUA) in the Department of Crop Science and Production, SUA Pest Management Centre (SPMC) and Department of Veterinary Pathology.

3.2 Preparation of *N. mitis* and Rearing of Insects

3.2.1 Collection and rearing of insects colonies

Five hundred (500) adults *P. truncatus* were collected from milling machines and from SUA Pest Management Centre laboratory in Morogoro. The insects were put in two glass jars of 1 liter capacity each. The glass jars were kept in a room maintained at optimum conditions of 25-29 ^{0}C and 65 – 70% relative humidity as per Shires (1980). The brims of the containers were covered with perforated lids. Three hundred adults of *P. truncatus* of both sexes at a ratio of male to female (1:2) and of different ages were introduced in to the jars with kernels of maize variety (Staha) and left for 7 days to allow them to lay eggs. The adult insects were then removed and discarded. The media containing eggs was left undisturbed until adults emerged. After 25 days the adult insects were removed from the large plastic containers by sieving and were used in the experiment.

3.2.2 Processing and preparation *N. mitis*

Dried chips /slices of *N. mitis* tubers were ground and sieved with a 0.2 mm mesh to obtain a fine powder commonly used by farmers. The process of pulverization and sieving of the tubers continued until no more powder was released. Both *N. mitis*

powder and liquid were used in this experiment. The *N. mitis* powder was applied at six levels or concentrations of 0, 1, 2, 5, 7and 10 g in 50g of maize grains (Othira *et al*, 2009).

The extracts were prepared according to Chitra *et al.* (1993) with some modifications. The extraction was done by immersing 1, 2, 5, 7 and 10 g *N. mitis* powder separate into 20ml of distilled water. The mixtures were stirred for 30 minutes by a magnetic stirrer at 6000 (rpm) and left to settle for next 24 hours. The mixture was then filtered through a fine cloth and re-filtered in Whatman No.1 filter paper. The filtrate was collected in the round bottom flasks five (5) ml of each solution was taken and used to treat the maize grains.

3.3. To compare the Effectiveness of Two Formulations (Powder and Water Extract) of *N. mitis* in Controlling Storage Pests

One thousand seven hundred and fifty (1 750g) maize grains at the moisture content of 14% were collected and placed in 35 bottles. Then *N. mitis* was applied at the rates of %w/w for powder and %v/w for liquid of 0, 1, 2, 5, 7and 10 in 50g of maize grains. The bottles were vigorously shaken to ensure proper mixing of maize grains with *N. mitis*. Twenty (20) *P. truncatus* adult (1-7 days old) were starved for 24 hours and then confined in the bottles contain the mixture of *N. mitis* and maize grains for 14 days. The bottles with the mixture of *N. mitis* and *P. truncatus* were labeled and covered with perforated lid to provide ventilation. Actelic Super Dust was used as a standard control at a rate of 0.05g in 1kg of maize. The bottles were then kept in a room maintained at a temperature of 28 – 29^{0}C and 65 – 70% relative

humidity as described by Shires, (1980). The layout of the experiment was a split-plot arrangement in a Complete Randomized Design (CRD) in which the *N. mitis* concentrations were the main plot and time (days) were the subplots with five (5) replications. Data collected include the number of dead insects, the number of damaged grains and grain weight loss. Analysis of Variance (ANOVA) using MSTAT C computer statistical package. Means were separated by Duncan Multiple Range Test at 0.05 level of significant.

3.4 Determination of Mode of Action of *N. mitis* Against *P. truncatus*

The experiment was conducted to determine the basic mode of action of *N. mitis* that is whether it is a repellent, stomach poison, contact poison or Anti-oviposition or development inhibition.

3.4.1 Repellency tests

Repellency material drives the insects away after exposure to the plant without necessarily feeding. The treatment involved grams of dried powder of *N. mitis* applied at the rates of 1, 5, 10, 15 and 20% w/w *N. mitis* in 50g of maize grains according to the method adopted after McDonald *et al.* (1970), Talukder and Howse, (1994) but with some modifications. Twenty five (25) Petri dishes 9 cm diameter each was divided into two equal portions; one portion was supplied with treated grains while the second portion was supplied with untreated grains for each concentration listed above (Figure 6). Ten (10) *P. truncatus* adults were released at the centre of each Petri dish which then covered. The exercise was repeated at interval of 1 day for 3 days. The insects that settled on each half of the petridish

were counted at 1 hour interval for 5 hours per day. The average of the counts was converted to the percentage repellency (PR) using the formula of Talukder and Howse (1995) that is PR = 2(C - 50), where, C is the percentage of insects on the untreated half of maize grains in the Petridish. Positive (+) values expressed repellency and negative (-) values expressed attractant.

The repellent classes were categorized as;

Class I 0.1% - 20 % Non repellent material

Class II 21% - 40 % slightly repellant materials

Class III 41% - 60 % Repellent material

Class IV 61% - 80% highly repellent material

Class V 81% - 100 % repellence (Mc Govern *et al.*, 1977)

The layout of the experiment was a split-plot arrangement in a complete Randomized Design (CRD) in which the *N. mitis* concentrations were the main plot and days were the subplots with five (5) replications (Table 10).

Figure 7: Experiment set up for repellency test

3.4.2 Contact mode of action

One hundred (100) adult insects 1-7 day old *P. truncatus* were introduced into two containers, one containing *N. mitis* powder and the other containing fine white sand as described by Rahman *et al.* (2003) with some modification. In each container,

100 adult insects were left for one hour, removed and placed in rearing bottles and allowed to feed on 300 untreated maize grains. The number of dead adults was recorded daily for one week (7days). Seven observations were made (n = 7). Student's t or t-test was applied to determine the difference in paired means of the numbers of dead adults.

3.4.3 Anti-oviposition or development inhibition

Fifty (50) grams of maize grains at moisture content of 14% were placed in 12 cm high x 6.5 cm diameter glass jars. The treatment involved dried powder of *N. mitis* applied at the rates (%w/w) of 1, 2, 5, 7 and 10 in 50g of maize grains according to the method of Othira *et al.* (2009) with some modification. The *N. mitis* powder was thoroughly mixed with 50g of maize grains in each jar. Actelic super dust was used as a standard control at a rate of 0.05g in 1kg of maize. Three replicates were provided for each treatment. A mixture of twenty five (25) 1-7 day old newly collected male and female *P. truncatus* were introduced in to each jar and covered with perforated lid. The female adults were allowed to oviposit on the grains for 48 hours, after which, they were discarded. A direct examination of the grains was done with the aid of a dissecting microscope. Since the presence of eggs could not be determined, the presence of larval tunnels was used as a basis for counting the number of deposited eggs. The eggs are laid in batches of the 20 in one tunnel and covered with finely chewed maize dust (Bell and Watters 1982). According to Maribet *et al.* (2008) a larval tunnel indicates egg deposition. Absence of larval tunnel suggests that no egg was deposited, hence this was the basis for anti-oviposition effect of the test materials. The examined grains were kept separately in

properly labeled and covered 12 cm high x 6.5 cm diameter glass jars for adult emergence.

Data collected

(a) The total development period of the test insects [the number of days when the egg was laid up (a day after removal of parent weevils from the jar) to adult emergence].

(b) The number of F_1 adults which emerged in each bottle (from day 27 – 42 days after the setup of experiment).

(c) The reproduction inhibition rates RIR% = [(Cn - Tn) / Cn] x 100, where Cn = Number of insects in control bottles and Tn = the number of insects in treated grains in the bottles (Talukder and Howse, 1994)

All the data gathered were analyzed using ANOVA following one factor Randomized Complete Block Design (RCBD) in MSTAT C computer program. Means were compared using the Duncan Multiple Range Test ($P<0.05$).

Figure 8: a and b. Experimental set-up for adult *P. truncatus* ant- oviposition test

3.5 Determination of Mammalian Toxicity of *N. mitis*

3.5.1 Preparation of *N. mitis* powder

Dried chips /slices of *N. mitis* tubers were ground into small particles or course powder and sieved with a 0.2 mm mesh to obtain a fine powder commonly used by farmers. The process of pulverization and sieving of the tubers continued until no more powder was released. Known weights of *N. mitis* powder were mixed with known weights of broiler mash to obtain six portions of 7g each containing increasing concentrations of *N. mitis* ranging from 0% to 75% as shown in Table 4.

Table 4: Portions of *N. mitis* broiler mash mixture containing concentrations of *N. mitis* ranging from 0% to 75%

Animals (rat)group number	Portions of *N. mitis*-broiler mash mixture	Weight of broiler mash (gm)	Weight of *N. mitis* (gm)	Weight of the mixture (gm)
I	0.00	7.00	0.00	7
II	5%	6.65	0.35	7
III	12.5%	6.12	0.88	7
IV	25%	5.25	1.75	7
V	50%	3.5	3.5	7
VI	75%	1.75	5.25	7

3.5.2 Animal trapping and maintenance in captivity

Fifty (50) rodent *Mastomys natalensis* were trapped from Mzinga farm using Sherman traps baited with peanut butter. The captured animals were caged and kept in an animal experimental room for two months. During the course of captivity the animals were daily fed with broiler mash and clean water for the whole period. At the end of the experiment, 36 animals were selected at random and were treated with carbonyl (5%) to remove ecto parasites (fleas, mites, lice and tick). The purpose of removing ecto parasites from the animals was to ensure maximum animals health as diseased animals could have interfered with clinical signs as well as

histopathological examinations results. Prior commencing the experiment pilot test was conducted using laboratory bred animals then followed by wild animals (*Mastomys natalensis*) aimed at refining the experiment.

3.5.3 Treatment of experimental animals

Prior to carrying out the experiment, sex and weight of each animal were determined and recorded. The animals were divided in to 6 groups with 6 animals each with male and females mixed at random, using a modified method of Cruz *et al.* (2006). All the animals were starved overnight prior to starting the experimental feeding. Each animal in each group was caged separately. Seven gram portions of *N. mitis*-broiler mash mixture containing concentrations of 0, 5, 12.5, 25, 50 and 75% w/w *N. mitis* were daily fed to the rats in groups I, II, III, IV.V and VI respectively for fifteen days. The Seven (7) grams were taken as a standard feeding ration for an adult rat per day (Cait, 2008). The rats in group I received 7g of broiler mash only while each of those in groups II, III, IV, V and VI received a 7g *N. mitis*-broiler mixture with specific amounts of *N. mitis* and broiler mash as shown in Table 1.

3.5.4 Estimation of the amount of *N. mitis* + broiler mash mixture consumed by the experimental rats per day

The leftovers from the 7g mixture given to each group were weighed and recorded daily. The actual weight of each constituent i.e. broiler mash and *N. mitis* powder consumed per day was obtained by taking the total mixture provided and subtracting the left over to obtain an average amount of *N. mitis* consumed per day (g/kg b. w). The average amount of *N. mitis* bait mixture consumed up to death (g/kg b. w) was

obtained by multiplying the amount of *N. mitis* consumed per day by the total number of days the rat survived after the treatments.

3.5.5 Number of rats which survived during the experimental period

The number of rats used in this particular experiment was 36. The total number of rats which survived up to the end of experiment was 19 of which 12 animals were from groups II and III, 3 animals were from group IV and 1 animal was from group VI. Three (3) rats were sacrificed as control to those that died during the experimental period.

3.5.6 Calculation of the lethal dose of *N. mitis*

The lethal dose of *N. mitis* that is amount of *N. mitis* which killed 50% of the experimental rats within 15 days under specific laboratory conditions of this study that is 27°C and 50-80 relative humidity was determined by the graphical method of Miller and Tainter (1937). The observed percentage of rats mortality was converted into probit by referring to the probit table (Appendix 13). The values thus obtained were plotted against log dose. The LD_{50} value and its standard error were determined from the graph (Table 12). Transformation of percentages to probits was done based on the table of probits (Appendix 13). The dose corresponding to 50% or probit 5 was taken as LD_{50}.

3.5.7 Clinical examination

All the experimental animals were kept under clinical examination for fifteen (15) days, during this period the animals body weights, feeding behaviors and body appearances were recorded.

3.5.8 Statistical analysis

The results on mortality rates and changes in body weights caused by different concentrations of *N. mitis* were subjected to the analysis of Variance (ANOVA) using a split-plot arrangement in a complete Randomized Design (CRD) in which the *N. mitis* concentrations were the main plot and time (days) were the subplots with six (6) replications. MSTAT C program was used in the analysis of data at 5% levels of significance. Duncan Multiple Range Test was used to detect mean differences between treatments.

3.5.9 Post mortem examination

Each rat which died in the course of the experimental period was dissected and examined for gross pathological abnormalities in comparison to the rat in group I (control), which was sacrificed, dissected and examined in a similar way. All the rats which remained alive up to day 15 of the experiment were sacrificed, dissected and examined for pathological abnormalities.

3.5.10 Histopathological examination

Tissue samples from the liver, kidneys and lungs in each rat that was dissected were collected and fixed in 10% neutral buffered formalin. The fixed samples were subjected to tissue processing, embedded in paraffin wax and sectioned to obtain 5μm thick sections. The sections were stained with haemotoxylin and eosin (H&E) and examined under the light microscope.

CHAPTER FOUR

4.0 RESULTS

4.1 Efficacy of *N. mitis* Formulations (Powder and Water Extract) against *P. truncatus*

4.1.1 Efficacy of powder formulation

The results showed that there was no significant difference in the effect of concentration of *N. mitis* powder on mortality of *P. truncatus* ($P<0.05$) (Table 5). The results obtained from this study showed that all *N. mitis* formulation had some effect on *P. truncatus*.

Table 5: Effect of *N. mitis* powder on maize grains damage, weight loss and mortality of *P.truncatus* for 14 days of storage

N.mitis concentration(g/kg)	Number of dead insects	Number of damaged grains	Grains weight loss(g)
0	2.66a	8.46a	1.11a
20	2.93a	6.1b	1.08a
40	3.20a	5.40b	0.99ab
100	3.26a	5.06bc	0.93ab
140	3.73a	4.53bcd	0.87ab
200	4.46a	2.73cd	0.60ab
0.05Actelic	8.06b	2.26d	0.52b
S.E	**0.55**	**0.79**	**0.16**
LSD	**1.61**	**2.31**	**0.49**
CV (%)	**51.27**	**10.95**	**42.89**

Means in the same column with the same letter(s) are not significantly different following separation by Duncan's Multiple Range Test ($P<0.05$)

The treatment of maize with *N. mitis* powder affected the number of dead insects, number of damaged seeds, and weight loss as opposed to the untreated maize grains

(Table 5). There was a significant difference in the number of damaged grains and weight loss (P<0.05) at different concentrations (Table 5). The study revealed that a high concentration of *N. mitis* resulted in high mortality of *P. truncatus* but it was not statistically different (Table 5). Actelic Super Dust at a concentration of 0.05g in 1kg of maize grains outperformed *N. mitis* in terms of effectiveness as it significantly (P<0.05) reduced number of live insects, number of damaged grains, and grains weight loss (Table 5). The lowest number (2.26) of damaged grains was recorded in maize grains treated with Actelic Super Dust (Table 5)

Table 6: Effect of storage time (days) on average number of dead insects under different *N. mitis* treatments

N. mitis (g/kg of maize grains)	Day 2	Day 7	Day 14
0	1.2 a	1.8c	5.0d
20	1.6 a	1.8c	5.4d
40	1.2 a	2.4c	6.0cd
100	1.6 a	2.2c	7.4bc
140	1.6 a	2.6c	5.6d
200	1.2 a	4.4b	7.8b
0.05 Actelic	2.0 a	9.4b	12.8 abc
LSD	1.518	1.518	1.518
S.E	0.535	0.535	0.535

Means in the same column with the same letter(s) are not significantly different following separation by Duncan's Multiple Range Test (P<0.05).

It was also observed that, the interaction of concentration and the storage time (days) on mortality of *P. truncatus* statistically (P<0.05) is significantly different (Table 6).The study indicated further that the number of dead insects and the amount of grain damaged were proportional to the days of storage and were statistically different (Tables 6). The maximum concentration used in this study induced mean mortality of 4.4 of *T. truncatus* (Appendix 16).

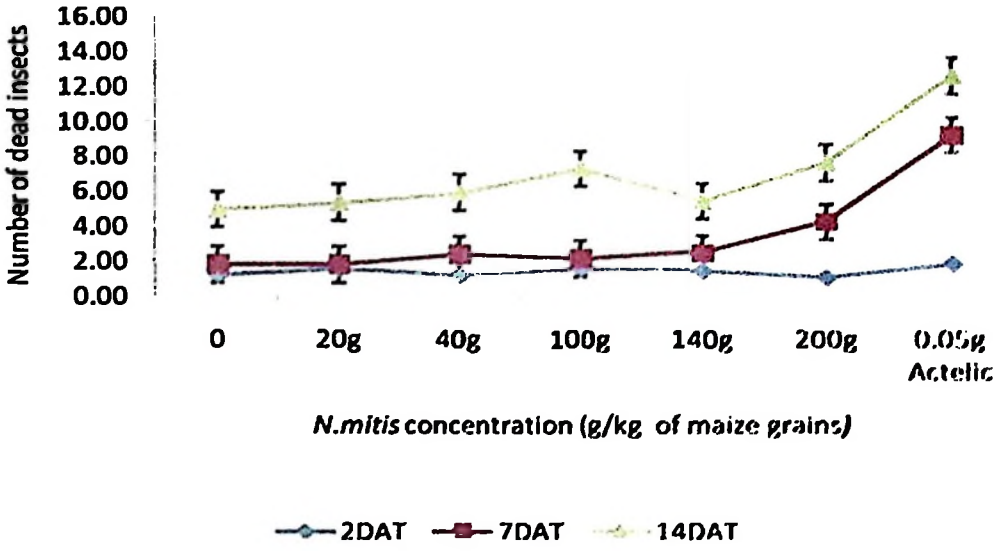

Figure 9: Mortality of *P. truncatus* in maize grains after treatment with varying concentration of *N. mitis* powder and Actelic Super Dust for 14 days

The study showed that *N. mitis* powder is slow in acting. High mortality was observed in day 14 followed by day 7 and ultimately day 2. In day 2, the number of insects which died was relatively low even at high concentration dosages (Figure 8). The results showed further that the number of damaged grains differ significantly at different concentrations of *N. mitis* powder (Table 7).

Table 7: Effect of storage time (days) on average number of grains damaged caused by *P. truncatus* under different *N. mitis* treatments

N. mitis (g/kg of maize grains)	Day 2	Day 7	Day 14
0	6.6a	8.6a	10.2a
20	4.6b	6.6b	7.2b
40	3.d	5.4d	6.0c
100	4.2c	5.8c	6.2c
140	3.2e	5.0e	5.4d
200	1.4f	3.4f	3.4e
0.05 Actelic	1.6f	2.6 g	2.6f
LSD	0.395	0.395	0.395
S.E	0.139	0.139	0.139

Means in the same column with the same letter(s) are not significantly different following separation by Duncan's Multiple Range Test ($P<0.05$)

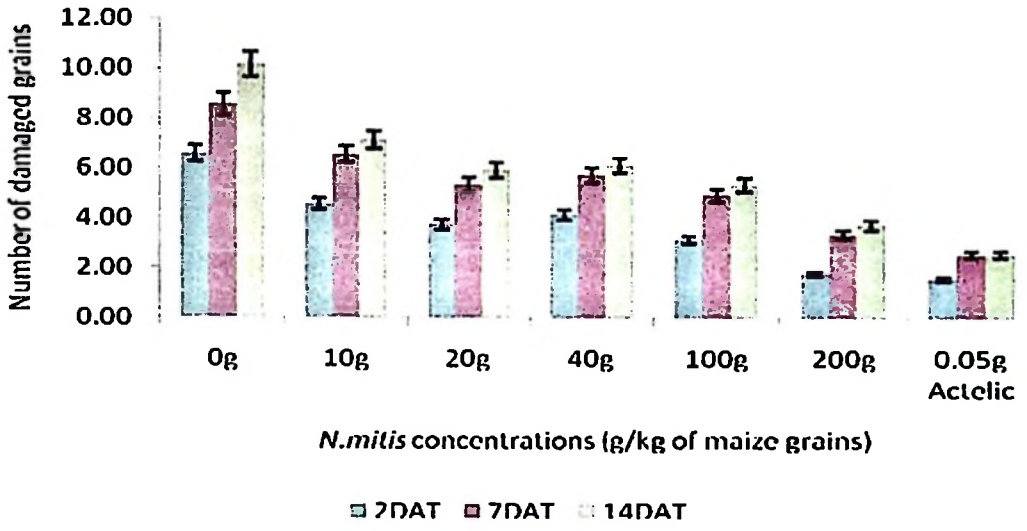

Figure 10: Maize grain damage after treatment with varying concentrations of *N. mitis* powder formulation for 14 days of storage

The concentration of 0g/kg, 20g/kg and 40g/kg recorded higher numbers of damaged grains (8.06, 6.13 and 5.40) day 2,7 and 14 as compared to concentrations of 100,140 and 200g/kg with (5.06, 4.53 and 2.73) number of damaged grains respectively (Figure 10).

4.1.2 Water extract formulation

The finding showed that insects mortality did not differ significantly at different *N. mitis* water extract concentrations (Table 8). However, the number of damaged grains differed significantly (Table 8). The results indicate that the number of maize grains damaged was high (23.22) in untreated and in the samples treated with minimum *N. mitis* concentration (22.33g/kg) (Table 8).

Table 8: Effect of *N. mitis* on maize grains damaged, weight loss and number of dead insects for 14 days of storage.

N.mitis concentration(g/kg of maize grains)	Number of dead insects	Number of damaged grains	Weight loss (g)
0	0.44b	23.22a	1.45a
20	0.33b	22.33a	0.73b
40	0.33b	20.22ab	0.60b
100	0.66b	19.78ab	0.54b
140	0.88b	18.89ab	0.46b
200	0.88b	15.0 b	0.59b
0.05Actelic	9.33a	7.00 c	0.53b
LSD	**4.398**	**4.908**	**0.6303**
S.E	**1.427**	**1.593**	**0.2025**
CV (%)	**29.56%**	**4.96%**	**47.39%**

Means in the same column with the same letter(s) are not significantly different following separation by Duncan's Multiple Range Test ($P<0.05$)

The number of grains damage was also lower and significantly different ($P<0.05$) for the treated grains as compared to the untreated ones (Tables 8). The effects of different concentrations of *N. mitis* water extracts on mortality of *P. truncatus* are presented in Figure 10. The highest number of damaged grains was recorded in untreated samples with an average value of 23.22. The samples which were treated with highest concentration (200g/kg) of *N .mitis* water extract gave value of 15.0 damaged grains.

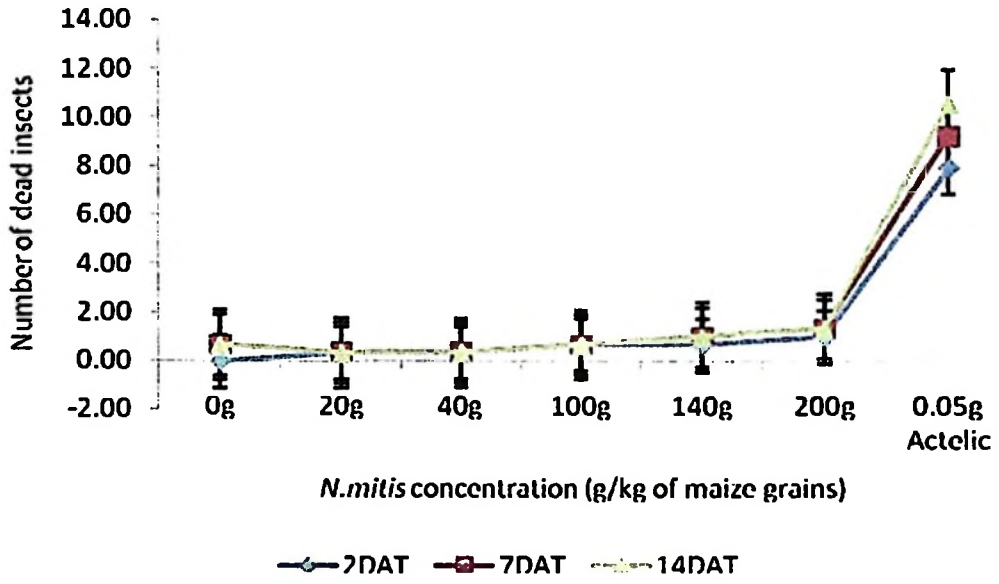

Figure 11: Cumulative mean mortality of *P. truncatus* in maize grains treated with water extract doses of *N. mitis* for 14 days of storage

The study showed that the mortality of *P. truncatus* was low and concentration dependant. Actelic Super Dust seemed to outperformed *N .mitis* in number of insects mortality (Figure 11).

4.1.3 Comparison of performance of *N. mitis* powder and water formulation against *P. truncatus*

The results show that maize grains treated with *N. mitis* powder formulation resulted to higher mortality of adults *P. truncatus* (4.4) followed by water extract formulation with (1.33) under the concentration of 200g (Fig12). Water extract formulation gave the lowest number of insects mortality at 14 days after treatment (Figure 12).

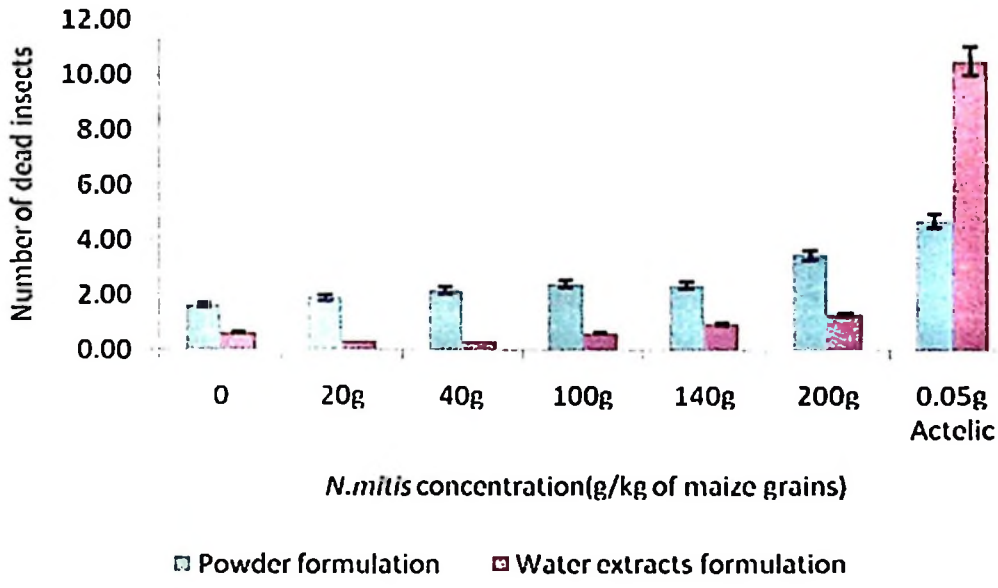

Figure 12 Average mortality of *P. truncatus* in maize grains treated with powder and water extract of *N. mitis* formulation for 14 days of storage

Actelic Super Dust at a concentration of 0.05g/kg of maize grains performed better by having a higher number of dead *P. truncatus* than either powder or water extracts formulations (Figure 12).

4.2 Mode of action of *N. mitis* against *P. truncatus*

4.2.1 Repellency

The results and statistical analysis of the repellency rate of tested plant extracts at different hours after treatment (HAT) are presented in Table 9. The repellency effect of *N. mitis* powder against *P. truncatus* showed significant difference ($p<0.05$) at different concentrations (Table 9).

Table 9: Effect of *N. mitis* concentration and time on repellency of *P. truncatus* after three (3) days of treatments

N. mitis conc. (g/kg of maize grain)	Effect of concentration (g/kg)	Effect of time (Hours)
20	30.62 c	45.40 a
100	39.25 bc	38.59 a
200	35.85 bc	38.75 a
300	47.83 ab	47.49.a
400	56.28 a	39.60 a
LSD	15.12	13.06
S.E	4.63	4.56
CV %	42.15	42.15

Means in the same column with the same letter(s) are not significantly different following separation by Duncan's Multiple Range Test ($P<0.05$).

The highest mean repellency rate was recorded at a concentration of 400g, with a percentage repellency value of 56.28 and the lowest at a concentration of 20g of *N. mitis* with a percentage repellency of 30.62 (Table 9). The study showed no statistical significant difference ($P<0.05$) in the percentage repellant with respect to time (Table 9).

Table 10: Repellency effect (%) of *N. mitis* concentrations against *P. truncatus* at different hours after treatments (HAT)

		N.mitis concentration (g/kg of maize grains)				
DAYS	TIME	20g	100g	200g	300g	400g
DAY 1	1HAT	11	55	40	50	60
	2HAT	50	25	20	33	40
	3HAT	11	25	50	55	80
	4HAT	11	25	50	55	80
	5HAT	50	25	20	55	80
	Mean	**26.6**	**31**	**36**	**49.6**	**68**
DAY 2	1HAT	20	33	50	60	77
	2HAT	33	43	40	40	55
	3HAT	33	33	25	20	20
	4HAT	33	43	40	40	55
	5HAT	33	33	25	20	20
	Mean	**30.4**	**37**	**36**	**36**	**45.5**
DAY 3	1HAT	50	75	25	50	25
	2HAT	11	33	40	60	55.5
	3HAT	33	25	50	50	71
	4HAT	25	55	42	71	85.5
	5HAT	55	60	20	80	40
	Mean	**34.8**	**49.6**	**35.4**	**62.2**	**55.4**

On the basis of mean repellency rate, it was found that the concentration of 20g, 100g and 200g of *N. mitis* in 1kg of maize were in the same repellency class i.e. II while the concentration of 300g and 400g were in repellency class III. The findings revealed that the rate of repellency increased with increase of dose level (Table 10).

4.2.2 Contact mode of action

The mortality of adults *P. truncatus* exposed to the grains treated with *N. mitis* and control grains did not differ significantly. The results indicate that *N. mitis* powder has less contact effect against *P. truncatus.*

Table 11: Contact effect of *N. mitis* powder against *P. truncatus* for Seven days of storage

Days	Number of insects used	Mean number of treated insects died	Mean number of untreated insects died
1	50	10	8
2	50	0	1
3	50	1	4
4	50	0	0
5	50	0	0
6	50	0	0
7	50	0	0

The number of treated insects died day 1 was 10 while for untreated ones was 8. In day 4, 5, 6 and 7 no mortality of insects recorded in both treated and untreated insects (Fig 12). The number of dead untreated insects recorded day 2 and day 3 was higher than the one recorded in treated insects (Table 12). The number of treated insects died within seven days of storage was 11 while for untreated insects was13 (Table 11).

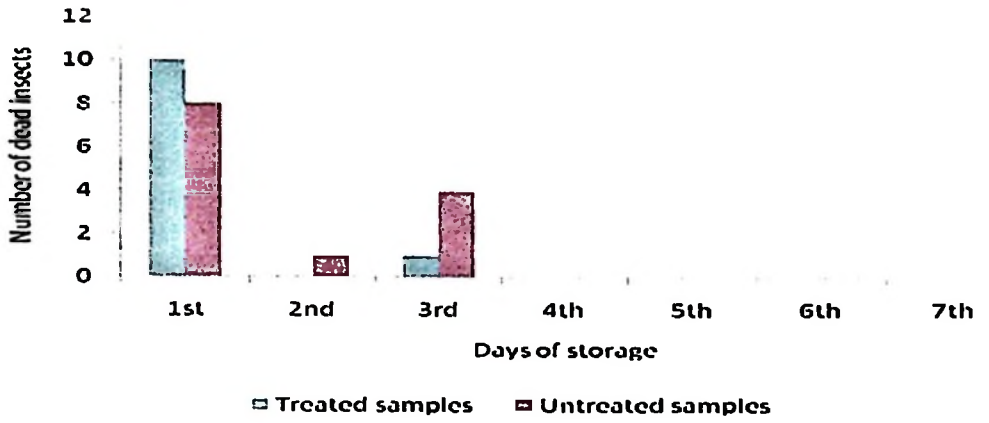

Figure 13: Contact effect of *N. mitis* powder against *P. truncatus* for Seven days of storage

4.2.3 Antioviposition or development inhibition

The results show that there was a significant difference ($p<0.05$) on F_1 of *P. truncatus* which emerged from grains under different treatments of *N. mitis* (Table 12). High adults emergence was recorded in the maize grains treated with low concentration of *N. mitis*. The significant effect was observed in adults development period (total development period) between the treated and untreated samples (Table 12).

Table 12: Effect *N. mitis* powder on oviposition of *P. truncatus* insect pest

N.mitis concentration (g/kg of maize grains)	Number of F_1 adults emerged	Reproduction inhibition rate (%)	Total development period (days)
0	16.00 a	24.33 b	32.00 b
20	12.33 ab	40.17 ab	39.67 ab
40	10.00 abc	49.37 ab	43.33 a
100	8.00 ab c	48.90 ab	43.33 a
140	7.33 bc	73.93 a	43.33 a
200	5.66 bc	80.57 a	43.00 a
0.05Actelic	3.33 c	81.43 a	43.33 a
LSD	7.80	29.54	3.18
S.E	2.53	9.58	1.58
CV (%)	47.73	29.15	6.54

Means in the same column with the same letter(s) are not significantly different following separation by Duncan's Multiple Range Test ($P<0.05$)

The Actelic Super dust used as control outperformed *N. mitis* in the reduction of adults emergence (3.33) and inhibition rate as compared with (16.00) recorded from untreated grains (Table 12). Reproductive inhibition rate was low in untreated samples as compared to the treated ones (Table 12).

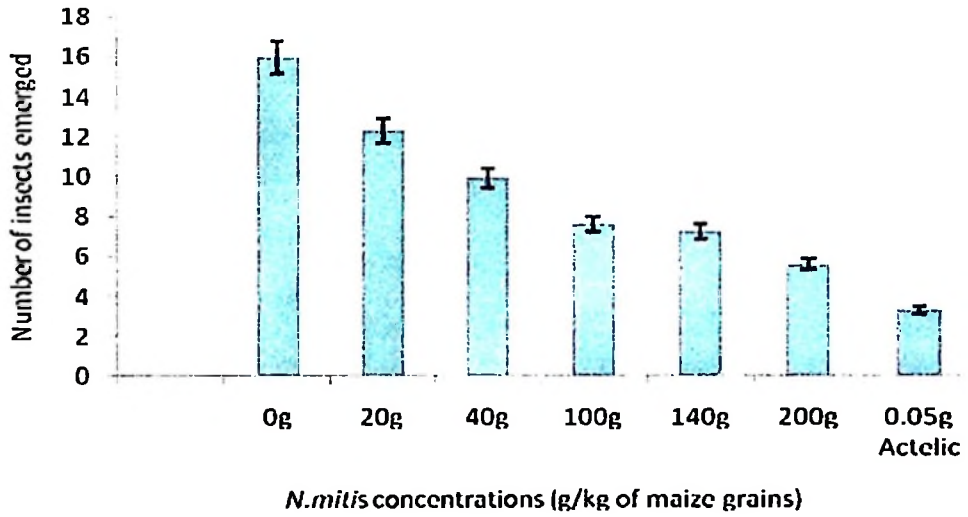

Figure 14: Effect of *N. mitis* powder on adults emergence of *P. truncatus* for 45 days of storage

4.3 Toxic Effect of *N. mitis* in Mammals

4.3.1 Amount of *N. mitis*-broiler mash mixture consumed by the experimental rats

The amount of *N. mitis* broiler mash mixture consumed by rats in each group is shown in Table 13. Results in this table show that the experimental rats started dying when the average amount of *N. mitis* consumed by each rat per day reached 0.77g. At this dose 50% out of six rats died on day 10 of the experiment. The table indicates further that, rats in group number IV and VI which were fed 25% and 75% *N. mitis* respectively in the broiler mash died earlier as compared to those in rest groups (Table 13).

Table 13: Amount of *N. mitis* broiler mash mixture consumed by rats in each group

Group Number	*N. mitis* (%)	Initial weight of *N. mitis* and Broiler mash provided (g)	Weight of leftover of *N.mitis* and Broiler mash mixture (g)	Mean weight of total *N. mitis* and Broiler mash consumed (g)	Mean amount of *N. mitis* consumed per day (g/kg)	Mean days of survival	Mean amount of *N. mitis* consumed up to death (g/kg)
I	0	105	28.50	76.50	0.00	15	0.00
II	5	105	40.88	64.12	0.21	15	3.15
III	12.5	105	48.05	56.95	0.47	15	7.05
IV	25	86.33	48.18	38.15	0.77	10	7.70
V	50	57.17	38.77	18.4	1.00	12	12.00
VI	75	68.17	49.98	18.19	1.3	10	13.00

4.3.2 Lethal doses of *N. mitis*

Results presented in Table 14 shows that the experimental rats started dying when the average amount of *N. mitis* consumed by each rat per day reached 0.77g or 770mg. At this dosage, three rats out of six died. The log dose of *N. mitis* in this study has been found to be 2.88 (Table 14). Estimation of LD_{50} gave a value of 0.77g/kg or 770mg/kg in rats (Table 14).

Table 14: LD_{50} determination by graphical method of Miller and Tainter

Group No	*N. mitis* Conc. mg/kg	Log conc.	No of dead rats	Percent No of dead rats	Corrected mortality (%)	Probit
I	0	0.00	0.00	0.00	0.00	0.00
II	210	2.32	0.00	0.00	0.00	0.00
III	470	2.67	0.00	0.00	0.00	0.00
IV	770	2.88	3.00	50.00	50.00	5.00
V	1000	3.00	6.00	100.00	100.00	8.09
VI	1300	3.11	5.00	90.00	90.00	6.28

4.3.3 Clinical examinations

All rats in group I (controls) had good body conditions with smooth hairs and were active in feeding on broiler mash supplied to them. The average body weight of rats in this group increased by 7.09g between day 0 and 15 of the experiment (Table 15).Rats in group II that were receiving 5% *N. mitis* in their diet appeared to have good body conditions during the first 7days of the experiment, but there after they developed rough skin and erected hairs. Rats in group III receiving 12.5% *N. mitis* developed signs of poor body conditions earlier than the rats in group II. These rats were emaciated with rough skin, erected hair and bending of their backbones. Rats in group IV that were receiving 25% *N. mitis* had similar but more pronounced clinical signs than the rats in group III. The average body weights of rats in this group decreased by 8.24g between days zero and 15 days of the experiment (Table 15). Three rats of this group died at day 10 of the experiment. Rats in group V that were receiving 50% *N. mitis*, showed a more pronounced rough skin and emaciation than that rats in group IV. Five rats of this group died at day 12 of the experiment. Rats in group VI that were receiving 75%*N. mitis* had more or less clinical signs exhibited by rats in group V. However, they presented a more marked state of emaciation as evidenced by the higher decrease in their average body weights of 16.65g between day 1 and 15 of the experiment (Table 15).

Table 15: Average body weights recorded in rats which were fed varying concentrations of *N. mitis* at different time

Group number	*N. mitis* concentrations (%)	Initial body weight (before treatments) (g)	Body weight after 7 days (g)	Final body weight (g)	Change of body weight (g)
I	0	32.68	35.52	39.77	7.09
II	5	30.23	31.70	34.05	3.82
III	12.5	27.67	28.07	28.48	0.81
IV	25	31.87	28.80	23.63	-3.07
V	50	31.32	27.38	21.50	-9.82
VI	75	36.68	31.37	20.03	-16.65

The decrease in rats body weights was more pronounced only at high dose levels. The comparison of treatment groups II and III with I also exhibited a significant decrease in mean body weights (Table 16).

4.3.4 Mortality rate

The analysis of variance for the effect of *N. mitis* powder in the mortality of rats showed significant different ($p<0.05$) between treatments (Table 16). However, further computation with DMRT showed that rats mortality in group I (untreated) was significant different from that of rats in groups IV, V and VI ($p<0.05$) (Table 16).

4.3.5 Change of rats body weight

Treatment caused a significant reduction in body weights of rats in group IV, V and VI ($p < 0.05$) as compared to controls (Table 16)

Table 16: Effect of *N. mitis* powder on mortality and body weight of rats

Portions of *N.mitis* in the mixture	Number of rats died	Rats body weight (g)
0	1.00 c	35.86 a
5%	1.00 c	32.13 ab
12.5%	1.00 c	27.67 b
25%	1.50 b	28.41 b
50%	2.00 a	26.88 b
75%	2.00 a	27.98 b
LSD	0.246	5.395
S.E	0.91	1.85
CV (%)	15.78	25.08

Means in the same column with the same letter(s) are not significantly different following separation by Duncan's Multiple Range Test ($P<0.05$)

4.4 Postmortem Examinations

Marked postmortem changes (gross pathological changes) were seen in the lungs, liver and kidneys of rats that died after receiving *N. mitis* doses higher than 0.47g/kg body weight. The lungs tissues consisted of red, gray and yellow colours giving it an appearance similar to a marked pattern. The liver and kidneys were redder than in the controls.

4.5 Histopathological Observations

Histological sections of lungs, liver and kidney from the experimental animals presented discussed below.

4.5.1 Lung sections

Sections of lungs tissue from rats in group I (control) were normal having empty discrete alveolar duct, alveolar sacs and alveoli lined by simple columnar epithelium. However, lung tissues from rats in group II, III and IV showed

accumulation of blood (congestion) in the pulmonary vessels as well as in the interlobular capillaries. The appearance of free red and white blood cells in the tissue, which indicated hemorrhage and inflammation were also observed in the rats in groups V and VI.

4.5.2 Liver sections

The sections from rats in group I (Control) showed intact liver cells containing eosinophilic cytoplasm and around nuclei with distinct dark blue clumps of chromatin separated by clear spaces. The death of cells (necrosis) increased with the increase in the concentration of *N. mitis* in the diet such that liver sections from rats fed with 50% and 75% revealed replacement of the dead cells with fibrous tissue(fibrosis) especially around the portal triads.

4.5.3 Kidney section

Kidney sections of rats that were fed with *N. mitis* revealed changes such as loss of epithelial cells lining the renal tubules, congestion of intertubular vessels, and hemorrhages in the renal corpuscles. The severity of these changes increased with the increase in the concentration of *N. mitis*

CHAPTER FIVE

5.0 DISCUSSION

5.1 Powder and Water Extract Formulations

5.1.1 Comparison of the effectiveness of *N. mitis* powder and water extract in Controlling *P. truncatus*

The study showed that both powder and water extracts have some effect on storage pests. However, the powder formulation was more effective in causing mortality and reducing damage grains than the water extract. It is possible that the powder reduces eggs adherence on the treated grains hence weakens the oviposition by *P. truncatus.* Low insects mortality and higher number of damaged grain in the water extract as opposed to the powder formulation was possibly due to the fact that water is not a good solvent for extraction of active parts of the *N. mitis.* The outcomes of this study are similar to the findings from the other researchers who have reported that *N. mitis* plant composes insecticidal properties that can be used in controlling storage pests (Sheto and Mkoga, 1995; Mulungu *et al.*, 2007). It is further elaborated in their reports that maize grains treated with *N. mitis* powder performed better than the untreated maize grains by having less number of damaged grains and live insects.

The finding recorded low mortality of *P. truncatus* in both formulations as opposed to the synthetic insecticide (Actelic Super Dust) used as a control. Due to low insects mortality recorded in both formulations it was hardly to establish lethal dose (LD_{50}) of *N. mitis* against *P. truncatus* using probit analysis method, because the maximum dosage of *N. mitis* used in this study caused only 22.5% insect mortality. In other hand no sigmoid curve observed in relationship between the concentrations of *N. mitis* against insects mortality.

However, no further response of insects observed even when *N .mitis* concentration doubled. Probably, *N. mitis* is effective against *P. truncatus* by affecting other biological activities and not direct stomach toxicity. This finding was in agreement with earlier reports that most plant extracts/powder which are thought to have insecticidal properties do not have direct toxicity but can control pests through affecting other biological activities (Schmutterer, 1995; Mostafa *et al.*, 1996; Musabyimana *et al.*, 2001).

The effects of *N. mitis* against storage pests *Sitophilus oryzae* and *P. truncatus* in wheat and maize were reported by Chimbe and Galley (1999). Also Fivawo, (2008) reports that *N. mitis* powder formulation was effective against beans bruchids *Zabrotes subfasciatus* and *Acanthoscelides obtectus(L)* as opposed to other botanical insecticides.

5.2 Mode of action of *N. mitis* against *P. truncatus*

5.2.1 Repellency effect

The repellency effect of *N. mitis* powder against *P. truncatus* showed a significant different at different concentration levels. There were positive and significant correlations among the investigated variables between *N. mitis* concentration with the number of insects that repelled. The use of *N. mitis* against insects is probably interfering with host orientation and selection hence prevents the grain from being attacked by insects. The bioactivity of *N. mitis* against *P. truncatus* may also depend on chemical composition, insects' susceptibility and variation in insect behaviour. The significant mortalities and oviposition deterrencies could be attributed to the

presence of highly pungent phenolic secondary metabolites or other compounds such as alkaloids, flavonoids, etc. These possibly repel the insects and influence their locomotion, oviposition, feeding behaviour, developmental and physiological processes, as well as general behavioural pattern.

The findings indicated that a concentration in the range of 20-100g *N. mitis* powder in 100kg of maize grains can induce repellency of 30.62 up to 39.14 which can prevent maize grains against storage pests. In related experiments, Binggeli (1999) reported that ground plants of *L. camara* when mixed with the produce or placed in between the produce as protective layers (Sandwich method) can protect grain legumes against bruchids and potato tuber moth *Phthorimaea operculella* (L) for about 6 months by acting as repellant. Similarly Philip *et al,* (2009) indicated that many plants have repellent properties against certain insects. Roy *et al.* (2005) demonstrated the effectiveness of a botanical leaf extract of *Blumea lacera* against the lesser grain borer, obtaining the highest repellency percentage of 57.41 at 3% extract concentration. The doses of 20g, 100g, 200g, 300g and 400g (w/w) *N. mitis* used in this particular study nevertheless, are too high from the economic point of view, and not used much in practical application. The doses were chosen as the highest limit dosage, especially in order to observe possible effect of the material.

5.2.2 Contact mode of action

The finding indicated that no contact mode of action of *N. mitis* against *P. truncatus.* Generally, no significant effect was observed when the two paired means of treated and untreated samples were tested. The number of insects mortality recorded both in

treated and untreated insects were relatively the same. High number of insects mortality was recorded day 1 after setting up an experiment, probably this was caused by the shock imposed to the insects due to the change of living medium. However, the number of mortality of untreated insects was relatively higher than the treated ones. The results of the present investigation are in accordance with those of other researchers who previously reported that plant products have poor contact toxicity so they must be ingested by pests to be effective (Gahukar, 1998).

5.2.3 Anti-oviposition or development inhibition

The finding showed that insects development and emergence were adversely affected by *N. mitis* treatments. It is assumed that the presence of powder particles of *N. mitis* some how alters the ambient surface characteristics of maize grains rendering it unsuitable as breeding habitat for *P .truncatus* or due to the presence of chemical which alters the behavior and physiology of the insects affecting adversely the egg laying and F_1 emergence.

A similar study conducted by Tapondjou *et al.* (2002) showed that the dry ground leaf of *Chenopodium ambrosioides* inhibited F_1 progeny production and adult emergence of the *C. chinensis* and *C. macul.* Likewise the reproduction inhibition rate was significantly different at different concentrations levels. From this study it was observed that in all the parameters, the higher the concentration of *N. mitis* showed the lower the infestation of *P. truncatus*. This means that the least number of F_1 adults emerged for which the seed damage rate were less. The reduction in adult emergence could either be due to egg mortality or larval mortality or even reduction in the hatching of the eggs. The mechanism or process involved in this reaction is

not known. It has been reported that the larvae which hatch from the eggs of *P. truncatus* species must penetrate the seeds to survive (FAO, 1999). The larvae are unable to do so unless the eggs are firmly attached to the seed surface. The *N. mitis* powders might thus have inhibited the male *P. truncatus* from making tunnel and the female laying eggs in the maize grains due to its repellency properties. It was reported that the repellent and antifeedant effects are often connected with pest reduction or oviposition deterrence effect (Deka *et al.*, 1998; Pavela and Herda 2007). The finding of the present investigation is in accordance with those of other researchers who previously reported plant powders reduce oviposition of bruchids, which include neem kernel powder (Sowunmi and Akinusi, 1983).

5.3 Mammalian Toxicity of *N. mitis*

5.3.1 Amount of *N. mitis* which caused death of the rats

In this study, administering of *N. mitis* to rats produced dose dependent multiple organ toxicities including the kidneys, the liver and the lungs. The lethal dose obtained from this study is of immense importance, in view of the large-scale human consumption of this plant and should be a matter of concern. However, LD_{50} is not a very important criterion in the determination of toxicity of the material as there is wide variations in formation on what system failure lead to death. LD_{50} in conjunction with clinical signs and the Histopathological observations give a good picture of the toxicity characteristics of the plant. Wild animals (*Mastomys natalesis)* had been used in this study because were strong enough to withstand

environmental shocks imposed by *N .mitis* toxin during the experiment as compared with laboratory bred animals.

5.3.2 Clinical signs shown by the experimental rats

The main clinical signs revealed by the rats following consumption of different amount of *N. mitis* were rough hair coat, erected hairs, arched backbones, difficult breathing (dyspnoea), reluctance in feeding (anorexia) and loss in body weights (emaciation). According to this study, a change in the quality of the hair coat was the earliest clinical sign that appeared seven days after the experimental rats were exposed to daily *N. mitis* dose of 0.21g/kg body weight. Rough hair coat and erected hairs are non specific clinical signs that occurred in different conditions associated with disturbances of metabolism due to the infectious and non infectious causes. One of the non infectious causes is panthothenic deficiency reported in albino rats (Oshima *et al*, 1966). In the study by Oshima *et al*., (1966) the researchers observed that abino rats receiving diets deficient in panthothenic acid failed to grow and presented rough hairs. It is evident from the results of the present study that the toxic principle of *N. mitis* induced metabolic disturbance that resulted in to similar signs caused by panthothenic deficiency

After the first *N. mitis* treatment, there was a slight decrease in the weight of animals relative to the dosage administered. However, in the control group, the animals gained weight with the passage of time. This decrease in weight in treated groups could be due to stress induced by *N. mitis* consumed. Lakkawar *et al.* (2004) have also noticed that in addition to typical clinical signs of Cypermethrin in female

rabbit, there was a decrease in body weight in the treated groups due to stress as opposed to control group.

The intensity of the change in the quality of the hair coat increased with the increase in the dose of *N. mitis* and was also associated with other clinical signs including: arching of backbones, difficulty breathing and loss in body weights. Arching of backbone started when the dose of *N. mitis* reached 0.47g/kg body weight. This was more likely a reflection of the toxic effect of *N. mitis*, which caused pain of the abdominal organs including the stomach and kidney. Difficulty in breathing and loss in body weights were clinical signs, which were markedly seen at *N. mitis* higher doses in which all the animals died. These signs pointed out to serious damage of the lungs and liver that resulted into respiratory arrest and hepatic failure.

5.3.3 Gross and Histopathological changes of selected organs caused by *N. mitis*

Marked postmortem changes (gross pathological changes) were seen in the lungs, liver and kidney of rats that died after receiving *N. mitis* doses higher than 0.47g/kg body weight. In these organs, a change in colour was prominent with the normal pale brown of lungs and pink red of liver and kidneys in the controls being replaced by a mixture of red and brown in the lungs and dark red in the liver and kidney of the rats which consumed *N. mitis*. The above colour changes indicated circulatory disturbances in the organs. A variety of endogenous metabolic and toxic conditions can cause injury to a tissue leading to vascular response. Evidence of tissue injury and vascular response in the lungs of rats that consumed *N. mitis* was

histopathologically revealed in this study through observation of the distortation of the lining epithelium of alveoli, free erthrocytes were phagocytosed and their haemoglobin iron deposited in the macrophages as hemosiderin.

Histopathological demonstration of lungs tissue injury is in agreement with the clinical findings that rats, which consumed higher amount of *N. mitis* suffered an acute respiratory distress syndrome (ARDS), which undoubtly was the primary cause of death of rats in this study. Likewise the study has also demonstrated histopathological injury in the liver and in the kidneys of experimental rats comprising of mainly necroses. The clinical manifestation of loss in body weight of the rats was undoubtedly a reflection of hepatic deficiency due to death of number of a hepatocytes evidenced by increased eosinophilia of the cytoplasm, disappearance or fragmentation of nuclei as well as replacement of lost hepatocytes by fibrous tissue.

Although the toxic principal of *N. mitis* was not determined in this study, Philip *et al.*, (2009) reported that *N. mitis* plants have retenoids and retonone in high concentrations with cellular respiratory enzyme inhibitor. Furthermore, retonone is also found in botanical insecticides and is associated with stomach poisoning in animals (Jembere, 2002). Based in vitro studies, the mechanism of toxicity of retonone has been shown to be based on mitochondria cellular oxidation impairment (Sherer *et al.*, 2003; Tretter *et al.*, 2004; Testa *et al.*, 2005).

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

This study has confirmed the potential of *N. mitis* in the control of storage insects. This work has provided indicative results on the effectiveness of the materials, safety to human (mammals) beings, mode of action and issues for further research. Both powders and extracts tested were effective to some degree in reducing the oviposition / eggs laying by *P. truncatus* hence increased the number of undamaged grains treated with *N. mitis*.

The powder formulation of *N. mitis* was found to be more effective in control of *P. truncatus* as opposed to liquid formulation. The concentration in the range of 20-100g *N. mitis* in 1kg of maize can be used in grains to prevent storage pests through repellency. The finding also shows that it is desirable to apply the material before infestation by insect pests due to their nature of slow killing. Basing on this study, it was observed that the reduction of emergence and repellency against *P. truncatus* are the most probable modes of action of the material. However, there was no significant effect observed in the effect of *N. mitis* against *P. truncatus* through contact mode of action. Finally the study revealed the presence of toxic in *N. mitis* against the experimental rats, the observed signs pointed out to serious damage of the lungs and liver that resulted in to respiratory arrest and hepatic failure. Therefore the use of *N .mitis* in treatment of foodstuffs should be a matter of concern.

6.2 Recommendations

The cause for a considerable protection of maize grains against the attack by *P. truncatus* by the powders of *N. mitis* in the current investigation could be due to the presence of different chemicals which interfere with the feeding habit of the pest or the reduction in adult emergence due to egg mortality or larval mortality or even reduction in the hatching of the eggs. Identification of the chemicals responsible should be an immediate research agenda.

This study has demonstrated the tissue toxicity of *N. mitis* in rats. This has been observed in rats which were fed varying concentrations of *N. mitis* and broiler mash for 15 days. However, the observed signs pointed out to serious damage of the lungs and liver that resulted in to respiratory arrest and hepatic failure therefore care should be exercised during the preparation and handling of the food staffs treated with *N. mitis*.

Further studies are required to isolate the specific component(s) of the plant responsible for the toxicity in order to come up with a suitable formulations as well as dose standardization due to regional, growth stage and seasonal variations of *N. mitis* aimed at improving the plant preparation for maximum post harvest storage purposes.

Study to improve the effectiveness of botanical derivatives as insecticides will benefit agricultural sectors, particularly in developing countries as the materials are not only of low cost, are abundant and can be multiplied and made available to resources limited farmers.

REFERENCES

Adda, C. C., Borgemeister, W. G., Meikle, R. H., Markham, I., Olaleye, K. S. and Zakari, M. O. (1996). First record of the larger grain borer, *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae), in the Republic of Niger. *Bulletin of Entomological Research* 86: 83-85.

Ahmed, S. and Grainge, M. (1986). Potential of the Neem tree (*Azadirachta indica)* for pest control and rural development. *Economic Botany* 40(2): 201-209.

Akob, C.A. and Ewete, F.K. (2007). The efficacy of ashes of four locally used plant materials against *Sitophilus zeamais* (Coleoptera: curculionidae) in Cameroon. *International Journal of Tropical Insect Science* 27: 21-26.

Ashimogo, G. (1995). Peasant grain storage and marketing in Tanzania: A case study of maize in Sumbawanga District. Ph.D. Thesis, Berlin University, German. 369 pp.

Banwo, O. and Adamu, R. S. (2003). Insect pest management in African agriculture: challenges in the current millennium. *Archives of Phytophathology Plant Protection* 36: 59- 68.

Bell, R.J. and Watters, F.L. (1982). Environmental factors influencing the development and rate of increase of *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae) on stored maize. *Journal of Stored Products Research* 18: 131-142.

Belmain, S. R., Neal, G.E., Ray, D.E. and Golob, P. (2001). Insecticidal and vertebrate toxicity associated with ethnobotanicals used as post-harvest protectants in Ghana. *Food Chemicals Toxicology.* 39: 287–91

Berger, A. (1994). Using natural pesticide: Current and future perceptive, Swedish University of Science, 56pp

Binggeli, P. (1999). *Lantana camara* L. (Verbenaceae). [http://www. members.lycos.co.uk/WoodyPlantEcology/docs/web-sp6.htm]Site visited on 25/10/2009.

Boeke, S.J., Barnaud, J.A., van Loon, D.K., Kossou, A. and Dicke, M. (2004). Efficacy of plant extracts against the cowpea beetle, *Callosobruchus maculatus*. *Introductory Journal for Pest Management* 50: 251-258.

Borgemeister, C., Meikle, W.G., Scholz, D., Adda, C., Degbey, P. and Markham, R.H. (1997). Seasonal and meteorological factors influencing the annual flight cycle of *Prostephanus truncatus* (Coleoptera; Bostrichidae) and its predator, *Teretriosoma nigrescens* (Coleoptera: Histeridae), in Benin.*Bulletin of Entomological Research* 87: 239–246.

Borgemeister, C., Adda, C., Setamou, M., Hell, K., Djomamou, B., Markham, R.H., and Cardwell, K.F. (1998). Timing of harvest in maize: effects on post harvest losses due to insects and fungi in central Benin, with particular reference to *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae). *Agriculture Ecosystem Environment*. 69: 233–242.

Borgemeister, C., Tchabi, A. and Scholz, D. (1998). Trees or store? The origin of migrating *Prostephanus truncates* collected in different ecological habitats in southern Benin. *Entomologia Experimentalis et Applicata* 87: 285–294.

Borgemeister, C., Holst, N. and Hodges, R.J. (2003). Biological control and other pest management options for larger grain borer *Prostephanus truncatus.* CABI Publishing, Oxford UK, pp. 311-328.

Buss, E.A and Park – Brown, S.G. (2002). Natural Products for Insect Pest Management. Chapman Hall, London [http://www.jsdafrica.com/Jsda/Spring2006PDF/ARC_Efficacy%20of%20the%20botanical%20pesticide.pdf] Site visited 20/10/2010.

Cait, M. K. (2008). How much do mice eat and drink? [http//www. fancy mice. info/feeding 1. Htm] Site visited 8/10/2010

Childs, F.J., Chamberlain, J.R., Antwi, E.A., Daniel J. and Harris, P.J. (2001). Improvement of neem and its potential benefits to poor farmers. Department of International Development.U.K., 32 pp.

Chimbe, C. M. and Galley, D.J. (1999). Evaluation of materials from plants of medical importance in Malawi as protectants of stored grain against insects *Crop Protection* 15: 289-294.

Chitra, K.C., Rao, S.J., Rao, K.P.and Nagaiah, K. (1993). Field evaluation of certain plant products in the control of pest. *Indian Journal of Entomology* 55(3): 237-240.

Cotton, R.T., and Wilbur, D.A. (1982). Insects in storage of cereal grains on developmental stages of stored products insects. *Environmental Entomology* 6: 181-184.

Cruz, R. C., Meurer, C.D., Silva, E.J., Schaefer, C., Santos, A.R, Bella-Cruz, A. and Filho, V. (2006). Toxicity evaluation of *Cucurbita maxima* seed extract in mice. *Pharmacy Biology* 44 (4): 301-303.

Deka, M. K., Singly, K., Handique, R. (1998). Antifeedant and repellent effect of pongam (*Pongamia pinnata*) and wild sage (*Lantana camarata*) on tea mosquito bug (*Helopeltis theivora*). *Indian Journal of Agricultural Sciences* 68: 274–276.

Demianyk, C. J., Sinha, R.N. (1988). Effect of infestation by the Larger grain borer, *Prostephanus truncatus* (Horn), and the Lesser grain borer, *Rhyzopertha dominica* (F.) (Coleoptera:Bostrichidae), on stored grain. *Environmental Entomology*, 16: 618-624.

Espinal, R., Markham, R.H. and Wright, V.F. (1996). *Honduras – summary of research activities on the larger grain borer and storage pest status in meso-America*. pp109–124

Facknath, S. (2006). Combination of neem and physical disturbance for the control of four insect pests of stored products. *International Journal of Tropical Insect Science* 26(1): 16-27.

FAO, (1983). Prevention of post harvest food losses. Training series No. 10 (122) Food and Agricultural Organization of the United Nations. Rome, 120 pp.

FAO, (1999). Botanical oils as grain protectants, In: The use of spices and medicinals as bioactive protectants for grains. FAO Agricultural Services Bulletin No. 137. FAO, Rome, Italy.

Fivawo, B. C. (2008). The efficacy of some Botanical Insecticides against common bean bruchids (*Zabrotes subfasciatus* and *Acanthoscalide obtectus*). Dissertation for Award of MSc degree at Sokoine University of Agriculture, Morogoro, Tanzania, 87 pp.

Franzen, H. (1993). Need for development of new strategies for locust control. In: New strategies for locust control. (Rembold, H. ed.) ATSAF. Bonn. 89: 9 - 13 .

Gaskins, M. H., Martin, F.W., and White, G.A. (1972). *Tephrosia vogelii* use source of rotenoids for insecticidal and pesticidal use. *U.S. Department of Agriculture Technology* 38 pp.

Gahukar, R. T. (1998). Commercial and industrial aspects of neem-based pesticides. *Pestology* 22(10):5-41.

Giles, P.H. and Leon, O. (1975). Infestation problems in farm-stored maize in Nicaragua. *Proceedings of the 1st International Working Conference on Stored Products* Georgia, USA, pp 68-76.

Golob, P. and Webley, D. J. (1980).The use of plants and minerals as Traditional Protectants of stored products .[https://tspace.library.utoronto.ca/retrieve/2794/jb03086.pdf] Site visited on 22/09/2009.

Golob, P. and Kilminister, A. (1982). The biology and control of *Zabrotes subfascsiatus* (Boh). (Coleoptera: Bruchidae) infesting red kidney beans. *Stored Produces Res*erve 18: 95-101.

Golob, P. and Hodges, R.J. (1982). A study of an outbreak of *Prostephanus truncatus* (Horn) in Tanzania. *Tropical Products Institute Report.* 23 pp

Golob, P., Moss, C., Dales, M., Fidgen, A., Evans, J. and Gudrups, I. (1999). The use of spices and medicinals as bioactive protectants for grains. [http://redalyc.uaemex.mx/redalyc/pdf/437/43713059007.pdf] Site visited on 18/01/2009.

Henkes, C. (1992). Investigation in to insect Dynamics, Damage and losses of stored maize. *An approach to IPM On small farms in Tanzania with special references to P. truncutus* (Horn) (Colleoptera Bostrichidae). Homburg Germany. 124pp.

Hodges, R.J., Dunstan, W.R, Magazini., I, Golob. P. (1983). An outbreak of *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae) in East Africa. *Protection Ecology* 5(2):183-19.

Hodges, R.J. (1994). Recent advances in the biology and control of *Prostephanus truncatus* (Coleoptera:Bostrichidae). [http://ddr.nal.usda.gov/bitstream/10113/36706/1/IND44300035.pdf] Site visited on 20/11/2009.

Holst, N. and Meikle, W.G. (2003). *Teretrius nigrescens* against larger grain borer *Prostephanus truncatus* in African maize stores: biological control at work? *Journal of Applied Ecology* 40 (2): 307-319.

Isman, M. B. (2000). Plant essential oils for pest and disease management. *Crop Protection.* 19: 603-608.

Isman, M. B. (2007). Botanical insecticides: for richer, for poorer. *Pest Management Science.* 64 (1): pp 8-11.

Jackobson, M. (1982). Plants, insects, and man: their relationship. *Economic Botany* 36 (3): 346-354.

Jacobson, M. (1989). Botanical pesticides, past present and future. In: Insecticides of plant origin. Proceeding of the American Chemical Society, Washington, D.C. pp 1-10.

Jembere, B. (2002). Evaluation of the Toxicity Potential of *Milletia ferrginea* (Hochst) Baker Against *Sitophillus zeamais* (Motsch.) *International Journal of Pest Management* 48 (1): 29-32.

Joseph, C. C., Ndoile, M.M., Malima, R.C. and Nkunya, H.H. (2003). Larvicidal and mosquitocidal extracts, a coumarin, isoflavonoids and pterocarpans from *Neorautanenia mitis*. *Journal of Ethnopharmacology* 72(1-2): 207-217.

Kabungo, D., Mkoga, A. and Shetto, M.A. (1998). On farm evaluation of Botanical insecticide for the control of storage insects pests on maize, Wheat and Beans, Report. pp 4-6.

Kabungo, D.A. (2004). Botanical insecticide as a control of crop insect's pest in the Southern highlands of Tanzania. Zonal plant health services. *Training work shop paper on IPM. Workshop*, held at the Youth Centre, Mbeya, pp1-4.

Key, G. E. and Mungereza, R.A. (1988). *An information book for all people concerned with Dumuzi* (*Prostephanus trancatus*) (Horn) *control.* T. M. P. Printers, Dar es salaam. 27pp.

Koona, P. and Njoya, J. (2004). Effectiveness of soyabean oil and powder from leaves of *Lantana camera* Linn. (verbenaceae) as protectants of stored maize against infestation by *Sitophilus zeamais* Motsch. (Coleoptera: Curculionidae). *Pakistan journal of Biosciences* 7(12): 2125-2129.

Lakkawar, A. W., Chattopadhyay, S. K. and Somvanshi, R. (2004). Experimental cypermethrin toxicity in rabbits. A clinical and patho-anatomical study. Folia Veterinaria, 48(1): 3-8.

Makundi, R. H., Uronu, B.E. and Mbise, W. (2006). *Biology, Ecology and management of infestation of the Larger Grain Borers* (*P.truncatus).* In Makundi R.H. (Ed); Management of selected Crop Pests in Tanzania. Tanzania Publishing House Limited Dar es Salaam. pp 205-219.

Makundi, R.H., Misangu, R.N, Reuben, S.O., Kilonzo, B.S., Mwatawala, M.W; Sikira, A, Lymo, H, and Maumba, M. (2007). Reduction of crop losses through improved storage for food security at village level in Tanzania.In: Proceedings of the first annual PANTIL Research 25-27th September 2006. Morogoro. pp174-179.

Maribet, L. and Aurea, C.R. (2008). Insecticidal action of five plants against maize weevil. *Sitophilus zeamais* Motsch. (Coleoptera: Curculionidae). *Journal of Science and Technology* 8 (1):24-38.

Markham, R.H., Wright, V.F., Rios, R.M., (1991). A selective review of research on *Prostephanus truncatus* (Col.: Bostrichidae) with an annotated and updated bibliography. *Ceiba* 32 (1):1–90.

McDonald, L. L., Guy, R. H and Speirs, R. D. (1970). Preliminary evaluation of new candidate materials as toxicants, repellents and attractants against stored-product insects. Agricultural Research Service, US Department of Agriculture, Washington DC, Marketing Research Report No. 882.

Meikle, W.G., Adda, C., Azoma, K., Borgemeister, C., Degbey, P., Djomamou, B., Markham, R.H., (1998). The effects of maize variety on the density of *Prostephanus truncates* (Coleoptera: Bostrichidae) and *Sitophilus zeamais* (Coleoptera: Curculionidae) in post harvest stores in Benin Republic. *Journal of Stored Produces Res*erve 34 (1): 45–58.

Mkoga, Z. J., Shetto, R. M., Kabungo, D.A. and Ndegeulaya, D. (1999). An inventory of insecticidal plants for field crops and grain storage protection in the Southern Highlands of Tanzania. [http://www.researchintouse.com/nrk/RIUinfo/PF/CPP01.htm] Site visited on 8/10/2009.

Miller, L. C., and Tainter, W. I. (1937). Estimation LD_{50} or ED_{50} values and their error using Log. Probit graph paper. 57: 264.

Mohan, S. and Fields, P.G. (2002). A simple technique to assess compounds that are repellent or attractive to stored insects. *Journal of Stored Products Research* 38: 23-31.

Mostafa, T.Y., Mahboub, S.M., Ahmed, M.S. (1996). The efficiency of certain plant powders against cowpea weevil *Callosobruchus maculates* (F.) (Coleoptera: Bruchidae). *Egypt Journal of Agriculture Reserve 74*: 307-319.

Mulungu, R. L., Elise, N.L., Shazia, O. W., Reuben, S.O. and Robert, N. M, (2007). Effectiveness of Local Botanicals and Protectants of Stored Beans (*Phaseolus vulgaris* L.) Against Bean Bruchids (*Zabrotes subfasciatus* Boh). *Journal of Entomology* 4 (3):210-217.

Musabyimana, T., Saxena, R.C., Kairu, E.W., Ogol, C.P and Khan, Z.R. (2001). Effects of neem seed derivatives on behavioral and physiological responses of the *Cosmopolites sordidus* (Coleoptera: Curculionidae). *Horticulture Entomology* 94: 449-454.

Nang'ayo, F.L., Hill, M.G., Chandi, E.A., Chiro, C.T., Nzeve, D.N. and Obiero, J.W. (1993). The natural environment as a reservoir for the larger grain borer, *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae) in Kenya. *African Crop Science Journal* 1: 39–47.

Nang'ayo, F.L.O. (1996). Ecological studies on the larger grain borer in savanna woodlands of Kenya. PhD. Thesis, University of London, 120pp.

Nchimbi-Msolla, S. and Misangu. R.N.(2002). Seasonal distribution of common beans (*Phaseolus vulgaris* L.) bruchid species in selected areas in Tanzania Beans/Cowpea collaborative Research Support Program-East Africa.
[http://sustainableseedsystems.wsu.edu/proceedings/Nchimba.pdf]
Site visited 13/03/2010.

Novo, R.J., Viglianco, A. and Nassetta, M. (1997). Actividad repelente de diferentes extractos vegetales sobre *Tribolium castaneum* Herbst. *Agriscientia* 14:31-36.

Nsemwa, L.T. and Lyimo, N. (2005). Improving farmers and other stakeholders Access to Quality. Information and products for pre and post-harvest maize systems management in Southern Highlands of Tanzania. [http://www.fao.org/docs/eims/upload/agrotech/R8406_FTR.pdf] Site visited on 18/01/2009.

Ofuya, T. I and Lale, N. E. (2001). *Pests of stored cereals and Pulse in Nigeria.* Biology, Ecology and control. Dave Collins Publication Nigeria. 147 pp.

Ogendo, J.O., Deng, A.L, Belmain, S.R., Walker, D.J., Musandu, A.O and Obura, R.K. (2004). Pest status of *Sitophilus zeamais* Motschulsky, control methods and constraints to safe maize grain storage in Western Kenya. *Egerton Journal Science Technology* 5(1): 175- 193.

Ogendo, J.O., Omolo, E.O., Deng, A.L, Matasyoh., J.C, Tabu, I.M. (2006). Field grains losses and insect pest management practices in subsistence agriculture. *Farmers perceptions Journal* 8 (1): 24- 42.

Onwueme, I.M., and Sinha, T.D. (1999). *Field crop production in Tropical Africa.* Technical [http://ejeafche.uvigo.es/index.php?option=com_docman&task=doc_view&gid=314Centre for Agricultural and Rural cooperation. 480pp] Site visited 03/09/2009

Oparaeke, A.M. and Kuhiep, G.C. (2006). Toxicity of powders from indigenous plants against *Sitophilus zeamais* Motsch on stored maize grains. *Journal of Entomology* 3(3): 216-221.

Oshima, Y., Morita, H., Kanno, Y., Tachizawa, H. (1966). Biological studies on pantethine (III): Teratogenic effects of pantethine in the experimental animals. Vitamins 34(1): 32-36.

Othira, J.O., Onek, L.A., Deng, L.A. and Omolo, E.O. (2009). Insecticidal potency of *Hyptis spicigera* preparations against *Sitophilus zeamais* (I) and *Tribolium castaneum* (Herbst) on stored maize and grains. *African Journal of Agriculture Research* 4(3):187-192.

Pavela, R., Herda, G. (2007). Repellent effects of pongam oil on settlement and oviposition of the common greenhouse whitefly *Trialeurodes vaporariorum* on chrysanthemum. *Insect Science* 14: 219–224.

Pavela, R. (2009). Effectiveness of some botanical insecticides against *Spodoptera littoralis* Boisduvala (Lepidoptera: Noctudiae), *Myzus persicae* Sulzer (Hemiptera: Aphididae) and *Tetranychus urticae* Koch (Acari: Tetranychidae). *Plant Protection Science*, 45: 161-167.

Peddy, M.U. and Peddy. G.S. (1987). Effectiveness of selected plants material protectants against insects infestation and nutrients composition during storage of food commodities. *Bulletin of Grain Technology* 25:48-57.

Pingali, P.L. (2001). Impact assessment research in the CGIAR, 1970-1999. With an annotated bibliography of impact assessment conducted in the CGIAR, 1970-1999, Prepared by M.P. Feldman. [http://www.docstoc.com/docs/85131349/Project-Proposals-Farmer-Organisations] Site visited 12/07/2010.

Philip, C .S. (2009). Southern African Pesticidal Plants (SAPP) Project. Optimizing the use of pesticidal plant [http://www.nri.org/projects/sapp/docs/sapp_ftr.pdf] Site visited on 09/07/2010.

Prakash, A. and Rao, J. (1997). Botanical pesticides in agriculture. CRC Press Inc.461pp [http://www.bioassay.org.br/articles/3.4/BA3.4.pdf] Site visited on 10/08/2009.

Purseglove, J.W., (1990). Tropical *Crops*. Monocotyledons. Long mans Crop Ltd. London

Rahman, A. and Talukder, F. A. (2006). Bioefficacy of some plant derivatives that protect grain against the pulse beetle, *Callosobruchus maculatus*. *Journal of Insect Science* 25: 60: 103.

Raja, N., Albert, S., Ignacimuthu, S., Dorn, S. (2001). Effect of plant volatile oils in protecting stored cowpea (*vigna inguiculata*) L. (Walpers) against *Callosobruchus maculatus* (F.) (Coleoptera:Bruchidae) infestation. *Journal of Stored products* 37: 127-132.

Ramirez-Martinez, M., De Alba, A. A. and Ramirez, Z.R. (1994). Discovery of the larger grain borer in tropical deciduous forests in Mexico. *Journal of Applied Entomology* 118: 354–360.

Rembold, H. (1994). Advances in invertebrate reproduction. *Elsevier Science Publishers* 3: 481-491.

Roy, B., Amin, R., Uddin, M.N., Islam A.T., Islam M.J. and Halder, B.C. (2005). Leaf extracts of Shiyalmutra (Blumea lacera Dc.) as botanical pesticides against Lesser Grain Borer and Rice Weevil. *Journal of Biological Sciences* 5 (2): 201 – 204.

Roux, P. W. (1999). Larger grain borer: further developments. *Plant Protection News* 55: 3-4.

Rugumamu ,C. P. (2005). Influence of simultaneous infestations of *Prostephanus truncatus* and *Sitophylus zeamais* on the reproductive performance and maize damage. *Tanzania journal of science*, 33:123-143

Rwamugira, W. (1996). Development and application of Soil moisture model for analyzing crop production conditions in Tanzania. Ph. D. Thesis Submitted at Agricultural University of Norway, Norgas land Brukshog skde Norway, 130 pp.

Saxena, R.C. (1987). Antifeedants in tropical pest management. *Insect Science Applied* 8: 731-736.

Schmutterer, H. (1990). Properties and potential of natural pesticides from the neem tree, Azadirachta indica. *Annual Review Entomology* 35: 271 – 277.

Schmutterrer, H. (1995). The neem tree *Azadirachta indica* A. Juss. and other Meliaceous plants. VCH Publishers, Weinheim, Germany; pp. 696.

Sherer, T.B., Kim, J.H., Betarbet, R., Greenamyre, J.T. (2003). Subcutaneous rotenone exposure causes highly selective dopaminergic degeneration and alpha-synuclein aggregation. Exp Neurol 179: 9-16.

Shetto, R. M. and Mkoga, Z. J. (1995). Post harvest grain systems project (Tanzania). Centre File: 3-p-88-0305. Report submitted to IDRC, Nairobi, pp. 41.

Shires, S.W. (1977). Ability of *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae) to damage and breed on several stored food commodities. *Journal of Stored Products Research* 13(4): 205-208.

Shires, S.W. (1980). Influence of temperature and humidity on survival, development period and adult sex ratio in *Prostephanus truncatus* (Horn) (Coleoptera, Bostrichidae). *Journal of Stored Product Research* 15: 15-10.

Sighamony, S., Anees, I., Chandrakala, T.S. and Kaiser, J. (1990). Indigenous plant products as grain protectants against *Sitophilus Oryzae* (L) and *Rhizopertha Dominica* (F) *Journal of Stored Products Research* 22: 21-23.

Stevenson, C.P. (2007). Southern African Pesticidal Plants (SAPP) Project. *Caesalpinioid woodlands of Southern Africa: optimizing the use of pesticidal plants.* Number – 9 , 1-11pp.

Sowunmi, O. E., Akinusi, O. A. (1983). Studies on use of neem kernel in the control of stored cowpea beetle *C. maculatus. Tropical Grain Legume Bulletin* 27, 28-31

Sumani, A.J. and Ngolwe, A.R. (1996). The status of the larger grain borer in Zambia (1996). In: Proceedings of the East and Central African Storage Pest Management Workshop (Edited by Greathead, G.*et al.*), 14-19 April 1996 Naivasha, Kenya, 177-182pp.

Talukder, B.K. and Laker, B.P. (2006). Conservation Status and Prospects of Manas Biosphere Reserve. V. (In press)

Talukder, F.A. and Howse, P.E. (1994). Laboratory evaluation of toxic repellent properties of the pithraj tree, *Aphanamixis polystachya* Wall and Parker, against *Sitophilus oryzae* (L.). *Introductory Journal for Pest Management* 40: 274-279.

Talukder, F. A. and Howse, P. E. (1995). Evaluation of *Aphanamixis polystachya* as a source of repellant, antifeedants, toxicants and protectants in storage against *Tribolium castaneum* (Herbst). *Journal of Stored Products Reserve* 31(1): 55-61.

Tapondjou, L.A., Adler, C., Bouda, H., Fontem, D.A. (2002). Efficacy of powder and essential oil from *Chenopodium ambrosioides* leaves as post-harvest grain protectants against six-stored product beetles. *Journal of Stored Products Research* 38(4):395–402.

Tavares, M.A. and Vendramim, J.D. (2005). Bioatividade da erva-de-santa maria, *Chenopodium ambrosioides* L. sobre *Sitophilus zeamais* Mots (Coleoptera: Curculionidae). *Neotropical Entomology* 34: 319-323.

Temu, A., Nyange, D. Mbiha, E. R., Mdoe, N.S. and Duma, T. (1995). Analysis of baseline farming systems data for the Southern Highlands Regions of Tanzania Part I. *General reports* (Districts and Regions) *Final report*, 95pp.

Testa, C.M., Sherer, T.B., Greenamyre, J.T. (2005). Rotenone induces oxidative stress and dopaminergic neuron damage in organotypic substantia nigra cultures. 134: 109-118.

Tretter, L., Sipos, I., Adam-Vizi, V. (2004). Initiation of neuronal damage by complex I deficiency and oxidative stress in Parkinson's disease. *Neurochemicals Research* 29: 569-577.

Van Puyvedele, L., De Kimpe, N., Mudaheranwa, J., Gasiga, A., Schamp, N., Declercq, J. and Van Meersche, M. (1987). Isolation and structural elucidation of potentially insecticidal and acaricidal isoflavone-type compounds from *Neorautanenia mitis. Journal of Natural Products* 50(3): 349-356.

Verma, J., Dubey, N. K. (1999). Prospectives of botanical and microbial products as pesticides of tomorrow. *Current science* 76(2): 172-179.

Vongtau, H.O., Amos, S., Binda, I., Kapu, S.D., Gamaniel, K.S., Kunle, O.F. and Wabembe, C. (2000). Pharmacological effects of aqueous extract of *Neorautanenia mitis* in rodents. *Transactions of the Royal Society of Tropical Medicine and Hygiene 8*: 451-455.

Ware, G.W. (1982). Pesticides*: Theory and application.* Thompson publications, Fresno, California, 308pp.

Wekesa, P.W. (1994). Field and store ecology of the larger grain borer, *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae) in Kenya. PhD. Thesis, University of Leicester, UK. [http://ddr.nal.usda.gov/bitstream/IND23330679.pdf] Site visited on 04/07/2008.

Zettler, J. L. and Cuperus, G.W. (1990). Pesticide resistance in *Tribolium castaneum* (Coleoptera:Tenebrionidae) and *Rhyzopertha dominica* (Coleoptera: Bostrichidae) in wheat. *Journal of Economic Entomology* 83:1677-168

APPENDICES

Appendix 1: ANOVA for mortality of *P. truncatus* in maize grains after treatment with varying concentration of *N. mitis* powder and Actelic Super Dust for 14 days of storage

	Source	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob
1	Replication	4	4.381	1.095	0.2372	
2	Conc. (A)	6	313.562	52.260	11.3180	0.0000
-3	Error	24	110.819	4.617		
4	Days (B)	2	574.990	287.495	66.7485	0.0000
6	AB	12	133.810	11.151	2.5889	0.0083
-7	Error	56	241.200	4.307		
	Total	104	1378.762			

Appendix 2: ANOVA for damaged maize grains after treatment with varying concentration of *N. mitis* powder and Actelic Super Dust for 14 days of storage

Source		Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob
1	Replication	4	20.895	5.224	0.5530	
2	Conc. (A)	6	394.057	65.676	6.9528	0.0002
-3	Error	24	226.705	9.446		
4	Days (B)	2	95.314	47.657	162.7317	0.0000
6	AB	12	10.286	0.857	2.9268	0.0033
-7	Error	56	16.400	0.293		
	Total	104	763.657			

Appendix 3: ANOVA for mortality of *P. truncatus* after treatment with varying concentration of *N. mitis* liquid extract and Actelic Super Dust for 14 days of storage

Source		Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob
1	Replication	2	32.032	16.016	0.8737	
2	Conc. (A)	6	592.413	98.735	5.3863	0.0065
-3	Error	12	219.968	18.331		
4	Days (B)	2	3.556	1.778	6.0000	0.0070
6	AB	12	8.444	0.704	2.3750	0.0303
-7	Error	27	8.000	0.296		
Total		62	864.413			

Appendix 4: ANOVA for damaged maize grains after treatment with varying concentration of *N. mitis* liquid extract and Actelic Super Dust for 14 days of storage

Source	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob
1 Replication	2	49.556	24.778	1.0852	0.3688
2 Conc. (A)	6	1664.190	277.365	12.1474	0.0002
-3 Error	12	274.000	22.833		
4 Days (B)	2	26.889	13.444	16.7723	0.0000
6 AB	12	8.667	0.722	0.9010	
-7 Error	28	22.444	0.802		
Total	62	2045.746			

Appendix 5: ANOVA for repellency effect of *N. mitis* at different doses on *P. truncatus* using treated maize grains at different hours after treatment (HAT)

Source	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob
1 Replication	2	361.952	180.976	0.5616	
2 Conc. (A)	4	6190.395	1547.599	4.8026	0.0286
-3 Error	8	2577.924	322.240		
4 Hours (B)	4	1045.243	261.311	0.8350	
6 AB	16	3847.536	240.471	0.7684	
-7 Error	40	12517.437	312.936		
Total	74	26540.487			

Appendix 6: ANOVA for body weights recorded in rats which were fed varying concentrations of *N. mitis* for 15 days

Source	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob
1 Replication	5	226.505	45.301	0.7335	
2 Conc. (A)	5	1087.025	217.405	3.5200	0.0152
-3 Error	25	1544.074	61.763		
4 Days (B)	2	203.834	101.917	1.8220	0.1707
6 AB	10	842.203	84.220	1.5057	0.1603
-7 Error	59	3300.203	55.936		
Total	106	7203.84			

Appendix 7: ANOVA for total development period of *P. truncatus* treated with vary concentration of *N. mitis* for 45 days of storage

Source	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob
1 Replication	2	59.429	29.714	3.9661	0.0476
2 Conc. (A)	6	115.238	19.206	2.5636	0.0779
3 Error	12	89.905	7.492		
Total	20	264.571			

Appendix 8: ANOVA for the number of F1 adults of *P. truncatus* emerged after treated with vary concentration of *N. mitis* for 45 days of storage

Source	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob
1 Replication	2	47.524	23.762	1.3012	0.3080
2 Conc. (A)	6	324.286	54.048	2.9596	0.0519
3 Error	12	219.143	18.262		
Total	20	590.952			

Appendix 9: ANOVA for the reproduction inhibition of *P. truncatus* after treated with *N. mitis* powder for 45 days

Source	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob
1 Replication	2	2096.340	1048.170	3.8013	0.0526
2 Conc. (A)	6	8740.351	1456.725	5.2829	0.0070
3 Error	12	3308.900	275.742		
Total	20	14145.591			

Appendix 10: Correlations between concentration of *N. mitis* and repellency of *P.truncatus*

		Number	Concentration
Number of insects	Pearson Correlation	1	.656**
	Sig. (2-tailed)		.000
	N	25	25
Concentration of *N. mitis*	Pearson Correlation	.656**	1
	Sig. (2-tailed)	.000	
	N	25	25

**. Correlation is significant at the 0.01 level (2-tailed).

Appendix 11: Correlations between concentration of *N. mitis* and the number of live *P .truncatus*

		Concentration	Response
Concentration	Pearson Correlation	1	-.468**
	Sig. (2-tailed)		.000
	N	105	105
Response	Pearson Correlation	-.468**	1
	Sig. (2-tailed)	.000	
	N	105	105

**. Correlation is significant at the 0.01 level (2-tailed).

Appendix 12: Comparison of observed and expected mortality of rats

Log conc.	Observed Probit (Y)	Expected Probit (P)	No of rats tested (n)	Number of rats affected		Discrepancy (r-np)
				Observed (r)	Expected(np)	
0.00	0.00	0.00	6.00	0.00	0.00	0.00
2.32	8.74	0.00	6.00	0.00	0.00	0.00
2.67	9.58	0.00	6.00	0.00	0.00	0.00
2.88	10.10	50.00	6.00	3.00	3.00	0.00
3.00	10.38	100.00	6.00	6.00	6.00	0.00
3.11	10.64	83.33	6.00	5.00	4.99	0.01

Appendix 13: Transformation of percentages to probit

%	0	1	2	3	4	5	6	7	8	9
0.00		2.62	2.95	3.12	3.25	3.36	3.45	3.52	3.59	3.66
10.00	3.75	3.77	3.82	3.87	3.92	3.96	4.01	4.05	4.08	4.12
20.00	4.16	4.19	4.23	4.26	4.29	4.33	4.36	4.39	4.42	4.45
30.00	4.48	4.50	4.53	4.56	4.59	4.61	4.64	4.67	4.69	4.72
40.00	4.75	4.77	4.80	4.82	4.85	4.87	4.90	4.92	4.95	4.97
50.00	5.00	5.03	5.05	5.08	5.10	5.13	5.15	5.18	5.20	5.23
60.00	5.25	5.28	5.31	5.33	5.36	5.39	5.41	5.44	5.47	5.50
70.00	5.52	5.55	5.58	5.61	5.64	5.67	5.71	5.74	5.77	5.81
80.00	5.84	5.88	5.92	5.95	5.99	6.04	6.08	6.13	6.18	6.23
90.00	6.28	6.34	6.41	6.48	6.55	6.64	6.75	6.88	7.05	7.33

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Appendix 14: Repellency classes of *N. mitis* powder at different doses on *P. truncatus* using treated maize grains at different hours after treatment (HAT)

N.mitis (g/kg of maize grains)	1HAT	2 HAT	3 HAT	4 HAT	5 HAT	Mean repellency rate (%)	Repellency class
20	27.0	31.3	25.7	23.0	46	30.62	II
100	54.0	33.7	27.6	41.1	39.3	39.14	II
200	38.3	33.3	31.6	44.2	21.6	33.80	II
300	53.3	44.3	41.6	55.4	44.3	47.78	III
400	54.0	50.1	57.0	73.5	46.6	56.24	III

Appendix 15: Effect of *N. mitis* in contact against *P. truncatus*

		Ranks	
	Treatments	N	Mean Rank
Insects mortality	Insect died in treated	11	11.36
	Insect died in untreated	13	13.64
	Total	**24**	

Appendix 16: LD_{50} determination for *N. mitis* against *P. truncatus* by graphical method of Miller and Tainter

N. mitis Con.g/kg	*N. mitis* Conc. mg/kg	Log conc.	No of dead insects	Percent No of dead insects	Corrected mortality (%)	Probit
0	0	0.00	2.6	13	0.00	3.87
20	20000	4.3	2.9	14.5	1.7	3.92
40	40000	4.6	3.2	16	3.4	4.01
100	100000	5.0	3.2	16	3.4	4.01
140	140000	5.1	3.7	18.5	6.5	4.08
200	200000	5.3	4.5	22.5	12.2	4.23