BIOLOGICAL EFFECTS OF NEEM OIL AND NEEMAZAL AGAINST THE LARGER GRAIN BORER, *PROSTEPHANUS TRUNCATUS* (HORN) (COLEOPTERA: BOSTRICHIDAE) ON STORED MAIZE

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DEDICATION

To my family.

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With a sincere heart I thank the Lord God Almighty for seeing me through this work successfully. I am very grateful to my supervisors, Dr. D. Obeng-Ofori, Dr. R. K. Akuamoah and Dr. A. R. Cudjoe for their enormous support and contribution which enabled me to finish this work as scheduled. My gratitude goes to my family for their moral support they gave me during the work. Finally, I thank all my friends who helped me in diverse ways to carry out this work successfully.

ABSTRACT

The biological activity of neem oil and neemazal against the larger grain borer, *Prostephanus truncatus* (Horn) was assessed in maize grain in the laboratory. The bioassays carried out were toxicity of the products against adults and immature stages, effect of the products on progeny production, damage assessment, repellent action and persistency of the products in grain and their effect on seed viability. Neemazal was highly toxic to *P. truncatus* than neem oil. The highest dosage of 0.8 ml killed 100 % adults of *P. truncatus* after 72 h exposure but the same dosage of neem oil evoked 70 % mortality after 96 h. The products were also active against the eggs and larvae causing a significant mortality. Neemazal provided greatest protection of maize grains with no noticeable feeding holes on grains treated with 0.6 and 0.8 ml/gm. Both products were moderately repellent to *P. truncatus* causing less than 50 % repellency. Activity of the two products significantly declined in treated grain after 24 h of storage following treatment. Neem oil and neemazal increased water absorption by maize seeds. Furthermore, seed germination was not affected by neem oil but neemazal significantly reduced seed germination. For the control of *P. truncatus* in stored maize, neemazal offers greatest protection against both the immature stages and adults. The non-toxicity of neem products to man and other beneficial organisms emphasize the potential use of these products as protectants of farm gate grain storage of small holdings in rural communities in Ghana and other parts of Africa where the neem is abundant.

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CHAPTER ONE

1.0 INTRODUCTION

Cereals are major component in the diet of Ghanaians and constitute the most important source of carbohydrate. They are usually stored to provide a food reserve as well as seed for planting. The popular cereals widely consumed in Ghana are maize, rice, millet and sorghum (Biney, 1978).

According to the Policy Planning Monitoring and Evaluation Department of the Ministry of Food and Agriculture (PPMED) report of 1997, annual production levels of maize in Ghana are 1,007,000 and 1,020,000 metric tonnes for 1996 and 1997, respectively.

As in field crops, a wide range of insect pests attack all stored products, the commonest among them being beetles and moths (Munro, 1966; Bekele *et al*., 1996). Halstead (1986) reported that over 500 beetle species have been found associated with stored products of plant and animal origin which include cereals, pulses, milled products, manufactured foods, skins, wool, textiles, etc. By their nature and sizes, some of the insect pests are able to invade storage structures through small cracks, crevices, and holes in the structures. Apart from insects, other agents responsible for grain losses in storage are mites, rodents and birds as well as physical and environmental factors (Ayertey, 1986).

In the tropics, climatic and poor storage conditions are often highly favourable to insect growth and development (Sharaby, 1988). The storage facilities of traditional farmers in the developing countries are unsuitable for effective, conventional chemical control as most storage types are open to reinfestation by arthropod pests (Jembere *et al*., 1995).

Insects feed on the grain and render them useless for human consumption by boring into the kernel, removing the food materials and sometimes selecting the highly nutritive fractions. Also their presence either as living bodies or as body fragments, excreta, frass or the webbing they produce cause contamination and quality loss. In Ghana, Prempeh (1971) estimated that out of the total annual harvest of 250 - 300,000 tonnes of maize, about 20% was lost to insects. Using FAO production figures of 1980, Ayertey (1986) estimated that losses in cereals and grain legumes that could have sustained the whole of Africa's population for 16 months was lost mainly to insects in that year alone.

Until recently, *Sitophilus zeamais* (Mots.) (Coleoptera : Curculionidae) was the most serious pest of stored maize in Ghana but the larger grain borer, *P. truncatus* (Horn) (Coleoptera : Bostrichidae) which was largely confined to the central parts of South America, where it is occasionally a pest of farm- stored cob maize, has gradually become very important pest in the country.

This pest was accidentally introduced into parts of Tanzania in the early 1980s (Mushi, 1983). It was identified in Togo in 1984 and from where it spread to Benin by 1986 and Ghana by 1989 (Haines, 1991). In Ghana, the pest status of *P. truncatus* has tremendously increased, becoming established in all parts of the country after it was discovered in the Volta Region which shares a common border with Togo, the country where the pest was first reported in West Africa (Krall, 1984). Being a typical primary pest, it attacks whole grains on the cob both before and after harvest (Anon., 1987). The adults can bore into a wide range of foodstuffs and other materials (Mushi, 1983), although development appears to be supported only by maize and cassava. According to Hodges and Meik (1984), adult *P. truncatus* initiates its attack on husk maize by boring into the base of a cob and eventually gaining access to the grain via the apex after crawling between the sheaths.

The feeding activity of *P. truncatus* is such that weight losses of up to 40% have been recorded on farm-stored maize in Nicaragua over a 6-month storage period (Giles and Leon, 1975). Hodges *et al.* (1985) observed that 70% of dried cassava roots were reduced to dust or powder after only four months of farm storage. Because *P. truncatus* does well at low moisture levels, at which most other stored product pests will not survive for long, its introduction into the hot tropics poses a grave danger to maize production.

Control of storage insect pests relies heavily on the use of synthetic insecticides but the increasing cost of application and the erratic supply in developing countries as a result of foreign exchange constraints do not allow the poor peasant farmers to patronize them (Jembere *et al*., 1995). Other problems such as the development of resistance by some pests, toxic residues in food, water, soil, etc. and killing of non-target organisms make the use of insecticides as the sole control tool unacceptable (Poswal and Akpa,1991).

To help the peasant farmers control storage insects at a lower cost, attention has been focused on the use of indigenous plants as sources of locally available and cheap products for protecting stored foodstuffs against pest attacks. One of the plants that have gained recognition for use both in storage environment and the field is the neem tree, *Azadirachta indica* A. Juss.

The use of neem as a biopesticide is now gaining popularity in many parts of the world including Ghana. According to Föster (1998), no developing country has developed its neem resources for plant protection to a significant extent except India and Pakistan.

The processing of neem into pesticide can be simple and easy even though sophisticated technologies can be used. At the village level, crude neem water extracts or neem oil can be prepared with simple equipment, which makes resource-poor farmers able to afford. Home-made neem oil preparation is employed by local farmers as a cheap and locally available source of biopesticide for grain protection but they often do not know the correct rate to apply and the persistency of the neem oil to protect the grain against insect infestation. Commercial neem products are recent innovation and have been introduced into the Ghanaian market (Förster *et al*., 2000). They include neemol and neemazal. They store well and do not lose their potency as rapidly as the home-made products. Neemazal, a commercial neem-based oil product has 3 % azadirachtin, the most potent compound in neem and many other limonoids, which are all held together by natural additives (Anon., 2000). Neemazal is popularly used by Ghanaian farmers in vegetable production while a few trials have been done on cash crops such as cocoa, but its efficacy has not been evaluated against larger grain borer in maize grain.

The objectives of this study were:

1. to evaluate the biological activity and protectant potential of neem oil and neemazal against the larger grain borer,
2. to determine persistency and effective application rate of the products,
3. to determine the effect of the chemicals on germination of seed maize, and
4. to identify the biologically active fraction of the neem oil.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Importance of cereals in the tropics

Cereal grains constitute the most important source of carbohydrate in the tropics (Ezueh, 1983). They are usually stored to provide a food reserve as well as seed for planting (Baba, 1994). The most important cultivated cereals grown in the tropics are maize, rice, millet and sorghum.

With their potential to store very well for longer periods when conducive environment is provided, cereals serve as food for both livestock and man during and after other short persistent carbohydrate crops are cultivated. Also, the importance of cereals cannot be overemphasized as human population increase coupled with food insecurity, especially in the developing countries.

In Ghana for instance, Owusu-Akyaw (1991) reported that the annual production of maize was 750,000 tonnes and it is still on the ascendancy. According to the Policy Planning Monitoring and Evaluation Department (PPMED) of the Ministry of Food and Agriculture report of 1997, annual production levels of maize for the country were 1,007,000 and 1,020,000 metric tones for 1996 and 1997, respectively.

2.2 Grain losses in storage

Grain losses in storage are mainly caused by insects, mites, rodents, birds, and pathogens especially fungi as well as physical and environmental factors (Ayertey, 1986). Losses caused by insects are probably the most widely reported among post-harvest loss estimates (Adesuyi, 1982). Insects damage grain either by boring into the grain or by feeding on the grain surface. Apart from such visible damage, the presence of insects, either as living bodies or as body fragments, excreta, frass or the webbing they produce, are indices of loss (Ayertey, 1986). Damage results in weight loss, permits increased uptake of moisture and encourages the growth of fungi and mites. The presence of foreign matter and contaminants lead to quality loss. Selective feeding by some insect pests leads to nutritional loss. Where such selective feeding is concentrated on grain germ, there may be total loss in seed viability, although weight and nutritive loss may not be total (Ayertey, 1986). Furthermore, insect activity in produce leads to temperature and moisture increase, producing caking and resulting in quality loss.

In the tropics, the problem is exacerbated by highly favourable climatic and poor storage conditions that encourage insect growth and development (Bekele *et al.*, 1996) and fungal diseases which cause considerable damage in storage and constitute an obstacle to processing (Mutlu and Hountonji, 1990). The storage facilities of traditional farmers in the developing countries are also unsuitable for effective conventional chemical control as most storage facilities are open to reinfestation by arthropod pests (Jembere *et al.*, 1995).

In most tropical countries, post-harvest losses in cereals due to attack by insect pests have been estimated at 20-30% (Dichter, 1976; Dick, 1988). The loss of food grain during storage due to various insect pests is a very serious problem. For instance, in the developed countries like the USA and Canada, 20-26% of stored wheat was infested by stored product pests (White *et al.*, 1985). According to Wolpert (1967), an FAO study estimated world-wide annual losses in store as 10% of all stored grain, and it is believed that any reduction in losses between harvest and consumption would increase the availability of food grains and security of food supply. Food grain losses in storage in India at the farm level are about 10% of the production (Lal, 1988). In Sub-Saharan Africa, Dichter (1976) reported 25-40% food grain losses during storage at farm or village level and which was attributed to inadequate post-harvest management practices and poorly designed storage structures (Anon., 1989). In Nyanza, a district of Kenya, a survey conducted revealed that approximately 20% of maize cobs were infested with weevils at the time of harvest (Nyambo, 1993). In the Sahel, Alzouma (1990) reported that among the cereals, post-harvest losses are only significant for bagged sorghum and maize because of damage caused by insects and rodents. He suggested that the solution to the losses appears to be an integrated approach, based on a proper understanding of pest biology. In Ghana, Prempeh (1971) estimated that out of the total annual harvest of 250-300,000 tonnes of maize, about 20% was lost to insects. Using FAO production figures of 1980, Ayertey (1986) estimated that cereals and grain legumes that could have sustained the whole of Africa's population for 16 months were lost mainly to insects in that year alone. In Benin, Kodjo (1990) reported that *P. truncatus* has increased post-harvest losses from 5-10% in the south and 2-5% in the north to 17% after its introduction to the southwestern part of the country. Cereal production in North Africa is insufficient and can satisfy only 50-70% of their needs. This has been attributed to significant losses that occur during post-harvest operations particularly as a result of pest infestation during storage (Bartali, 1990).

2.3 Some important storage pests

As in field crops, several insect species attack stored products, the commonest among them being beetles and moths (Munro, 1966). Halstead (1986), reported that over 500 different beetles are associated with stored products of plants and animal origin, which include cereals, wool, textiles, etc. By their nature and sizes, some of the insect pests are able to invade storage structures through small cracks, crevices, and holes in the structures.

One or several species may be found attacking the same commodity at the same time. They may occur on the commodity as primary pests and others as secondary pests. Damage by primary insect pests renders grain prone to infestation by secondary insect pests, leading to succession of different insect species in the produce. In some cases, infestation is so severe that grains are reduced to empty husks and dust (Ayertey, 1986).
Most of these insects are cosmopolitan in nature. The most important Coleopteran storage insect pests belong to the families of Bostrichidae, Curculionidae, Bruchidae, Tenebrionidae, Dermestidae and Silvanidae. The most frequently encountered storage pests of grains in the tropics are the maize weevil, *S. zeamais* Mots., rice weevil, *S. oryzae* Linn., saw-toothed grain beetle, *Oryzaephilus surinamensis* Linn., rusty red flour beetle, *Tribolium castaneum* Herb., the lesser grain borer, *Rhyzopertha dominica* Fab.

and larger grain borer, *P. truncatus* Horn (Fatope *et al*., 1995). Others are cowpea beetle, *Callosobruchus maculatus* F., cigarette beetle, *Lasioderma serricorne* F., groundnut borer, *Caryedon serratus* Oliv., hides beetle, *Dermestes maculatus* Deg., bean beetle, *Acanthoscelides obtectus* Say, and khapra beetle, *Trogoderma granarium* Everts. The major lepidopteran storage pests include the rice moth, *Corcyra cephalonica* Stain., angoumois grain moth, *Sitotroga cerealella* Oliv., Indian meal moth, *Plodia interpunctella* Hubn., tropical warehouse moth, *Ephestia cautella* Walk., mediterranean flour moth, *E. kuehniella* Zell., and tobacco warehouse moth, *E. elutella* Hlib. (Davis and Harein, 1992; Haines, 1991; Saxena, 1995; Hodges *et al*., 1983; Golob, 1988).

2.4.1 *Prostephanus truncatus* (Horn)

The adult is 3-4.5 mm long and has the typical cylindrical bostrichid shape. The declivity is flattened and steep, and on its surface there are many small tubercles. The limits of the declivity, apically and laterally, are marked by a carina (Haines, 1991). The antennae are 10-segmented and have a loose three-segmented club with its stem being slender and clothed with long hairs and the apical club segment is as wide as, or wider than, the preceeding segments. The larvae are similar to those of *R. dominica* (Fab.) but the thoracic segments are considerably larger than those of the abdomen (Haines, 1991).

2.4.2 World-wide distribution of *P. truncatus*

Compared to some major stored pests, the distribution of *P. truncatus* is very discrete and delimited (Markham *et al*., 1991). Historically, *P. truncatus* has been found only in the Americas. Although, the insect was originally described from specimen collected in California, it appears to be indigenous to Mexico and parts of Central America where it sporadically achieves a primary pest status of local importance on maize (Markham *et al*., 1991). The insect was first reported from Mexico by Chittenden in 1895 (Chittenden, 1911) and its distribution map in Mexico was made by Ramirez *et al*.(1958).

The distribution in Central America includes Panama, Honduras, El Salvador (McGuire and Crandall, 1967), Nicaragua, and Costa Rica (Fisher, 1950). In South America, *P. truncatus* was first recorded in Brazil in 1937 (Cotton and Good, 1937). Other reports by stored product entomologists cited by Wright (1984) indicated that the insect was absent in Bolivia, Chile, Ecuador, Guyana, Jamaica, Uruguay and Venezuela.

In Africa *P. truncatus* was introducted into parts of Tanzania in the early 1970s (Mushi, 1983). The borer was identified in Togo in 1984 and from where it spread to Benin by 1986, Ghana by 1989 (Dick and Rees, 1989). To date, the pest has been confirmed to be present in Tanzania, Kenya, Burundi, Rwanda, Malawi and Zambia within the Eastern and Southern Africa outbreak areas, and in West African states as Benin, Togo, Sierra Leone, Guinea, Burkina Faso, Nigeria and Ghana (Hodges, 1994).

In Ghana, the pest status of *P. trunactus* has tremendrously increased, becoming established in all parts of the country after it was discovered in the Volta Region, which shares a common border with Togo, the country where the pest was first reported in West Africa.

2.4.3 Behaviour and economic importance of *P. truncatus*

Prostephanus truncatus is a serious primary pest of stored maize and dried cassava roots (Hodges *et al*., 1983, 1985). It also attacks whole grains on the cob both before and after harvest (Anon., 1987). The adults can bore into a wide range of foodstuffs and other materials (Mushi, 1983). According to Hodges and Meik (1984), adults of *P. truncatus* initiate their attack on stored maize cobs, with intact sheath, by boring into the base of the maize cob cores, although they eventually gain access to the grain via the apex of the cob after crawling between the sheaths.

Adults bore into the maize grains, making neat holes, and as they tunnel from grain to grain, they generate large quantities of maize dust. The feeding activities of *P. truncatus* are such that losses of up to 40% have been recorded for farm-stored maize in Nicaragua over a 6-month storage period (Giles and Leon, 1974). Hodges *et al*. (1985) observed that 70% of dried cassava roots were reduced to dust or powder after only four months of farm storage in Tanzania (Table 1).

Table 1: Recorded weight losses in stored maize and cassava due to *P. truncatus*

Produce	Storage Period (months)	% weight loss	Country	Reference
Cob-maize	6	40	Nicaragua	Giles & Leon, 1974
"	3-6	30	Honduras	Hoppe, 1986
"	3-6	34	Tanzania	Hodges *et al.*, 1983
"	6	17.9	"	Keil, 1988
"	8	41.2	"	Keil, 1988
"	8	44.8	Togo	Pantenius, 1988
Dried cassava	4	70	Tanzania	Hodges *et al.*, 1985
"	3	9.7	Togo	Wright *et al.*, 1993
"	7	19.5	"	Wright *et al.*, 1993

Life history

Adult female lays eggs in chambers bored at right angles to the main tunnels. Egg-laying on stabilized grain on maize cob is higher than on loose, shelled grain (Haines, 1991). Larvae hatch from the eggs after about three days at 27°C and thrive well on the dust produced by boring adults, where they also pupate. Development of the larva through to adult at the optimum, 32°C and 80% r.h takes 27 days. *Prostephanus truncatus* has the ability to develop in grain at low moisture content of about 9%, which many storage pest species occurring in the same ecological niche are unable to utilize and increase their number. Development on maize is more rapid than on cassava (Haines, 1991).

2.5 The use of botanicals for stored product protection

The use of plant materials believed to have protectant activity against stored product insect pests is an ancient practice. The number of plants known to be active against stored product insect pests is small and only a few are active against all major stored grain insects (Jacobson, 1983).

With the advent of synthetic pesticides, research on plant-derived pesticides diminished (Talukder and Howse, 1995), as synthetic pesticides were relatively cheap and had the most spectacular results in pest control. However, control of stored product pests by chemical insecticides has serious drawbacks (Sharaby, 1988). The indiscriminate use of chemical pesticides has given rise to many serious problems, including the development of genetic resistance by pest species, toxic residues in food, water and soil, increasing cost of application, pollution of storage environment, and other hazards from handling, (Ahmed *et al.*, 1981; Khanam *et al.*, 1990).

The strict requirements in ensuring safety for food commodities limit the choice of pesticides that can be used for protecting commodities in storage. Such stringent conditions according to Snelson (1979) have excluded the selection of several pesticides, employed on field crops, for use in storage. On the other hand, plant-derived pesticides have low mammalian toxicity and are more readily biodegradable, and are less likely to contaminate the environment (Freedman *et al.*, 1979). Also, botanicals usually contain a number of active ingredients which makes the development of resistance by pests very difficult (Főrster, 1998). These merits of botanicals have stimulated interest by various workers in the last two decades or so towards the age-old practices of using phytochemicals to protect stored products (Koul, 1982; Obeng-Ofori et al., 1997; Schmutterer, 1995).

In many areas of Africa and Asia, locally available plants and minerals are widely used to protect stored products against damage by insect pests, as an alternative to synthetic pesticides (Golob and Webley, 1980). Many plants manufacture chemicals that protect them against being fed on and the extract from these plants affects metabolism of species other than those attacking the plant from which the chemical was derived. Use of plant materials for pest infestation control in stored grains therefore seems to offer desirable solutions, especially in developing tropical countries where plants are abundant throughout the year (Talukder and Howse, 1995).

Over 200 plant species have been reported to have insecticidal properties (Baba *et al.*, 1992). According to Ignatowicz and Wesolowska (1995), some secondary metabolites are toxic to many pests while others act as anti-feedants or sterilants. To protect stored products, local farmers mix foodstuffs with different types and parts of plant materials. The materials used by different communities vary from place to place, and depend partly on the type and efficacy of suitable plant materials available in different localities (Obeng-Ofori, 1995). Many of these practices are undocumented and the scientific rationale for their continuous use has remained uninvestigated (Hassanali *et al.*, 1990).

In Africa, some of the widely studied plants for pest control include *Ocimum* sp.(Obeng-Ofori, 1997); *Nicotiana tabacum, Chenopodium ambrosioides* and *Tephrosia vogelii* (Delobel and Malonga, 1987), *Khaya senegalensis*(Aidoo, 1998) and *Azadirachta indica* (Schmutterer, 1985) . *Ocimum* species have shown good potential for use as repellent and toxic agents against the maize weevil, *Sitophilus zeamais*, the lesser grain borer, *R. dominca* and the angoumois grain moth, *S. cerealella* (Bekele *et al.*, 1996). According to Bekele *et al.* (1996), the ground leaves and essential oil extract of *Ocimum* species provided the greatest protection of maize and sorghum against feeding by the insects. Powders of *N. tabacum* have significant effect on egg development, causing up to 20 % mortality to fertile eggs of *Caryedon serratus* but when used against *C. serratus*, 14 % of fertile eggs were found dead 30 days after infestation (Delobel and Malonga, 1987). *C. ambrosioides* and *T. vogelii,* according to Delobel and Malonga (1987), reduce longevity of adult *C. serratus* by 98.8 and 90.0%, respectively, dying within 13 days with its main effect being reduction in fecundity. Crude extracts from *Chromolaena odorata* and *K. senegalensis* have shown good protentant potential against *S. zeamais* and *C. maculatus,* evoking high mortality and repellency, especially in *C. maculatus* (Aidoo, 1998).

A. indica is the most widely studied plant. Its derivatives are known to have distinct antifeedant and growth inhibitory effects, and have shown activity against a wide range of insect pests of many crops worldwide (Schmutterer, 1985; Saxena, 1989).

In Ghana, neem is traditionally used for the treatment of malaria (Abbiw, 1990) but local farmers also mix stored grains with products derived from it for protection against pest infestation in storage (Obeng-Ofori and Akuamoah, 1998). Research work done in different parts of Ghana has demonstrated the efficacy of neem extracts against storage pests of cereals and grain legumes (Obeng-Ofori and Akuamoah, 1998). The major target species include grain weevils, grain beetles, grain borers, cowpea beetles and several species of storage moths (Cobbinah and Appiah-Kwarteng, 1989; Owusu-Akyaw, 1991; Baba, 1994; Allotey and Dankwah, 1994; Obeng-Ofori, 1997).

Baba (1994) tested several species of plants including neem leaf and seed powders to determine their effectiveness as grain protectants against *P. truncatus* and *S. oryzae* in maize and wheat, respectively. Neem seed or leaf powder induced 100% mortality of *S. oryzae* and over 60% in *P. truncatus* within the 30 days experimental period. In a warehouse trial conducted in Togo, Zehrer (1984) found that white cowpea treated with 5% neem oil was protected from infestation by *C. maculatus* for up to 6 months. In Togo, Adhikary (1981) compared neem leaf and seed powder with conventional insecticides for protecting corn stored in sacks or unpeeled corncobs held in bins against *S. zeamais*, *Tribolium* spp., *R. dominica* and *Cathartus* spp. Neem seed powder together with the conventional insecticides gave significant results.

In Asia, Jotwani and Sircar (1967) reported in India that green gram, chickpea, cowpea and pea could be protected from damage by mixing powdered neem kernel with the grains at 1 or 2 to 100 parts. Field trials conducted in Pakistan demonstrated that grain storage bags treated with water extract of neem leaves at 20 % (w/v) or water extract of neem seed at 5 % (w/v) prevented the penetration of stored grain pests into the bags for at least 6 months during storage (Malik *et al.*, 1976). In Malaysia, neem extracts applied in different forms reduced significantly the infestation by *S. oryzae* and *R. dominica* and damage to paddy grain stored in 40 kg jute bags for 3 months (Muda, 1984).

Several other plant products have been tested for protection of stored grains against several insect pests in Asia. The sweetflag, *Acorus calamus* (L.), which occurs widely in Asia, has been established to have toxic and sterilizing effect on various stored product insects (Saxena and Mathur, 1976; Tikku *et al.*, 1978). The seeds of pithraj, *Aphanamixis polystachya*, have been shown to have strong repellent effects on *T. castaneum* and *S. oryzae* and high feeding deterrent to the later (Talukder and Howse, 1995). The rhizomes of turmeric, *Curcuma longa* (L.), have been shown to have repellent action against *S. granarius* and *R. dominica* (Ghulam and Su, 1983). In India, 13 plant materials thought to have insecticdal properties were obtained locally and their powder incorporated at 15 or 30 % (wt/wt) in a standardized culture medium which contained eggs of *Musca domestica* L. All the 13 plant species gave 100 % control of the pest (Ahmed *et al.*, 1981).

2.6.1 Botany of neem, *Azadiractha indica* A. Juss. (Meliaceae)

The neem tree, *Azadirachta indica* is native to Southeast Asia and grows in many countries throughout the world (Ascher, 1993). It is a fast growing plant that usually reaches a height of 15-20 m, and under very favourable conditions up to approximately 35-40 m (Schmutterer, 1995). It is an evergreen plant but under extreme conditions, such as extended dry periods, it may shed most or nearly all of its leaves. The branches spread widely with a fairly dense roundish or oval crown and may reach a diameter of 15-20 m (Schmutterer, 1995).

The trunk is relatively short, straight and may reach a girth of 1.5-3.5 m, with a hard bark, fissured or scaly, and whitish-gray to reddish-brown. The sapwood is grayish-white and the heartwood reddish when first exposed to air but turns reddish-brown after exposure (Schmutterer, 1995). The unpaired, pinnate leaves are 20-40 cm long and the medium to dark green leaflets, which number up to 31, are approximately 3-8 cm long with short petioles.

The tree bears seasonally white, fragrant flowers, which are auxiliary arranged. The inflorescences bear approximately 150, and occasionally up to 250 flowers. The glabrous fruits are oval-like drupes which vary in shape from elongated oval to nearly roundish and when ripe are 1.4-2.8 x 1.0-1.5 cm. They are green when young and yellowish-green to yellow when mature (Schmutterer, 1995).

2.6.2 Uses of neem

The neem tree has many medicinal uses. Notable among them are its use as an antiseptic, diuretic and anti-malaria drug. It has been used to cure many ailments from diabetes to syphilis, and is widely relied upon by herbalists in its native habitat (Koul *et al.*, 1990). The use of *A. indica* as a source of natural insecticide was discovered approximately 30 years ago (Ascher, 1993). In general, extracts of neem fruit, seeds, seed kernels, twigs, stem bark and root bark have been shown to possess insect antifeedant, insecticidal, insect growth disrupting, nematicidal, and fungicidal properties (Jacobson, 1989), bactericidal (Ara *et al.*, 1989), anti-inflammatory (Dhawan and Parmar, 1993), antitumor (Fujiwara *et al.*, 1982, 1984), immunostimulant (Van der nat *et al.*, 1987) as cited by Schmutterer (1995).

2.6.3 Bioactive compounds in neem

All biologically active neem compounds are suspected to be derived from one parent compound, the teracyclic triterpenoid tirucallol (Addae-Mensah, 1998). All other products formed are considered successive rearrangement and oxidation products of tirucallol (Ascher, 1993). The main constituents of the neem tree are also referred to as limonoids or meliacins. Several such compounds have been identified from neem and these include azadirachtin, azadiron, epoxyazadirdion, gedunin, nimbin, salannin, 1,3-diacetyl vilasinin, 1-tiglyl-3-acetyl vilasinin, 3-deacetyl salannin, salannol and salannol acetate. Others are nimbandiol, 6-acetyl nimbandiol, nimbainen, 6-deacetyl nimbinen, meliantriol and nimbidin[1-] (Schmutterer, 1995).

Azadirachtin (appendix 1) is the major component and generally accepted to be responsible for the majority of biological effects observed in organisms exposed to neem compounds (Mordue and Blackwell, 1993). It is estimated that azadirachtin accounts for up to 90% of the bioactivity of neem, but there is much synergism with other major and minor active components. Various modes of action of azadirachtin can be observed in target insect pests, notable among them are antifeedant effect, repellency, reduction or prevention of settling and oviposition, disturbance of metamorphosis, sterilization, reduction of activity or fitness and effects on the cell level (Schmutterer, 1998).

Evaluation of neem products from different parts of the tree (leaf, bark, seed) has shown that products derived from the seeds are more potent than from the leaves and bark (Forjoe, 1995; Ankra, 1998). About three to six times more leaves are needed to obtain equivalent amount of active ingredient in the seeds (Obeng-Ofori and Akuamoah, 1998). Fine extracts (in alcohol) are also more active than crude water extracts. Activity is usually dosage-dependent (Schmutterer, 1985).

2.6.4 Antifeedant and growth regulatory effects of Azadirachtin

Both primary and secondary antifeedant effects have been observed in azadirachtin (Ascher, 1993). Primary effects include the process of chemoreception by the organism, that is, sensory organs on the mouthparts which stimulate the organism to begin feeding whereas secondary processes include gut motility disorders due to topical application only (Ascher, 1993). The secondary antifeedant effect can be observed in many species after intake of neem-contaminated food. It results in reduced food consumption, slow growth and reduced ability to convert plant material into body substance. Small adults and low fecundity may be additional consequences of this striking effect (Schmutterer, 1998). The inhibition of feeding behaviour by azadirachtin results from blockage of input receptor cells or both (Mordue and Blackwell, 1993). Sensitivity between species to the antifeedant effects of azadirachtin is profound. According to Mordue and Blackwell (1993), the order Lepidoptera appear most sensitive to azadirachtin's antifeedant effects, with Coleoptera, Hemiptera and Homoptera being less.

A metamorphosis-disturbing effect is observed in larvae of numerous insect species after oral intake of azadirachtin, sometimes also after topical application of neem products. This mode of action is based on a disturbance of the hormonal system of the target insects. As a consequence, the release of neurosecretory hormones from the brain and the production and or release of the moulting hormone from the prothoracic gland is reduced or delayed (Schmutterer, 1998).

2.6.5 Neem in Ghana

The introduction of neem into Ghana has many divergent views. According to Addae-Mensah (1998), it was introduced to the northern part of the then Gold Coast, now Ghana, sometime between 1919 and 1927 from India by the then colonial Governor, the late Brigadier-General Sir Frederick Gordon Guggisberg who brought some seeds from India and planted as shade and ornamental trees in the dry north. The credence of this origin of the plant into Ghana, and its uses in the country is supported by the folkloric or local Ghanaian and West African names (Table 2).

Table 2: Folkloric or local names of neem in some West African countries

Country	Tribe	Local name(s)
Ghana	Twi	Dua gyane
	Fanti	Aboodua or Aboodee
	Ga	Dangme, Kingtsoho or Bodetsho
	Nzema	Anwe egyane
	Ewe	Liliti, Linigbe or Kiniti
Nigeria	Hausa	Dogonyaro or Kurnan nasar
Mali	Diula	Goo-gay
Cote d'Ivoire	,,	,,
Guinea	,,	,,

Source: Adapted from Addae-Mensah (1998)

The Fante word "aboodee" could be a corruption of the phrase arbour day, a day set aside for the planting of shade or arbour trees. The Ga name "Kingtsoho" means the king's tree and the tree might have earned this name since the Governor represented the king in the colony, and he is the one who normally ordered the arbour or tree planting day. The Togolese ewe name "Kiniti" is obviously a corruption of king's tree (ti in Ewe means tree or plant). The Hausa word "Kurnan" could also be a corruption of king. The Diula name "Goo-gay" is said to be a corruption of Governor Guggisberg, who is believed to have introduced the plant to the northern part of Ghana from where it probably spread to the Sahel regions of Burkina Faso, Mali, Guinea and Niger (Addae-Mensah, 1998).

According to Cobbinah (1998) on the other hand, neem is believed to have been introduced into Ghana by Indian settlers during the first quarter of the century. It was planted extensively as an amenity tree and can be found lining roads in Accra, Takoradi, Cape Coast, Tamale, Kumasi and Ho.

Schmutterer(1998) is also of the view that neem was introduced during the first quarter of the 20th Century but also said it was not quite clear whether Indian immigrants or the former British governor Guggisberg brought the first seeds to this country.

2.6.6 Commercial neem products

During the past two decades the biological activity of neem extracts has been investigated intensively since the discovery of its potential use as biopesticide almost a century ago (Schmutterer, 1995). Due to the quick degradation of the active ingredient, azadirachtin, in aqueous extracts, there is little hope of using these simple but effective extracts for longer treatment duration in stored grain products (Schmutterer, 1995).

There are several commercial neem pesticides in the world market and a number are still at the experimental stage (Mordue and Blackwell, 1993). The first commercial neem insecticide was Margosan-O, which was registered by the Environmental Protection Agency (EPA) for non-crop use in the United States in July 1985 (Jacobson, 1989). Since that time, other commercial neem insecticides have been developed worldwide (Ascher, 1993). Some of the commonly used commercial neem products include Turplex (formerly Azatin), Neemguard, Margosan-O, Wellgro, RD-Repelin, Bioneem, Neemol, Neemesis, Neemazal and Neemark (Larson, 1993; Mordue and Blackwell, 1993). Most of these are used for organic vegetable and fruit production. Table 3 shows a list of neem commercial products.

Table 3: Commercial neem-based products

Trade name	Country of production
Neemark	India
Neemol	India
Margosan-O	USA
Neemguard	USA
Wellgro, RD-Repelin	India
RH-9999	India
AD 1000	USA
Neemazal	USA, India, Germany
Neemix 4.5	USA, Israel, Egypt
AMBAC/ FORTUNE	USA
Neem oil and Emulsifier	Germany
Formulated neem oil	Kenya, Thailand, Dominican Republic
Neemros	Kenya
Neemroc EC oil	Kenya
Neemsar 'O'	Kenya
SADAO THAI 111	Thailand
SADAO THAI 222	Thailand
Semilla Molida (SM)	Dominican Republic
Torta Molida (TM)	Dominican Republic
Torta Nim	Nicaragua
Aceita de Nim	Nicaragua
Organ Nim	Nicaragua

Source: Adapted from Larson (1993); Mordue and Blackwell (1993); Förster and Moser (2000); Förster *et al.* (2000).

Neemazal

In Ghana, neemazal, which was recently introduced, is mainly used by farmers for vegetable and fruit production. Neemazal contains 10,000 ppm azadirachtin, the most potent molecule in neem and over 60 other limonoids. The limonoids are held together with natural additives making its usage safe to users and consumers (Anon, 2000). It acts as an anti-feedant, insect growth regulator, anti-resistant, oviposition deterrent, ovicidal against pests and also as non-phytotoxic (Anon, 2000). Few documentation has been done on the use of neemazal, a neem seed oil product, in the country. A preliminary study in laboratory and field tests on neemazal for cocoa capsid control in Ghana was conducted at Tantro, near Tafo in the Eastern Region. In this work, a number of commercial neem formulations namely neemazal, neemol, super neemol and potenised systemic neem oil, as well as crude plant extracts including neem seed extracts, were screened for the control of cocoa capsids. Neemazal emerged as the most effective product causing mean mortality of 95.8 % and 90 % at 5 % v/v and 3 % v/v concentrations, respectively but the other commercial products being only slightly better than the crude neem extract (Padi and Adu-Acheampong, 1999).

2.6.7 Utilisation of neem as pesticide in developing countries

Plants which possess insecticidal properties and would be suitable for integrated pest management (IPM) have to fulfill certain criteria. These criteria envisage that the active ingredients derived from these plants must first of all be effective against a broad spectrum of target pests. Secondly, it must have no or only slight side-effects on non-target organisms, human beings and the environment, and thirdly, it must be easy to derive, to formulate and to apply (Förster, 1998).

It has been proved by many research carried out that the extract from the neem tree, fulfils these standard requirements better than any other plant known so far (Förster, 1998). The reasons why neem is the most promising source of raw material for natural pesticides are the following. The neem tree is available throughout the tropics (Schmutterer, 1998).

The processing of neem into pesticide can be simple and easy even though sophisticated technologies can be used. Even at village level, crude neem water extracts or neem oil can be prepared with simple equipment, which makes resource-poor farmers able to afford.

The neem tree is not toxic to mammals and has only slight side-effects on beneficial organisms compared to other pesticidal plants. According to Schmutterer (1998), none of the other plant extracts investigated so far is non-toxic against mammals but the neem is used as a medicine.

Neem-based pesticides have low risk for the development of pest resistance because neem contains more than 100 active ingredients. Besides the major component azadirachtin, more than 8 other ingredients show strong insecticidal or repellent or insect-growth-inhibiting effects in various ways thereby making it least possible for pests to develop resistance (Förster, 1998).

The neem tree is a perennial plant making it possible to obtain various parts for use throughout the year. The tree bears fruits at least once every year and the seeds are more potent than other parts of the plant (Forjoe, 1995; Ankra, 1998).

Another attribute that makes the neem tree the most important is the possibility to put it to many other uses. It is used in afforestation, as shade tree, fuelwood, contraceptives and other medicines, cosmetics, animal feed, etc. (Schmutterer, 1995).

Even though neem has so far proved beyond doubts as the best source of biopesticide, there are numerous constraints to its effective utilization in developing countries. Except for India and Pakistan, no developing country has developed its neem resources for plant protection and storage to a significant extent (Förster, 1998).

The main reasons preventing greater use of neem include a lack of knowledge about neem, harvesting and processing times coinciding with other labour peaks, commercial neem products being expensive, and doubts about the efficacy of neem products especially concerning its knock-down effect. Also, neem pesticides are more difficult to apply than synthetic pesticides, and have inferior social prestige. There are also technical problems when processing neem, and have short shelf-life under tropical conditions.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Culturing of insects

Adults of *Prostephanus truncatus* were obtained from a stock culture at Agricultural Research Station, Kpeve and maintained in an insectory at the Plant Protection and Regulatory Services Directorate (PPRSD), Pokuase at a temperature of 27±2 ^{0}C and 70-80 % r.h. One hundred and fifty of *P. truncatus* adults of were placed in glass jars containing 500 g of maize for three weeks for egg laying after which the adults were removed. The infested maize was kept in the conditions of the laboratory and the adults that subsequently emerged were used for the various bioassays.

3.2 Neem products used

Home-made product

Matured neem fruits were harvested at the campus of the University of Ghana, Legon. Depulping was done by hand after soaking the fruits in water for a day, in order to allow fermentation to occur. The kernels were dried in the sun for 6 hours followed by 4 weeks drying in the shade (Schmutterer, 1995). The dried kernels were cracked and the seeds were milled into powder using a laboratory mill of speed 250 r/m for 10 mniutes. Five hundred grams of ground seeds were placed in a 1500 ml conical flask and petroleum ether was added till it reached the thousand millilitres mark. The mixture was well stirred and kept in a dark room for 24 h after which the liquid portion was decanted and filtered. The residue was again treated in similar manner two more times. The filtrate was then concentrated at 35 ^{0}C using the rotary vacuum evaporator (Plate 1) until all the petroleum ether had been driven off. The oil extract was kept in a refrigerator at temperature of 10°C until use. The extract was applied at dosage rate of 0.2, 0.4, 0.6 and 0.8 ml (equivalent to 0.18, 0.36, 0.54 and 0.72 g of oil, respectively) per 200 g of grain.

Five hundred grams of ground seeds were also pressed using a clamp and the oil drips were collected. The oils obtained from the two processes were analysed for their similarity and differences using thin layer chromatography (TLC).

Neemazal was purchased from the Ministry of Food and Agriculture chemical shop in Accra and was also applied at dosages of 0.2, 0.4, 0.6 and 0.8 ml active ingredient in 4 ml petroleum ether per 200 g of grain in glass jars and mixed thoroughly with a glass rod. Control was untreated grain.

Plate 1: Rotary evaporator

3.3 Assay of mortality and progeny emergence assays

Neem oil and neemazal were mixed separately with 200 g of maize in glass jars at the four different dosages indicated above. Untreated grains acted as the control. Twenty adults of *P. truncatus* of three-seven days old were introduced into the jars containing the treated and untreated grain and covered with wire mesh held in place with the lids. Four replicates of each treatment were set up in the experimental laboratory maintained at $27 \pm 2\ ^{0}C$ and 70-80 % r.h. The number of dead insects in each jar was counted after 24, 48, 72 and 96 h to estimate mortality. Insects were presumed dead if they remained immobile and found not to respond to three probing with a blunt dissecting probe after a five-minute recovery period.

In a similar experiment, 20 adults of *P. truncatus* were introduced into treated and control grains and after three weeks oviposition period, the parent adults were removed. Insects subsequently emerging were counted to estimate frist filial (F1) progeny. Counting began after 30 days and stopped after 54 days to avoid overlapping of generations.

3.4 Damage assessment

The Thousand Grain Mass (TGM) method was used to assess damage caused by *P. truncatus* during the period of storage. Four samples of 200 g of maize were weighed from the maize stock before treatment. Two subsamples of about 20 g each were taken from each sample to determine the moisture content of the grain. The remaining grains in each sample were weighed and counted. This was used to determine the TGM of the grain at the start of the storage period using the formula, M= 10 m (100-H) / N

Where: m is the wet weight of sample,

H is percentage moisture content,

N is number of grains in the sample.

Weight loss assessment was carried out on treated and untreated (control) grains after nine weeks of storage period to cover only the F1 progeny. Twenty adults of *P. truncatus* were introduced into treated and control grains. After three weeks oviposition period, the parent adults were removed. Insects subsequently emerging were removed regularly until a period of six weeks following the removal of first adults. There were four replicates for each treatment.

Similarly , the TGM was calculated for the treated and untreated grains. The percent weight loss was calculated as follows:

$$\% \text{ weight loss} = M_1 - M_x / M_1$$

Where: M_1 is the TGM of the grain at the start of the storage,

M_x is the TGM of the grain at storage time x.

3.5 Product persistence in grain

The persistence of the neem oil and neemazal were assessed by treating the grains separately in glass jars with the dosages indicated above. Untreated grains acted as control. Twenty three-to-seven days old beetles were exposed to treated and untreated maize, which have been stored for 1, 10, 30 and 60 days. Each treatment was replicated four times. Mortality counts were made after 24 h exposure.

3.6 Effect on hidden eggs and immature stages

Batches of 200 g of maize were each infested with 20 adults (three to seven days old) of *P. truncatus* to allow egg laying. The parent adults were removed after five days. One day after the removal of adults, neem oil and neemazal were mixed separately with maize grains at the dosage rates indicated above to determine their effect on eggs. Thereafter, these treatments were repeated one, two and three weeks after adult removal to assess their effect on early larvae, late larvae and pupae, respectively. The adults that subsequently emerged were counted for a period of six weeks following the removal of first adults. There were four replicates for each treatment.

3.7 Repellency test

The repellent action of the products was assessed in an olfactometer (Plate 2) consisting of two 0.5-*l* glass jars connected together at their rims. Two hundred grams of maize were put into each of the two glass jars. The grains in one of the jars were treated with neem oil or neemazal at the four dosages indicated above and the other half acted as control. Twenty adults of *P. truncatus* were introduced into the jar through the circular hole by means of 5-cm diameter funnel. The number of insects present on control (Nc) and treated (Nt) jars were recorded after 30 minutes of exposure. After each test, the glass jars were thoroughly cleaned. The assay for each dose of test chemical was replicated four times. Percent repellency (PR) value was computed as: PR =[(Nc - Nt) / (Nc + Nt)] x 100 .

PR data was analysed using ANOVA after transforming them into arcsine values. All negative PR values were treated as zero (Hassanali et al., 1990).

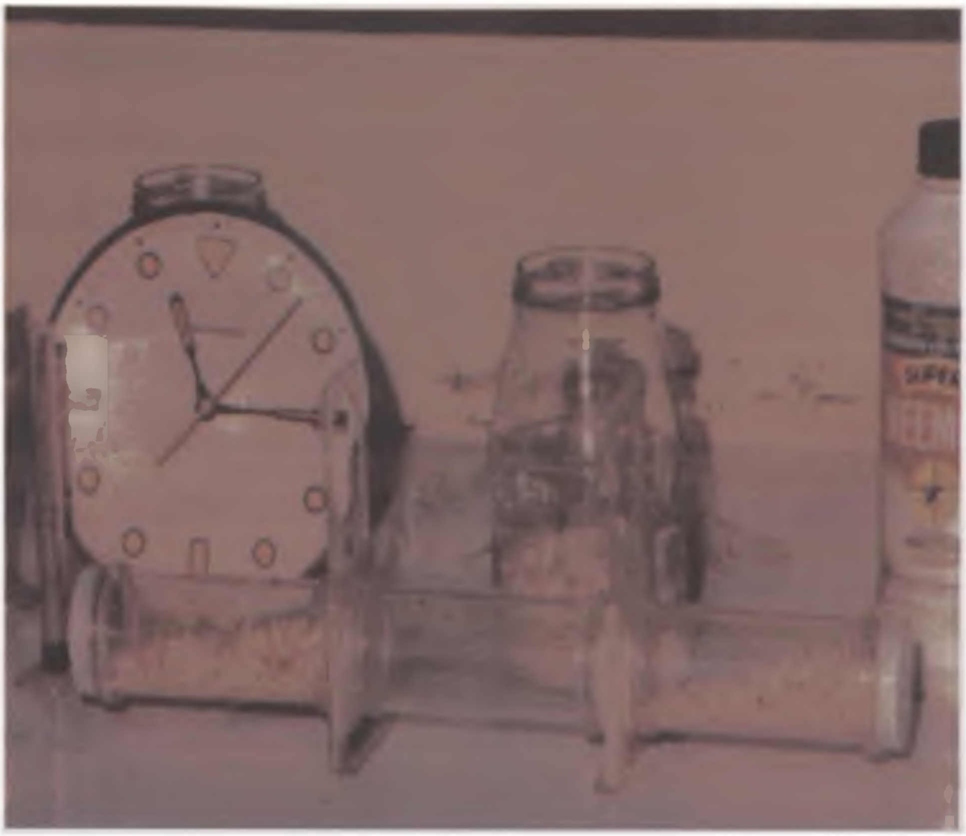


Plate 2: Olfactometer

3.8 Germination and water absorption tests

Grains were treated with 0.2, 0.4, 0.6 and 0.8 ml of the neem oil and neemazal separately. The treated grains were kept free from infestation in the laboratory. Germination tests were carried out 30 and 60 days after treatment. Twenty seeds of each grain type were placed in separate glass petri dishes each lined with two layers of Whitman No. 1 filter paper soaked with water. Percentage germination of each treatment was recorded after four days. Untreated seeds acted as control.

To determine the amount of water absorbed by the seeds, 20 g samples of treated and untreated seeds were submerged in water for 1, 6 and 24 h. The grains were then dried with paper towels and reweighed to estimate water absorption. There were four replicates of each treatment.

3.9 Biological activity of fractions of neem oil

Two fractions were prepared by means of column chromatography (Plate 3) from neem oil and were assessed for their toxicity and repellency action by treating the grains separately in glass jars. Two hundred grams of silica gel was added to 1000 ml of petroleum ether and stirred continuously. The mixture was then poured into a column tube sealed at the base with cotton wool. The tap on the tube was opened to allow some of the petroleum ether to drain till it got to about 5 cm above the gel. Ten grams of the neem oil was poured into the solution in the column. Thousand millilitres of petroleum ether was put into a separating funnel and allowed to flow under gravity into the column. The tap on the column was opened to allow the solvent flow under gravity and was collected into test tubes using fraction collector. Various mixtures collected into separate test tubes were spotted on the silica/ alumina gel plates and run in petroleum ether to obtain the first fractional component of neem oil. After the first fraction, A was obtained, chloroform was poured into the mixture in the column tube to wash down the rest of the components together to obtain a second fraction, B. The two different mixtures were then concentrated separately at 35°C using the rotary vacuum evaporator until all the solvents had been driven off.

Mortality and repellency tests were carried out using A and B fractions against *P. truncatus* as previously described in sections 3.5 and 3.7, respectively.

3.10 Analysis of data

The data were analysed using analysis of variance (ANOVA). Least significant difference (LSD) was used to separate means where the ANOVA was significant. The data on percentage adult mortality were corrected by Abbott's (1925) formula and transformed to arcsine values before ANOVA was done.

Arcsine transformation was done using the formula: $y = \text{Sin}^{-1}\ x / 100$.

Abbott's (1925) correction formula = [(%T- %C) / (100 - %C)] x 100,

Where %T = percent treated mortality, and %C = percent control mortality.

Plate 3: **Column Chromatography**

CHAPTER FOUR

4.0 RESULTS

4.1 Yield and comparison of oils

The yield of oil obtained from 500 g ground neem seeds through pressing and the use of petroleum ether is shown in figs. 1 and 2. The yield obtained from extraction through petroleum ether was 63 % (Fig. 2) whereas that obtained from pressing was 38 % (Fig. 1). This shows that extraction of neem oil through the use of petroleum ether was almost twice more efficient than that obtained from pressing.

Thin layer chromatography showed that the two oils were the similar (Plate 4).

4.2 Adult mortality in grain

Table 4 shows the percent mortality of *P. truncatus* in grain treated with different dosages of neem oil and neemazal. Neem oil was less toxic to *P. truncatus*. The highest dosage of 0.8 ml/kg of neem oil killed 70 % of *P. truncatus* after 96 h and this was not significantly different ($P<0.05$) from 0.6 ml/kg which evoked 71 % mortality after 96 h. With the neemazal, 0.6 and 0.8 ml/ kg of grain were highly toxic to *P. truncatus* evoking over 95 % mortality after 72 h of exposure.

Two fractions obtained from neem oil caused significant mortality to *P. truncatus*. Fraction A was less toxic to *P.truncatus* as the highest dosage of 0.8 ml/kg killed only 35 % of the adult beetles after 96 h of exposure. Fraction B, however, was more toxic than A evoking more than 50 % mortality from the highest dosage after 72 h (Table 4).

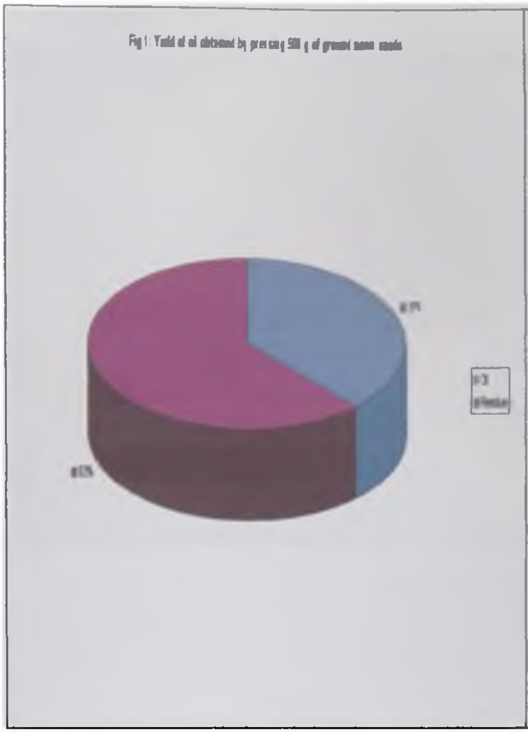


Fig 1: Yield of oil obtained by pressing g of ground neem seeds

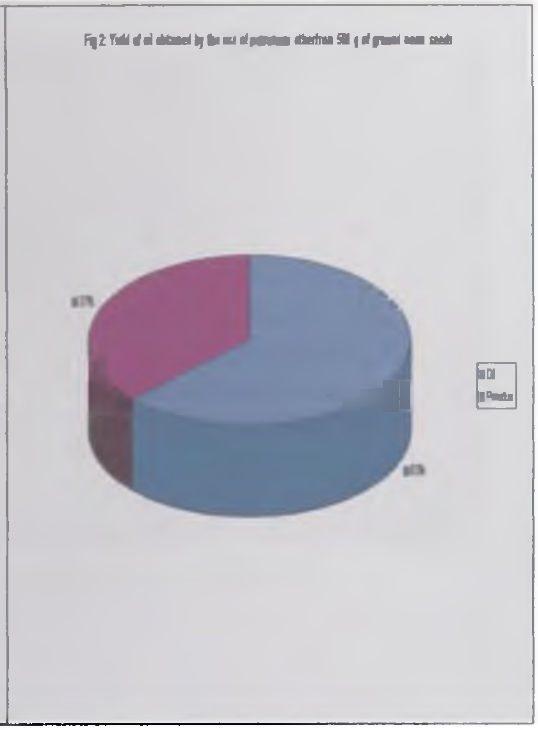


Fig 2: Yield of oil obtained by the use of petroleum ether from g of ground neem seeds

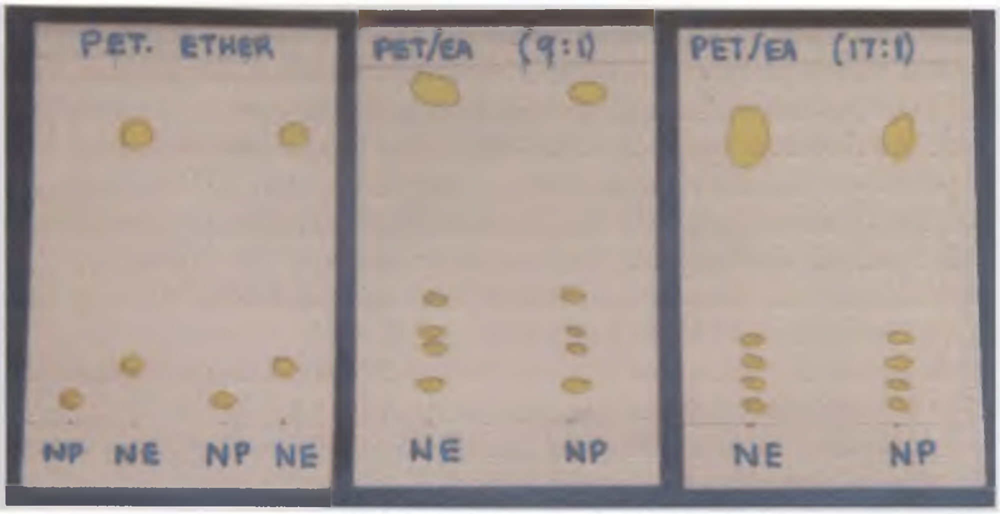


Plate 4: Thin Layer Chromatography alumina gel sheets

Table 4: Toxicity of neem oil and neemazal against *P. truncatus* in maize grain.

Treatment (ml/ 200 g of grain)	Mean adult Mortality (± S.E), h after exposure 24	48	72	96
(a) Neem oil				
0.2	11 ± 0.48^{b}	19 ± 0.25^{d}	26 ± 0.48^{d}	29 ± 0.63^{f}
0.4	26 ± 1.11^{d}	41 ± 1.32^{g}	54 ± 0.85^{i}	56 ± 0.75^{i}
0.6	39 ± 0.63^{e}	51 ± 0.85^{h}	66 ± 0.48^{k}	71 ± 0.25^{k}
0.8	41 ± 0.63^{f}	50 ± 0.71^{h}	59 ± 1.18^{j}	70 ± 0.91^{j}
(b) Neemazal				
0.2	1 ± 0.25^{a}	9 ± 0.63^{b}	14 ± 0.63^{b}	16 ± 0.41^{c}
0.4	16 ± 0.75^{c}	34 ± 1.75^{f}	46 ± 1.70^{g}	55 ± 1.58^{i}
0.6	71 ± 2.32^{g}	88 ± 1.26^{i}	96 ± 0.48^{l}	99 ± 0.25^{k}
0.8	78 ± 1.32^{h}	98 ± 0.50^{j}	100 ± 0.00^{m}	100 ± 0.00^{k}
(c) Fraction A				
0.2	9 ± 0.25^{b}	14 ± 048^{c}	14 ± 0.48^{b}	14 ± 0.48^{b}
0.4	9 ± 0.75^{b}	26 ± 0.48^{d}	26 ± 0.48^{d}	26 ± 0.48^{e}
0.8	24 ± 0.85^{d}	34 ± 0.48^{f}	35 ± 0.41^{e}	35 ± 0.41^{g}
(d) Fraction B				
0.2	10 ± 0.41^{b}	15 ± 0.41^{c}	20 ± 0.71^{c}	23±0.87d
0.4	11 ± 0.63^{b}	18 ± 0.65^{d}	24 ± 0.48^{d}	30 ± 0.41^{f}
0.6	24 ± 0.85^{d}	33 ± 0.96^{f}	40 ± 0.41^{f}	45 ± 0.41^{h}
0.8	33 ± 0.65^{e}	40 ± 0.91^{g}	51 ± 1.11^{h}	56 ± 1.11^{i}
Control	0 ± 0.00^{a}	0 ± 0.00^{a}	0 ± 0.00^{a}	0 ± 0.00^{a}
LSD	2.61	2.40	2.12	1.96

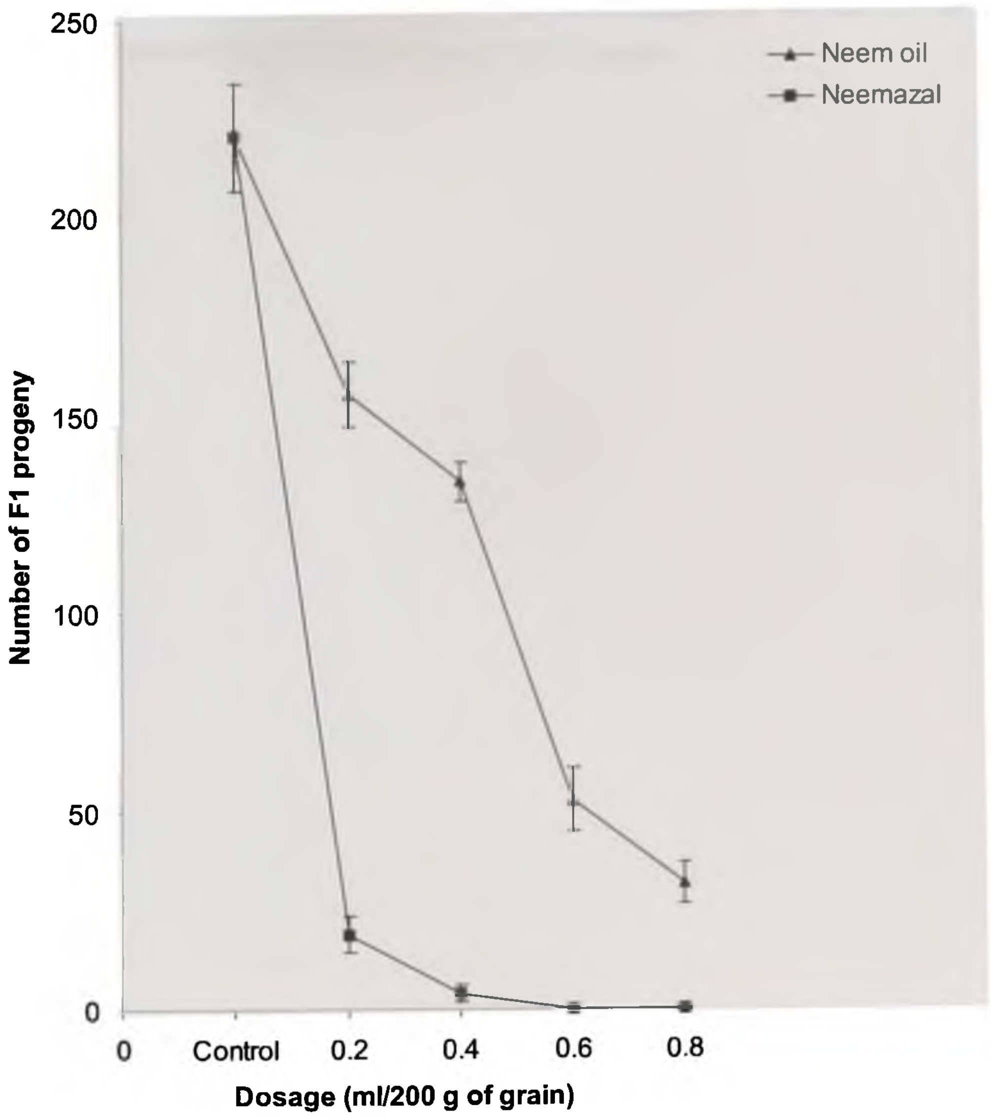
Mean of four replicates of 20 insects each. Mortality recorded after 24, 48, 72 and 96 h exposure. Column means followed by different letter(s) are significantly different at 0.05 level.

4.3 Progeny production

The number of F1 progeny produced by *P. truncatus* in untreated grains and grains treated with neem oil and neemazal are shown in Fig. 3. Neem oil applied at 0.6 and 0.8 ml caused significant reduction in the number of F1 progeny produced by *P. truncatus*.

Neemazal completely inhibited emergence of progeny production of *P. truncatus* in grain treated with 0.6 and 0.8 ml whereas control had high numbers of insects.

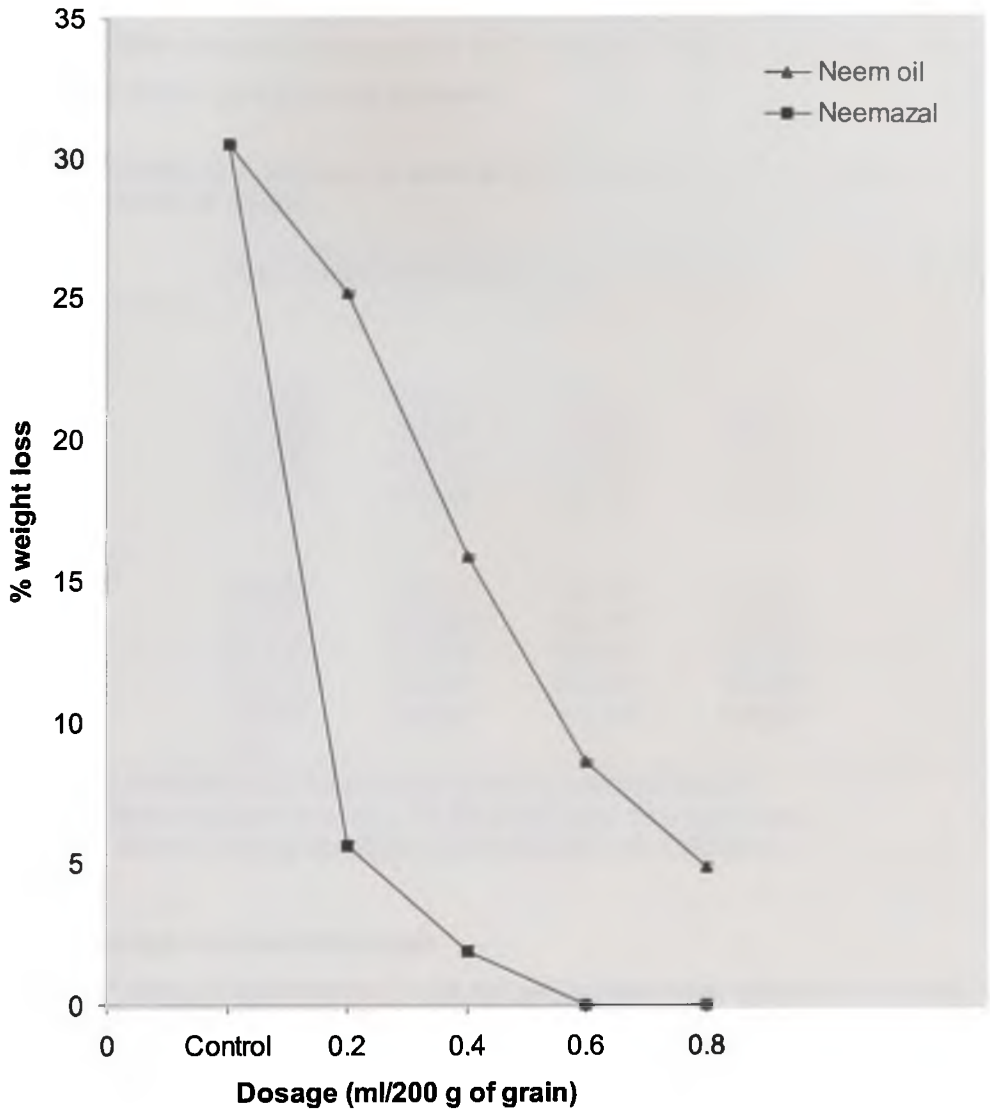
Fig. 3: F1 progeny produced by *P. truncatus* in maize treated with neem oil and neemazal.



4.4 Damage assessment

Weight loss caused by *P. truncatus* in untreated grains and grains treated with neem oil and neemazal are shown in Fig. 4. Both neem oil and neemazal applied to the grain protected them against damage by *P. truncatus* compared to the untreated grains. Grain treated with the neem products had observable holes but were significantly reduced with all dosages in a dosage-dependent manner. Neemazal applied at 0.6 and 0.8 ml completely protected the grains against damage by *P. truncatus*.

Fig. 4: Percent weight loss caused by *P. truncatus* in maize treated with neem oil and neemazal.



4.5 Persistence of neem oil and neemazal in grain

Toxicity of neem oil and neemazal in treated and untreated grains are shown in Table 5. Toxicity of both products rapidly declined significantly (P<0.05) in storage following application. Both chemicals were not toxic to *P. truncatus* after the treated grains were stored for 10 days or more following treatment.

Table 5: Mortality of *P. truncatus* in neem oil and neemazal treated grain after different intervals of storage.

Treatment	Mean % adult mortality, days after treatment			
(ml/ 200 g of grain)	1	10	30	60
(a) Neem oil				
0.2	1±0.25[a]	0±0.00[a]	0±0.00[a]	0±0.00[a]
0.4	6±0.48[b]	0±0.00[a]	0±0.00[a]	0±0.00[a]
0.6	9±0.48[b]	0±0.00[a]	0±0.00[a]	0±0.00[a]
0.8	29±0.85[e]	0±0.00[a]	0±0.00[a]	0±0.00[a]
(b) Neemazal				
0.2	6±0.48[b]	0±0.00[a]	0±0.00[a]	0±0.00[a]
0.4	10±0.41[c]	0±0.00[a]	0±0.00[a]	0±0.00[a]
0.6	16±0.48[d]	0±0.00[a]	0±0.00[a]	0±0.00[a]
0.8	45±1.29[f]	0±0.00[a]	0±0.00[a]	0±0.00[a]
Control	0±0.00[a]	0±0.00[a]	0±0.00[a]	0±0.00[a]
LSD	1.82			

Mean of four replicates of 20 insects each. Mortality recorded after 24 h exposure of treated grains stored for 1, 10, 30 and 60 days. Column means followed by different letter(s) are significantly different at the 0.05 level.

4.6 Effect on eggs and immature stages

The effect of neem oil and neemazal on the various developmental stages of *P. truncatus* is shown in Table 6. Both products had significant toxicity to the eggs and immature stages compared to the control. Although neem oil significantly affected the eggs and immature stages, it did not completely inhibit their development. Higher dosages of neemazal, however completely inhibited the development of eggs, early and late larvae of *P. truncatus* but was less toxic to pupae when applied at 0.6 and 0.8 ml. When applied at 0.2 and 0.4 ml, neemazal did not completely inhibit the development of eggs and immature stages of *P. truncatus*.

Table 6: Mean number of *P. truncatus* produced in grain treated with neem oil and neemazal at different times after oviposition period.

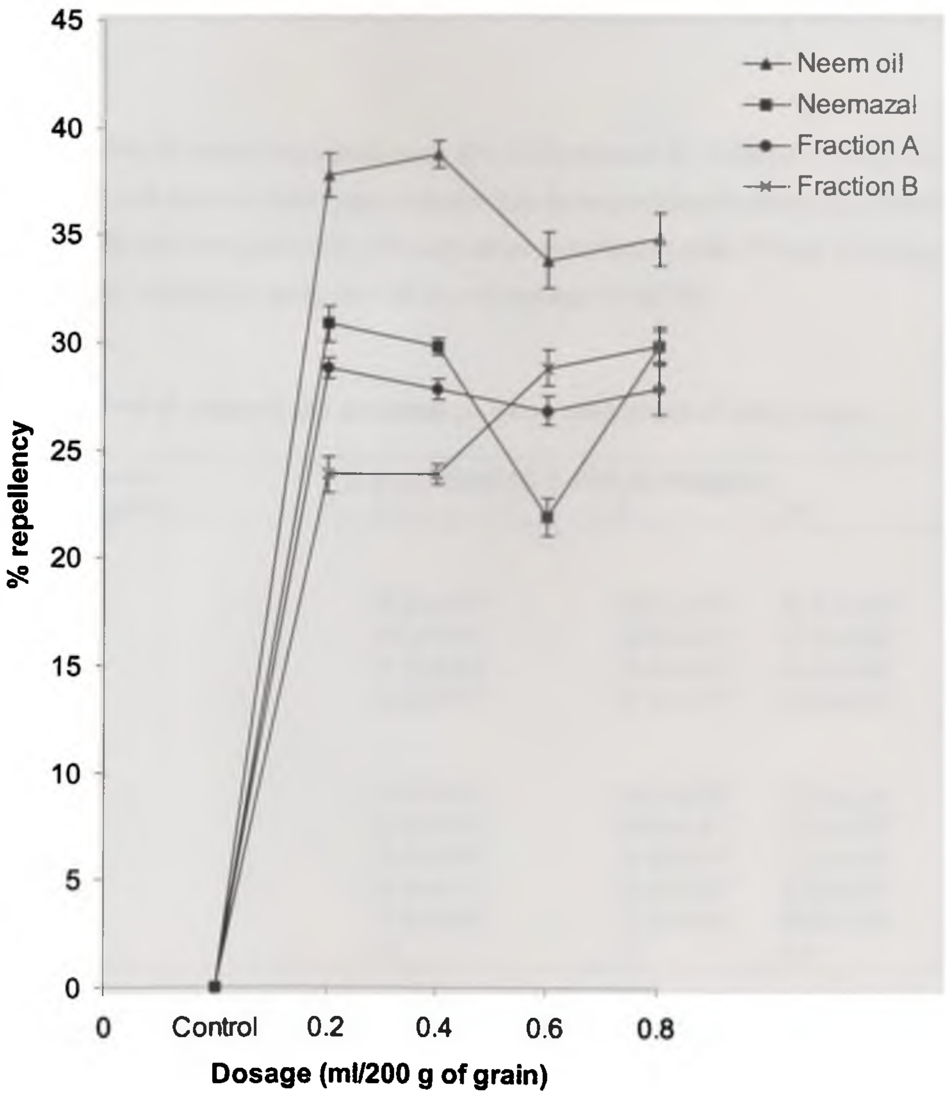
Treatment	Time of treatment			
(ml/ 200 g of grain)	24 h	1 week	2 weeks	3 weeks
(a) Neem oil				
0.2	15±4.70[a]	24±4.18[a]	45±6.69[b]	21±3.88[a]
0.4	10±3.10[a]	15±2.43[a]	28±4.92[ab]	18±1.44[a]
0.6	5±1.25[a]	12±2.69[a]	19±4.19[ab]	15±1.69[a]
0.8	2±0.86[a]	12±3.71[a]	16±4.00[a]	9±2.55[a]
(b) Neemazal				
0.2	9±1.47[a]	9±2.35[a]	29±5.09[ab]	48±5.28[b]
0.4	4±1.50[a]	6±1.70[a]	17±3.82[ab]	40±7.00[b]
0.6	0±0.00[a]	0±0.00[a]	0±0.00[a]	24±4.56[a]
0.8	0±0.00[a]	0±0.00[a]	0±0.00[a]	13±3.33[a]
Control	216±13.03[c]	216±13.03[b]	216±13.03[d]	216±13.03[c]
LSD	13.94	14.41	17.09	17.09

Mean of four replicates. Column means followed by different letter(s) are significantly different at 0.05 level.

4.7 Repellency

Fig. 5 shows the mean repellency values for neem oil and neemazal at different doses against *P. truncatus*. Analysis of variance indicated significant difference ($P<0.05$) between the responses of the beetle to the products. Repellency was not dosage-dependent. Neemazal and neem oil showed moderate repellency against *P. truncatus* as they caused less than 50 % repellency. The two fractions also showed some repellency against the beetles but their repellent action was lower than neem oil applied as unfractionated product.

Fig. 5: Mean percentage repellency for neem oil, neemazal, fraction A and B.



4.8 Water absorption and germination of seeds

Water absorption by maize seeds was increased significantly ($P<0.05$) by neem oil and neemazal (Table 7a). The rate of water absorption for seeds treated with neem oil and neemazal was generally dosage-dependent, with neem oil absorbing the greater amount of water.

The germination of maize was significantly ($P< 0.05$) reduced by neemazal (Table 7b). Seeds treated with neem oil had longer radicles than those treated with neemazal (Plate 5 & 6). Neem oil did not significantly ($P<0.05$) affect germination after 30 days of storage but reduced the viability of seeds after 60 days of storage (Table 7b).

Table 7a: Effect of neem oil and neemazal on water absorption of maize seeds.

Treatment dosage	% water absorbed, h after submergence		
(ml/ 200g of grain)	1	6	24
(a)Neem oil			
0.2	14.2±0.04^{d}	28.6±0.02^{b}	41.3±0.03^{b}
0.4	14.7±0.03^{f}	28.9±0.03^{b}	41.7±0.06^{c}
0.6	15.1±0.06^{g}	29.2±0.05^{b}	41.6±0.08^{c}
0.8	15.3±0.05^{h}	30.1±0.01^{c}	41.9±0.03^{c}
(b)Neemazal			
0.2	13.0±0.03^{b}	28.2±0.02^{b}	41.8±0.01^{c}
0.4	13.8±0.02^{c}	29.6±0.05^{c}	43.0±0.04^{d}
0.6	13.8±0.05^{c}	28.6±0.01^{b}	41.7±0.04^{c}
0.8	14.4±0.01^{e}	28.6±0.02^{b}	41.6±0.04^{c}
Control	9.4±0.04^{a}	24.5±0.04^{a}	40.0±0.05^{a}
LSD	0.1	1.0	0.3

Means of four replicates of 20 g of grains each. Column means followed by different letter(s) are significantly different at 0.05 level.

Table 7b: Effect of neem oil and neemazal on germination of maize seeds.

Treatment (ml/ 200 g of grain)	% germination, days after treatment 30	60
(a) Neem oil		
0.2	94±0.48[g]	78±1.19[e]
0.4	94±0.63[g]	78±0.29[e]
0.6	90±0.71[f]	76±0.75[e]
0.8	85±0.41[e]	53±0.65[b]
(b) Neemazal		
0.2	69±0.75[d]	64±1.11[d]
0.4	60±0.91[c]	53±0.65[b]
0.6	48±0.65[b]	58±1.04[c]
0.8	40±0.41[a]	34±1.11[a]
Control	94±0.48[g]	93±0.65[f]
LSD	1.81	2.53

Means of four replicates with 20 seeds each. Column means followed by different letter(s) are significantly different at 0.05 level.

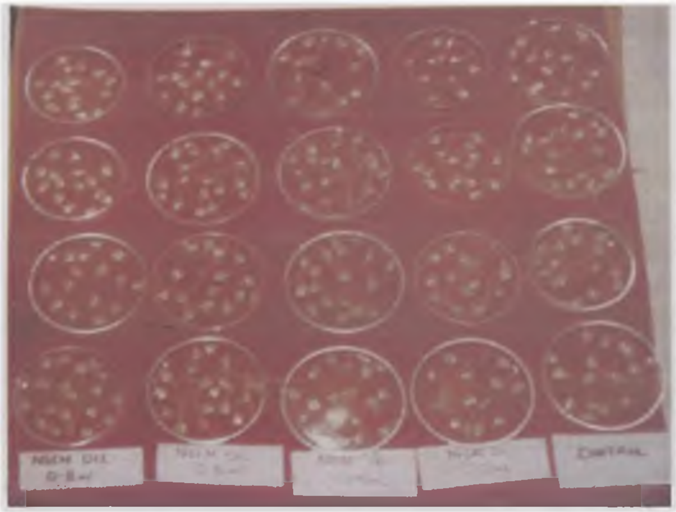


Plate 5: Germinated maize seeds treated with neemazal

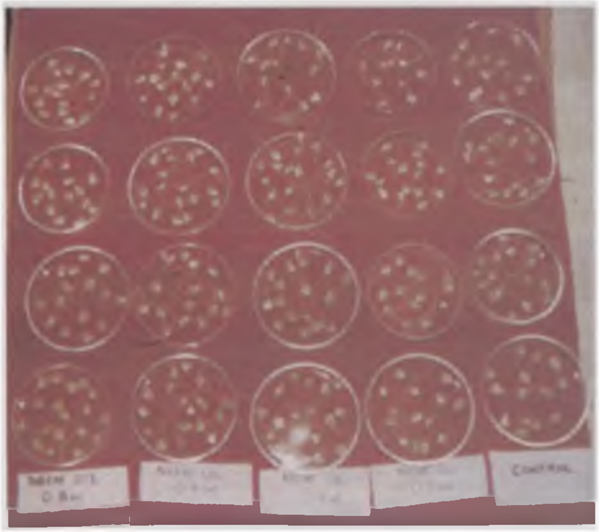


Plate 6: Germinated maize seeds treaded with neem oil

CHAPTER FIVE

5.0 DISCUSSION

5.1 Toxicity of extracts and fractions

In this study, home-made neem oil, fractions from oil and neemazal were highly toxic and caused significant mortality of *P. truncatus* after 48-hour exposure compared to untreated controls. Dead insects from oil-treated grain showed signs of rapid immobilization with their legs stretched out whereas those alive had their legs flexed. Beetles killed in the treated grains this way probably suggests contact toxicity and not due to ingestion of treated grains. The products were also active against the eggs and larvae of *P. truncatus*. Several previous studies have demonstrated the effectiveness of different plant oils in protecting grains against major stored product insect pests (Don-Pedro, 1989; Kumar and Okonronkwo, 1991; Obeng-Ofori, 1995; Obeng-Ofori and Reichmuth, 1999; Obeng-Ofori and Amiteye, 2000). Although the mode of action of plant oils is not clearly understood, Don-Pedro (1989) suggested that insect death caused by oils is due to anoxia or interference in normal respiration resulting in suffocation (Schoonhoven, 1978).

There was considerable variation in the toxicity of neem oil and neemazal. Neemazal with a dosage of 0.8 ml induced the highest mortality of 100 % after 72 hour exposure compared to neem oil, which induced mortality of 70 % after 96 %. Mortality of beetles increased with time. The action of the two products was dosage-dependent which confirms the findings of Schmutterer (1995) and Ankrah (1998).

The toxicity of neem oil and neemazal on eggs and immature stages was significant ($P< 0.05$). Higher dosages of 0.6 and 0.8 ml of neemazal completely inhibited the development of eggs, early and late larvae. The pupae that were exposed to the products developed into adults and this might be due to lack of penetration of the products through the pupal case.

Both fraction A and B were moderately toxic and caused significant ($P<0.05$) mortality of *P. truncatus*. The toxicity of the fractions reduced when applied separately compared to unfractionated neem oil. However, fraction B was more toxic than A, since the highest dosage of fraction B induced 56% mortality compared to 35 % in the case of fraction A, after 96 h exposure. The activity of the two fractions were also dosage-dependent. The low mortality caused by the two fractions when applied separately suggests probably that they may act synergistically.

5.2 Progeny emergence and damage assessment

Maize grains treated with neem oil or neemazal significantly reduced the number of progeny produced by *P. truncatus*. Neemazal applied at the rate of 0.6 and 0.8 ml completely inhibited progeny production by *P. truncatus*. The number of progeny produced in maize treated with neem oil and neemazal indicates that both products had moderate effect on egg development. This suggests that the products became less potent with storage period enabling adults that survived to lay eggs.

Both neem oil and neemazal applied to the grain protected them against damage by *P. truncatus* compared to the untreated grains. The damage caused by *P. truncatus* was significantly ($P < 0.05$) higher in grains treated with neem oil compared to neemazal. Neemazal protected the grains against feeding by the beetle, with no noticeable feeding damage on grains treated with 0.6 and 0.8 ml. Azadirachtin, the major active compound in neem is known to be antifeedant to most stored products (Ascher, 1993; Mordue and Blackwell, 1993; Schmutterer,1995).

5.3 Persistence of neem oil and neemazal

The activity of both products impregnated in maize grains declined rapidly with storage and did not cause any significant ($P<0.05$) mortality to *P. truncatus* after the treated grains had been stored for more than 24 hours except the highest dosage of 0.8 ml. Presumably, with storage, the chemicals were absorbed by the grain thereby reducing its availability for picking up by the beetles (Tembo and Murfit, 1995). This reaffirms the suggestion by Don-Pedro (1989) that insect death caused by oils is due to anoxia or interference in respiration resulting in suffocation (Schoonhoven, 1978). Thus the longer the duration of contact of treated grains to the beetles the higher the rate of mortality. Low persistence of the products may also be attributed to the rapid degradation of the active compounds, which occurs when exposed to high temperatures and UV light (Schmutterer, 1995). Mixtures of plant oils combined with synthetic insecticides used in stored product protection is known to enhance toxicity and persistency because the oils increases the uniformity of distribution of the toxicant over the grain surface (Amiteye, 1998). The possibility of using reduced levels of oils in combination with synthetic insecticides in simple mixtures as a means of making their use more attractive, effective and economical has been suggested by several workers (Don-Pedro, 1989; Obeng-Ofori, 1995; Obeng-Ofori and Reichmuth, 1999; Obeng-Ofori and Amiteye, 2000).

5.4 Repellency of neem oil, neemazal and fractions

Neem oil and neemazal were moderately repellent to *P. truncatus* relative to the control. Repellency was not related to dosage. The repellent action of neem may be due to secondary metabolites. Secondary plant chemicals attract or repel insects and influence their locomotion, oviposition, feeding behaviour, development and physiological processes as well as behavioural patterns (Beck and Reese, 1976). Fractions A and B obtained from neem oil were less repellent than neem oil.

The repellent action of the products, coupled with the inhibition of the development of eggs and larvae of *P. truncatus* hidden inside grain kernels as well as the significant reduction in progeny emergence in treated grain, increase the potential practical value of neem for grain protection against insect pest attack (Schmutterer, 1995).

However, it should not be mixed with grains which soak up the oil. Again it must be emphasized that poor quality oil from mouldy kernels may contain mycotoxins. Treated grains should be washed thoroughly to remove the oil from the seeds and avoid any bitter taste, especially when the treatment has been carried out less than four weeks before consumption. However, the bitter taste usually disappears with time (Schmutterer, 1995).

5.5 Germination and water absorption of treated grain

The study demonstrated that neem oil and neemazal significantly ($P<0.05$) increased the amount of water absorbed by maize grains. The increase in water absorption by the grains with time is likely to enhance the fineness of milled maize grain. This result is contrary to the findings of Amiteye (1998) who reported that plant oils significantly reduced the amount of water absorbed by maize grains. This might be due to some fraction component(s) in neem which may be hydrophilic.

Germination of seeds were not significantly ($P<0.05$) affected when treated with neem oil but neemazal significantly ($P<0.05$) reduced seed germination. Seeds treated with neem oil had longer radicals than that treated with neemazal. Thus it is not advisable to treat seeds destined for propagation with neemazal. Contrary to the findings of Don-Pedro (1989) and Obeng-Ofori (1995), who reported that plant oils adversely affects germination, neem oil did not affect germination. The differences observed by these workers may be attributed to differences in grain variety and the oil extraction techniques.

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

Neem oil and neemazal were evaluated to determine their effectiveness as grain protectants against *P. truncatus* on maize. Toxicity of both products were high and caused significant mortality of *P. truncatus*. Mortality of the beetles was dose-dependent and related to the duration of exposure. Toxicity of neem oil and neemazal rapidly declined in storage following application. Neemazal was more effective and gave better protection to grains than neem oil. Neemazal, applied at 0.6 and 0.8 ml/kg completely inhibited the development of immature stages of *P. truncatus* except when at the pupal stage, but neem oil only reduced the number of adults that emerged after different stages of treatment. Weight loss caused by *P. truncatus* was significantly reduced when treated with both neem oil and neemazal; neemazal when applied at 0.6 and 0.8 ml/kg gave 100 % protection. Both neem oil and neemazal were moderately repellent to *P. truncatus* causing about 50 % repellency.

The study also demonstrated that neem oil and neemazal significantly increased the amount of water absorbed by maize grains which will enhance the fineness of milling quality of maize grains.

Generally, neem oil and neemazal affected seed germination. Percentage germination of seeds treated with neem oil applied at 0.2 and 0.4 ml/kg were not different from untreated grains after 30 days of storage.

Neem oil obtained from pressing of the ground seeds and that obtained through the use of petroleum ether were similar but the yield of that obtained through the use of petroleum ether was almost twice as that from pressing.

The mode of action of neem oil and neemazal against the beetles needs to be studied in more detail to promote the development of more potent fractions for use as grain protectants. Also certain preservatives could be added to neem oil to prevent the rapid degradation of the active ingredients.

This work was carried out under laboratory conditions which may not simulate actual conditions in the storage environment. It is recommended, therefore, that this work be repeated under warehouse conditions to confirm the findings of this work in order to recommend neem oil and neemazal as grain protectants to farmers.

It is recommended that, these neem products be mixed with synthetic insecticides which are used in stored product environment to enhance their toxicity and persistency as a means of making their use more attractive , effective and economical.

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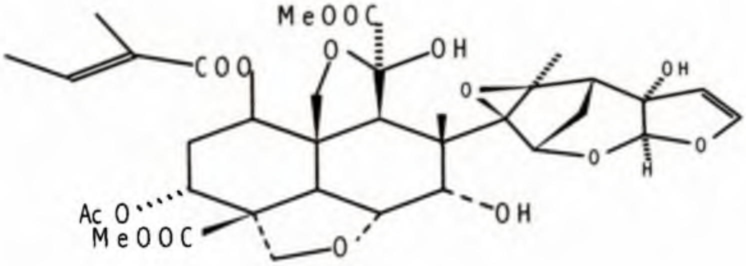
Appendix 1



Azadirachtin, $C_{35}H_{44}O_{16}$