**Influence of Derivatives of Neem Tree (*Azadirachta indica* A. JUSS.)
on the Biology and Behaviour of *Prostephanus truncatus* (HORN)
(Coleoptera: Bostrichidae) and its Predator,
Teretrius nigrescens (Lewis) (Coleoptera: Histeridae)**

DISSERTATION

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Dedication

To my departed brother
Albert Arms Jumah

and my departed nephew
Godfrey Okello Kasanda

Table of Contents

List of Figures

viii

List of tables

List of abbreviations

ø	Diameter
Adj. r^2	Adjusted coefficient of determination
ANOVA	Analysis of variance
CV	Coefficient of variation
d.f.	Degrees of freedom
DMRT	Duncan's multiple range test
FAO	Food and Agriculture Organisation of the United Nations
GTZ	Gesellschaft für Technische Zusammenarbeit (German Technical Cooperation Agency)
H	Heat production
ICIPE	International Centre for Insect Physiology and Ecology
IGR	Insect Growth Regulation
IPM	Integrated Pest Management
MVE	Maize volume equivalent
mW	Milli watt
NAPC05	NeemAzal® containing 5% azadirachtin
NAPCKG10	NeemAzal® containing 1% azadirachtin
ND	Non-detectable
NO	Neem oil
NSCP	Neem seed cake powder
p	Probability of an event occurring
r.h.	Relative humidity
RI	Response index
SE	Standard error
UNEP	United Nations Environmental Programme

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Finally, I wish to thank all that I may not have mentioned who supported me in many different ways for their material and moral support, advice and criticisms.

Summary

The larger grain borer *Prostephanus truncatus,* is a devastating storage pest of especially maize and cassava imported to Africa from Central America in the early 1980s. From its introduction points, it has continued to spread and has now been reported in 16 African countries in which it is now one of the most serious pests of stored products. Its control on small-scale farms, which is mainly by synthetic insecticides, is limited by low income and the the inaccessibility of these insecticides, risks of misuse resulting in toxicity to users and beneficial insects, environmental hazards and insect resistance development. Neem tree *(Azadirachta indica)* products have for long been used in many parts of the world especially Asia and Africa for the control of a wide range of insect pests. The tree is well adapted and is widely grown in many parts of Africa and could therefore serve as a source of safe, cheap and accessible pest control products for small-scale farmers. The objective of this study was to investigate the potential of various neem products, NeemAzal® PC KG 01 and NeemAzal® PC 05 from Germany and neem seed cake powder, leaf powder and oil from Kenya, to control *P. truncatus* and their effects on the pest's natural enemy, *Teretrius nigrescens*.

The lethal effects of neem leaf and seed powder and NeemAzal PC 05 on *P. truncatus* adults were generally not profound. Treatment of maize grains with neem oil resulted in *P. truncatus* mortality of 100% but this declined to non-significant levels in one month while treatment with NeemAzal® PC KG 01 attained 98% mortality, which persisted for more than six months. Both neem oil and NeemAzal® PC KG 01 were highly effective in preventing *P. truncatus* population increase; treatments with 20ml/kg and 6g/kg, respectively, resulted in 0% increase in insect population. Treatment with the same dosage of neem oil also resulted in a 73.6% reduction in the number of eggs laid by *P. truncatus* and nil adult emergence when the treatment was applied post-oviposition. Insect population increase was not significant in neem oil treatments of above 2.5ml/kg. The reproduction of *P. truncatus* was not affected by the neem oil volatiles.

The lethal effect of neem oil on *T. nigrescens* was generally lower than that on *P. truncatus*, 56% and 100% respectively, at 20ml/kg dosage level on maize grains. The predatory ability of *T. nigrescens* on *P. truncatus* was not significantly affected in the presence of neem oil although *T. nigrescens* could not reproduce without provision of additional *P. truncatus*

larvae. In the repellence experiments, both of *P. truncatus* and *T. nigrescens* were not repelled by neem oil volatiles. The volatiles however prevented the attraction of *P. truncatus* to both non-infested and *P. truncatus*-infested maize grains. In the contact repellence experiment, neem oil-treated grains resulted in a repellence response index of -0.62 at the 7.5ml/kg dosage against *P. truncatus.* The response index extends from –1 for compelete repellence to +1 for complete attraction. Where *P. truncatus* were allowed to escape from treated grains, 67.6% escaped from grains treated with the 7.5ml/kg dosage as compared to 8.8% that escaped from the control. A higher percentage of *P. truncatus* adults survived on neem oil-treated flour than on grains, while no first instar larvae survived on the treated flour. It was also observed that the persistence of neem oil on the surface of maize grains was influenced by the variety of maize used.

This study leads to the conclusion that the effect of neem oil and NeemAzal® PC KG 01 depends more on the formulation than on the content of azadirachtin. It can also be argued that the effect of neem oil on *P. truncatus* is mainly antifeedant, and this effect is more pronounced in larvae than in adults and may persist for more than six months. The predatory ability of *T. nigrescens* may not be significantly affected by the treatment of maize gains with neem oil since the resultant control of *P. truncatus* is not adversely affected. It is hereby suggested that neem oil may therefore be utilized, in combination with other pest management practices, in an integrated control strategy against *P. truncatus*.

Zusammenfassung

Der Große Kornbohrer *Prostephanus truncatus* verursacht große Schäden an Mais und Maniok, nachdem er um 1980 aus Zentralamerika nach Afrika vermutlich mit befallener Ware importiert worden war. Seitdem hat er sich in 16 afrikanischen Ländern als sehr bedeutender Vorratsschädling etabliert. Seine Bekämpfung im Farmbereich, die überwiegend mit synthetischen Insektiziden erfolgt, wird durch das niedrige Einkommen der Farmer, die unzureichende Verfügbarkeit von Insektiziden und das Risiko des mißbräuchlichen Gifteinsatzes begrenzt. Dadurch entstehen Gefahren der Vergiftung sowohl für den Anwender und Verbraucher als auch für nützliche Insekten sowie das Risiko der Resistenz bei Insekten und eine Gefahr für die Umwelt.

Produkte aus Teilen des Niembaums (*Azadirachta indica*) werden seit langem in vielen Teilen der Welt, besonders in Asien und Afrika, für die Bekämpfung verschiedener Schadinsekten verwendet. Der Baum ist weit verbreitet und wächst in vielen Regionen Afrikas. Deshalb stellt er eine sichere, preiswerte und leicht zugängliche Quelle von Schädlingsbekämpfungsmitteln für Kleinbauern dar.

In der vorgelegten Studie wird das Potential verschiedener Niem Produkte, NeemAzal® PC KG 01 und NeemAzal® PC 05 aus Deutschland und Niemexpellermehl, Neemblattpulver und Niemöl, als Bekämpfungsmittel gegen *P. truncatus*. und ihre Wirkung auf den natürlichen Gegenspieler des Schädlings, *Teretrius nigrescens*, untersucht.

Die letale Wirkung von Niemblattpulver, Niemexpellermehl und NeemAzal® PC 05 auf Imagines des Kornbohrers war nicht besonders ausgeprägt. Behandlung von Maiskörnern mit Niemöl führte zu 100% Mortalität, die Wirkung schwächte sich aber innerhalb eines Monats auf einen nicht signifikaten Wert ab. Der Einsatz von NeemAzal® PC KG 01 verursachte 98% Mortalität, die auch über 6 Monate Prüfzeit anhielt. Sowohl Niemöl wie auch NeemAzal® PC KG 01 unterdrückten vollständig den Populationsaufbau von *P. truncatus.* Die Behandlung mit Niemöl führte zu einer Verringerung der Anzahl der Eier von *P. truncatus* um 73,6%, sowie zur völligen Unterdrückung von Nachkommen, wenn die Behandlung nach der Eiablage erfolgte. Oberhalb einer Dosierung von 2,5 ml Öl/kg wurde der Populationsaufbau fast vollständig verhindert. Die Vermehrung von *P. truncatus* wurde durch die flüchtigen

Bestandteile des Öls nicht beeinflußt.

Generell war die abtötende Wirkung auf *T. nigrescens* geringer als auf *P. truncatus*, bei einer Dosierung von 20 ml/kg auf Mais bei 56% bzw. 100%. Neemöl schwächte die Wirkung des Gegenspielers nicht ab, allerdings fehlten wegen der Wirkung auf den Wirt Entwicklungsmöglichkeiten für *T. nigrescens*. Die flüchtigen Bestandteile des Öls hatten keine repellierende Wirkung auf *P. truncatus* und *T. nigrescens*, allerdings verhinderten sie die Anlockung von *P. truncatus* sowohl durch befallene als auch unbefallene Maiskörner. Bei den Kontakt-Repellent-Experimenten mit *P. truncatus* erreichten niemölbehandelte Maiskörner einen Repellenzindex von − 0,62 bei einer Dosierung von 7,5 ml/kg. 67,6% der Käfer verließen bei dieser Dosierung im Wahlversuch das Getreide, gegenüber nur 8,8% im Kontrollversuch. Die Verweildauer des Öls auf der Oberfläche der Maiskörner war deutlich von der jeweiligen Varietät des Maises abhängig. Ein höherer Prozentsatz der Käfer von *P. truncatus* überlebte auf niemölbehandeltem Maismehl als auf behandelten Körnern, wohingegen auf dem Mehl keine einzige junge Larve gefunden wurde.

Die vorgelegten Untersuchungen legen den Schluß nahe, dass eher die Formulierung als der Gehalt an Azadirachtin die Wirkung von Niemöl und NeemAzal® PC KG 01 bestimmen. Weiterhin scheint Niemöl auf *P. truncatus* überwiegend fraßhemmend zu wirken, wobei der Effekt auf Larven stärker ist als auf Imagines und länger als 6 Monate vorhält. Auch weil die Wirkung des Gegenspielers durch Verwendung von Niemöl nicht stark beeinflußt wird, sollte Niemöl auch in Kombination mit anderen Methoden der Schädlingsabwehr innerhalb eines integrierten Ansatzes verwendet werden.

1 Introduction

1.1 General

The larger grain borer (LGB), *Prostephanus truncatus* (HORN) (Coleoptera: Bostrichidae), has become one of the most important pests of stored products in Kenya and in many parts of Africa since its introduction from its natural home in Central America and Mexico in the early 1980s (Dunstan and Magazini, 1981). It has since established itself and continued to spread to many countries of Eastern, Central, Southern and Western Africa. Being a pest of mainly stored maize and cassava, it has become a major problem in most areas that produce these crops. In Kenya, *P. truncatus* is important mainly because of the country's dependence on maize as a staple food, the effect on which will affect the majority of the population. Average losses due to the occurrence of this pest in Kenya have been estimated at about 1.2 billion Kenya shillings (15 million US dollars) per year (Pierce and Schmidt, 1992). Even in the absence of these losses, the country is generally a net importer of maize for most of the years.

The damage caused by *P. truncatus* is enormous. Losses of up to 40 and 70% by weight have been reported in maize and cassava, respectively (Giles and Leone, 1975; Hodges, 1985; Anon., 1994). Although the total losses in cassava are generally low due to the farmers' practice of piecemeal harvesting, such losses cannot be completely ignored. In Kenya, the pest was first reported in Taita Taveta district, which borders Tanzania, in 1983 (Kega and Warui, 1983). Initial attempts to control its spread by legislative means failed mainly because of liberalisation of the maize market and the small-scale transportation of maize from infested to non-infested regions. The pest has now spread to many other regions that no single area can be said to be safely free from it, including the large-scale maize growing areas in Rift Valley and Western provinces. Its survival is highly favoured by the warm and humid climatic conditions in many parts of the country.

The pest is currently controlled mainly by synthetic insecticides (Golob *et al.*, 1985; Golob, 1988), which, although effective under large-scale production, have limitations normally associated with the use of such synthetic insecticides especially by small-scale farmers. Most of the maize consumed within Kenya is produced by these farmers who experience such limitations as unavailability of capital and inaccessibility of the chemicals in rural areas. Limited chemical utilisation skills also contribute to poor chemical handling practices and

misuse, which may result in harmful effects both on consumers and on beneficial and non-target species, contamination of the environment and pesticide resistance development (*Golob et al.*, 1990). The most commonly used insecticides today are combinations of organo-phosphates and pyrethroids (Biliwa *et al.*, 1987; Golob, 1988).

Most research is currently focused towards the establishment of an appropriate integrated pest management (IPM) strategy for the control of *P. truncatus*. Much research has been carried out towards this goal, but an IPM cannot be said to have been conclusively recommended. The most promising area of this IPM is the use of *Teretrius nigrescens*, the natural enemy of *P. truncatus,* which was first released in Kenya in 1992 (Giles *et al.*, 1996) for this purpose. Although this strategy was reported to be effective in the control *P. truncatus*, (Borgemeister *et al.*, 1997) recent reports indicate high loses even in areas where the predator has satisfactorily established (Meikle *et al.*, 2002) and show the need for additional measures.

Products of the neem tree, *Azadirachta indica,* a native to India and Burma, have been shown to affect a wide range of insects in various ways. Having been utilised in Asia for centuries in the control of insect pests and diseases, these products has been shown to affect almost all the insects on which they have been tested in different parts of the world, including close relatives of *P. truncatus* such as the lesser grain borer, *Rhyzopertha dominica*. Various products that have been tested include the leaves, bark, roots, seeds and even flowers. They have been found to contain a wide range and varying amounts of bioactive components. These results suggest that neem tree could play an essential part in the integrated management of *P. truncatus*.

The use of neem in pest control offers several advantages. The tree, if grown on the farm, would provide the farmers with products that would be cheap and readily available. Because neem products have been used for a long time, they may be considered to have low or no mammalian toxicity. Being natural products, they are likely to be safe to beneficial insects and the environment and readily biodegradable (Kleeberg, 1992). The tree is also tolerant to poor growth conditions such as low rainfall and low soil fertility. In a successful IPM approach, the safety of beneficial insects, and in the case of *P. truncatus* the safety of *T. nigrescens*, needs to be given high priority since this will determine the success of such IPM. Previous reports have indicated that *T. nigrescens* is even more susceptible to the chemicals used in the control of *P. truncatus* than the pest itself (Golob *et al*. 1990).

Little information exists on the effect of neem products on *P. truncatus* unlike for many other insects. The little information available concentrates on direct toxicity of the neem products to *P. truncatus* adults (Maredia *et al.*, 1992; Niber *et al.*, 1992; Niber, 1995) and does not provide a good basis for comparison since the materials used are not fully described especially with regard to their azadirachtin content. Since it is known that the major cause of bioactivity of neem products is azadirachtin (Ermel *et al.*, 1987; Singh, 1987) and that its content in the products differs with many factors including the agro-ecological origin of the material used, temperature, and humidity (Ermel *et al.*, 1987; Singh, 1987), it is important that further work is carried out to provide as specific information as possible on both direct and indirect effects of neem products starting with known contents of azadirachtin.

1.2 Problem description and study objectives

This study was intended to address the problem of *P. truncatus* as a pest of stored products, especially under small-scale maize production. The study addressed the constraints resulting from small-scale farmers' financial and location limitation of access to conventional pest control chemicals by investigating possible alternative means of control. The difficulty of comparison of the results on effects of different neem products on *P. truncatus* and other insect pests due to lack of standardization was also addressed by utilisation of products with pre-determined azadirachtin content. The study also addressed the problem of variability of results due to differences in environmental factors and cultural practices between regions by focussing on Kenya as the affected region as well as the source of the experimental materials. Although there was quite a significant amount of information on the effects of neem products on very many other insects, information on *T. nigrescens* was totally lacking and that on *P. truncatus* was very limited and non-specific. This study was hence intended also to provide the hitherto unavailable highly specific information on the effects of neem products on reproduction, development and feeding of *P. truncatus* as well as on its predation by *T. nigrescens*.

Hence the specific objectives of this study were:

- To evaluate the effects of various neem products on mortality, reproduction, and feeding of *P. truncatus*

- To determine the effect of neem products on the reproduction and performance of *T. nigrescens* as a predator of *P. truncatus*

- To evaluate the repellent effect of neem oil on both *P. truncatus* and *T. nigrescens*

- To evaluate the persistence of the effects of the neem products on *P. truncatus*

- To investigate the mode of action of neem oil on the larvae of *P. truncatus*

2   Literature Review

2.1   The importance of maize in Kenya

Maize is the most important food crop in Kenya on which the largest proportion of the population depends for nutrition. It is the major source of carbohydrates among all the cereals used and is grown on 90% of the farms (Mbithi and Huylenbroeck, 2000). Despite the country's production of about 2.3 million tonnes of maize per year (Groote, 2002), it remains a net importer because of this dependence. A summary of maize production and utilisation figures is given in Table 2.1. Normally, as illustrated over the given period, domestic utilisation increases steadily while production fluctuates. It has been estimated that maize production will have to grow by 3.0-3.5% annually to satisfy demand (Anon., 2000). This rate of growth substantially exceeds the 2.0% growth in production recorded over the past two decades. A rate of 3.5% cannot be achieved without expanding the maize producing area, as well as intensifying the production to increase yields and minimising losses especially due to diseases and pests.

Table 2.1: Maize production and utilization in Kenya

		Amount (million metric tonnes)				
Year		1997	1998	1999	2000	2001
Production	All cereals	2.7	2.9	2.7	2.6	3.1.
	Maize	2.2	2.4	2.3	2.2	2.7
Domestic utilisation	All cereals	3.8	3.8	4.0	4.2	-
	Maize	2.8	2.8	2.8	3.0	-

(Source: Anon., 2002)

Maize provides a large proportion of caloric needs (Table 2.2) for the majority of rural and urban consumers, although the contribution to the total food requirement is higher in rural areas since urban dwellers have alternative food sources such as bread and rice. The rural population makes up two thirds of the total population (Anon., 2002) although the urban proportion is constantly rising. About 75% of the maize is produced by small-scale farmers (Nyoro, 2002), most of whom retain a fraction of their production for food and seed while the rest is released to the market.

Table 2.2: Importance of maize in nutrient supply in Kenya

Source	Calorie	Protein	Fat
	Per capita supply (grams)		
Total	1,965	50.5	46.9
All cereals	976	26.2	8.6
Maize	705	18.6	7.6

(Source: Anon., 2002)

Maize is used mainly for food in different ways. It is either boiled alone or mixed with beans, or it is milled into flour, which is utilized by mashing into a thick paste called ``ugali´´ or for porridge. The flour is occasionally mixed with millet and/or sorghum flour for this purpose or with wheat flour for baking purposes. Figure 2.1 gives a breakdown of the most important uses and loses of maize in Kenya.
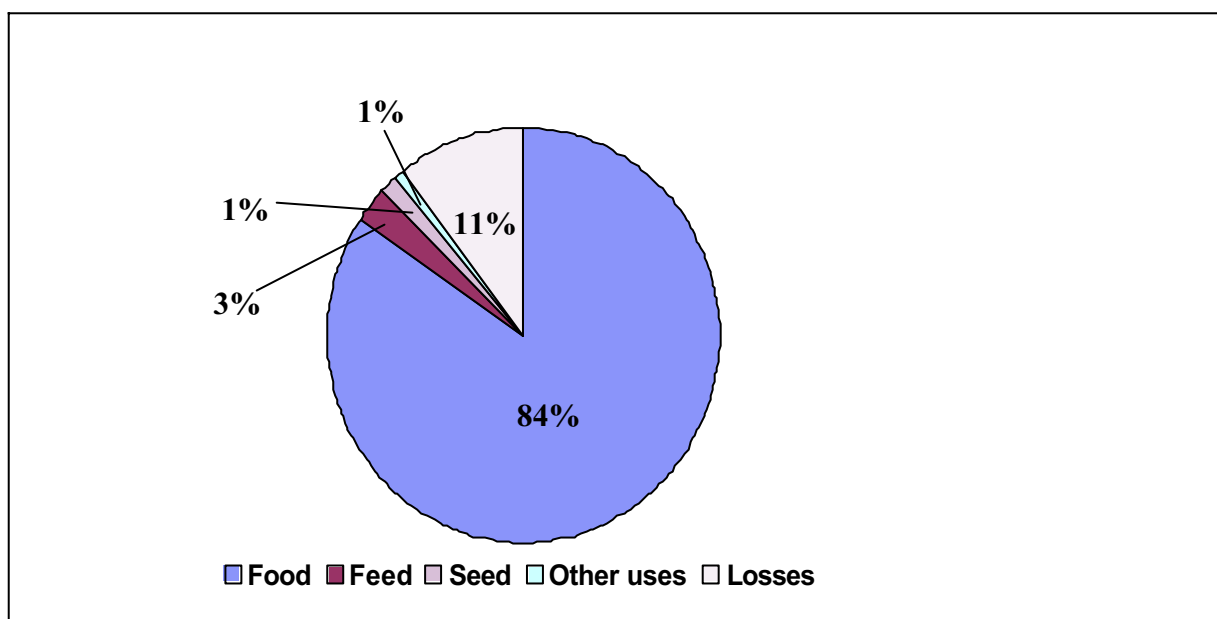
Figure 2.1: Maize utilization and post-harvest losses in Kenya (Source: Anon., 2002)

The above information clearly shows the importance of maize to Kenya and its economy. All efforts need to be made to ensure not only that production is increased but also that losses are minimised.

2.2   *Prostephanus truncatus*: origin, biology and economic importance

Common names and synonymy

Available literature indicates that *P. truncatus* was first described as *Dinoderus truncatus* Horn by Horn (1878). From there on, several related names have been used by different people of different countries and languages. Back and Cotton (1922) referred to it as *Stephanopachys truncatus* before Lesne (1897) suggested the name *Prostephanus* to accommodate this and other species which generally do not infest stored products. The currently common English name is larger grain borer, which was first used by Chittenden (1911). It is commonly abbreviated as LGB, which incidentally leads to confusion with its smaller relative, the lesser grain borer (*Rhyzopertha dominica*). Another English name, the greater grain borer has also been used and was common in Eastern Africa in the period following the pest's introduction (Sekyembe *et al.*, 1993) but is now not widely used since LGB was formally sanctioned by the Entomological Society of America in its list of common names (Sutherland, 1978).

In Spanish, the pest is called "barrenador de los granos" which translates to grain borer (Hodges, 1985). In Tanzania, the pest was originally known by the name "scania", which was officially replaced by "dumuzi", a word in the local Mwezi language meaning robber (Hodges, 1985) and which is now the official name in the Swahili language that is widely spoken in Eastern Africa. Most recently, the name "Osama" has been coined in Kenya.

Origin and distribution

P. truncatus is native to Central America and Mexico where it has been known for long to be a pest of stored maize (Lesne, 1897; Chittenden, 1911) and where it is known to cause minor losses. Giles and Leone (1975) were the first to quantify the losses caused to stored maize in Nicaragua when they reported losses of up to 40% in six months. There are records of the pest in many countries in the new world including USA and Mexico as reviewed by Wright (1984) and Hodges (1985). From this indigenous home, the pest has spread to many other parts of the world where it has either established or merely been reported. In Israel (Calderon and Donahaye, 1962) and Iraq (Al-Sousi *et al.*, 1970), the pest was only found in imported maize

but could not establish. It has also been listed among the pests of Thailand (Sukprakarn, 1976) and found in export grain in India (Verma and Lal, 1987).

The most significant introduction and establishment of *P. truncatus* outside its indigenous home occurred in Africa. The pest was first reported in Tanzania (Dunstan and Magazini, 1981) and it is believed to have been introduced through imported maize during the drought and famine of 1980. It spread rapidly within Tanzania and crossed the border into Kenya where it was first reported in Taita Taveta district (Kega and Warui, 1983), which borders Tanzania. Through international trade and normal beetle flight activity, the pest spread to other countries in the region, namely Burundi in 1984 (Schulten, 1987), Rwanda in 1993 (Bonzi and Ntambabazi, 1993) and Uganda in 1997 (Opolot and Odong, 1999).

The introduction of *P. truncatus* into Malawi in 1991 (Munthali, 1992) is likely to have been as a result of the spread of the pest from Tanzania since the two countries share a common border. In the Southern African countries, the pest has been reported in Zambia (Milimo and Munene, 1993), Namibia (Larsen, 1998) and most recently in South Africa (Roux, 1999).

Another relatively independent outbreak occurred in Togo in 1984 (Harnish and Krall, 1984) and it seems to have led to the spread of the pest in the following ten years to other six countries, namely Benin (Anon. 1986), Guinea (Kalivogui and Mück, 1991), Ghana (Dick and Rees, 1989), Burkina Faso (Bosque-Perez *et al*., 1991), Nigeria (Pike *et al*., 1992) and Niger (Adda *et al*., 1996). Figure 2.1 gives the current distribution of *P. truncatus* in Africa and confirms that many countries, which border the affected ones, are at risk of infestation by the pest. The pest is therefore viewed as a continental problem in Africa.
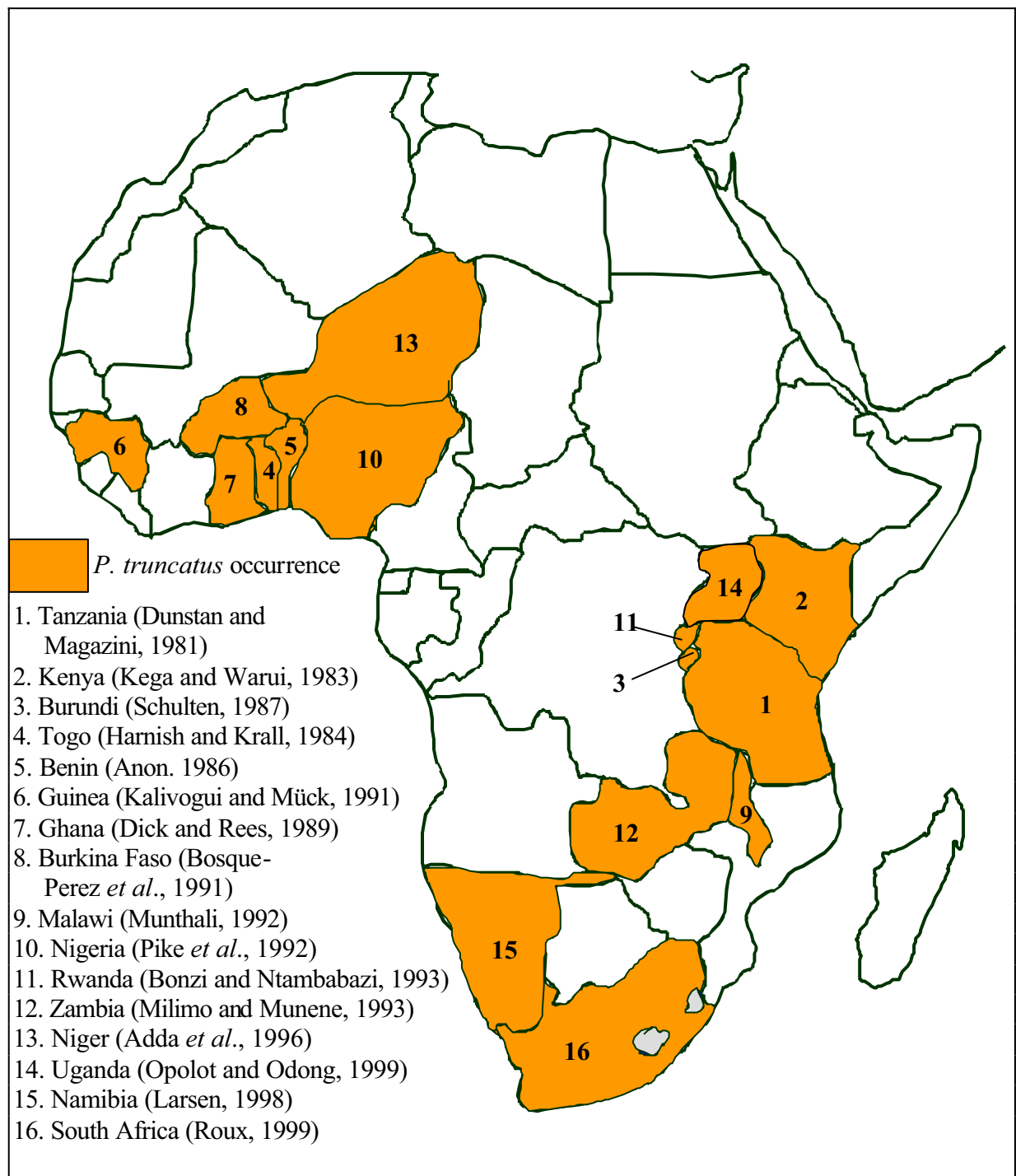
Figure 2.2: Occurrence of P. *truncatus* in Africa (Adapted from Farell, 2000)

1. Tanzania (Dunstan and Magazini, 1981)
2. Kenya (Kega and Warui, 1983)
3. Burundi (Schulten, 1987)
4. Togo (Harnish and Krall, 1984)
5. Benin (Anon. 1986)
6. Guinea (Kalivogui and Mück, 1991)
7. Ghana (Dick and Rees, 1989)
8. Burkina Faso (Bosque-Perez *et al*., 1991)
9. Malawi (Munthali, 1992)
10. Nigeria (Pike *et al*., 1992)
11. Rwanda (Bonzi and Ntambabazi, 1993)
12. Zambia (Milimo and Munene, 1993)
13. Niger (Adda *et al*., 1996)
14. Uganda (Opolot and Odong, 1999)
15. Namibia (Larsen, 1998)
16. South Africa (Roux, 1999)

9

Pest occurrence in Kenya

As described above, *P. truncatus* has been known as a pest of stored produce in Kenya since 1983. The first incidence of the pest was recorded in Taita Taveta district from where it spread to the neighbouring districts of Wundanyi, Voi, Kibwezi and Loitoktok. Ten years after its introduction, it had spread over an area of approximately 50,000 square kilometres, mainly in the low maize producing semi-arid parts of the country. Around this time, it started spreading into the maize surplus areas of Central and Western Kenya. Figure 2.2 shows the occurrence of the pest in 2002.
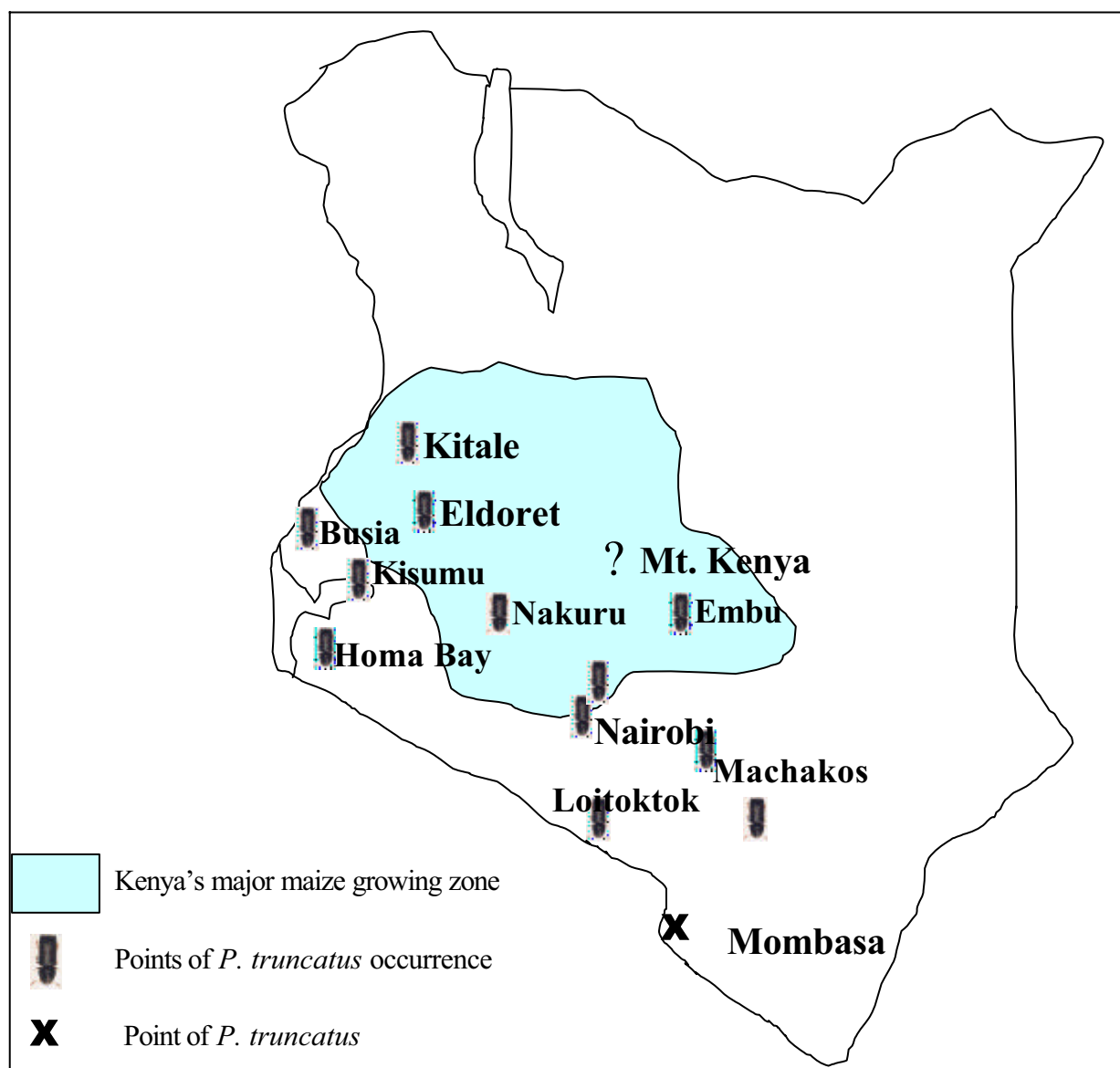
Figure 2.3: **Current spread of P.** *truncatus* **in Kenya (Source: Anon, 2002a)**

10

Characteristics of *P. truncatus*

Taxonomy

P. truncatus belongs to the order Coleoptera, which is the largest of all insect orders. In this order, it falls in the family Bostrichidae, which belongs to a larger group of families, referred to as Bostrichoidea. These families are normally associated with timber and their species are generally very destructive to timber and wood (Hill, 1994). Of the more than 400 species in this family, only less than ten are considered pests of agricultural importance and only three are important pests of stored products. These are *P. truncatus* (larger grain borer), *R. dominica* (lesser grain borer) and *Dinoderus spp.* (bamboo borer).

Morphology

The Bostrichidae adults are normally black or brown in colour, with cylindrically shaped bodies and short antennae with a loose club of three terminal segments (Hill, 1994). *P. truncatus* is easily confused with the other two important Bostrichidae because of the many similarities between them. There are however important features used to distinguish the three. *P. truncatus* is darker brown in colour and larger (3-4mm long) than *R. dominica* (2.5-3.5mm). The posterior end of both insects slopes back but that of *P. truncatus* has two pronounced lateral ridges with sharp edged corners (Figure 2.4), the pest's most distinguishing feature. The anterior end of the head cannot be seen from above, a feature that distinguishes it from *Dinodorus spp*.

The males and females are externally very similar, only very close examination by Shires and McCarthy (1976) revealed some differences in the size of, and the distance between, clypeal tubercles. Their description was used to sex the beetles with up to $86 - 97\%$ accuracy. The females exhibited larger tubercles ($\emptyset = 35.85\mu m$) that were more widely spaced ($118.57\mu m$) than those of the males ($\emptyset = 22.55\mu m$), which were closer together ($106.99\mu m$) (Figure 3.1).
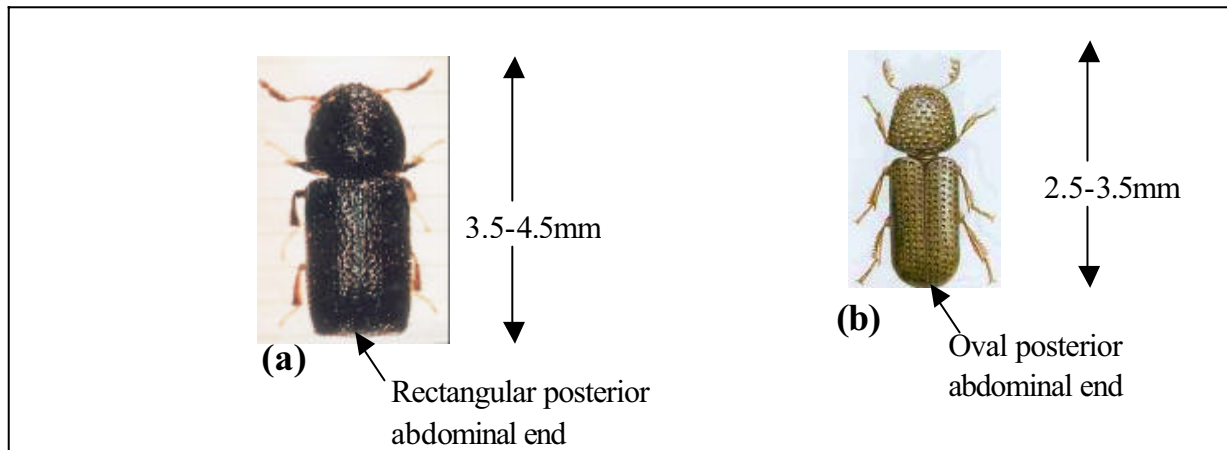
Figure 2.4: Body characteristics of: a) *P. truncatus*   **b)** *R. dominica***. Note the rectangular and oval posterior abdominal ends of the two, respectively**.

Biology and Ecology

The adult female *P. truncatus* lays about 50-600 eggs in maize kernels although the number depends on the prevailing temperature and the availability of food. The eggs hatch into larvae after about five days, which develop into pupae after 17 days and the pupae develop into adult beetles after another seven days (Figure 2.5). The duration of the life cycle has been reported to vary between 27 and 45 weeks (Chittenden, 1911; Genel, 1966; Shires, 1980; Hodges and Meik, 1984; Hashem, 1990; Sekyembe *et al.*, 1993) according to prevailing environmental conditions. There is an average pre-oviposition period of 10 days. The pest has, however, been shown to complete its life cycle even under extreme conditions of temperature and relative humidity such as 18°C at relative humidity of 70% and 25°C at humidity of 40% (Bell and Watters, 1982). This greatly extends the area under which the pest can spread in Kenya and may explain its recent detections in highland areas such as Kitale and Eldoret. Studies in Romania have shown that the pest is also able to survive in Europe (Beratlief, 1998). The insect develops through four larval stages. The first stage feeds on floury endosperm while the second and third prefer germ tissue. Competition is reduced by moving out of the grain (Vowotor *et al.*, 1998)

P. truncatus has also been reported to colonise regions where moisture content is too low to support large populations of other stored products pests (Dobie, 1988), and to survive on grains with as little moisture content as 9% (Pierce *et al.,* 1992; Hodges *et al.,* 1983). However, Shires (1980) also reported that although the pest is a long-lived species with rapid immature development, its finite rate of increase of 1.399 per week was quite low. It has been reported to considerably extend its life span by boring into wood and hence surviving

extended periods in empty grain stores (Detmers, 1990). In Kenya, it has been found widely distributed in woodlands in the Tsavo National Park (Nang`ayo *et al.*, 1993). The beetle has an average life span of 120 days and a maximum of $9^1/_2$ months (Pierce *et al.*, 1992). The lifetime egg production per female is about 430 eggs with their peak laying period occurring between the fourth and eighth week (Bell and Watters, 1982).
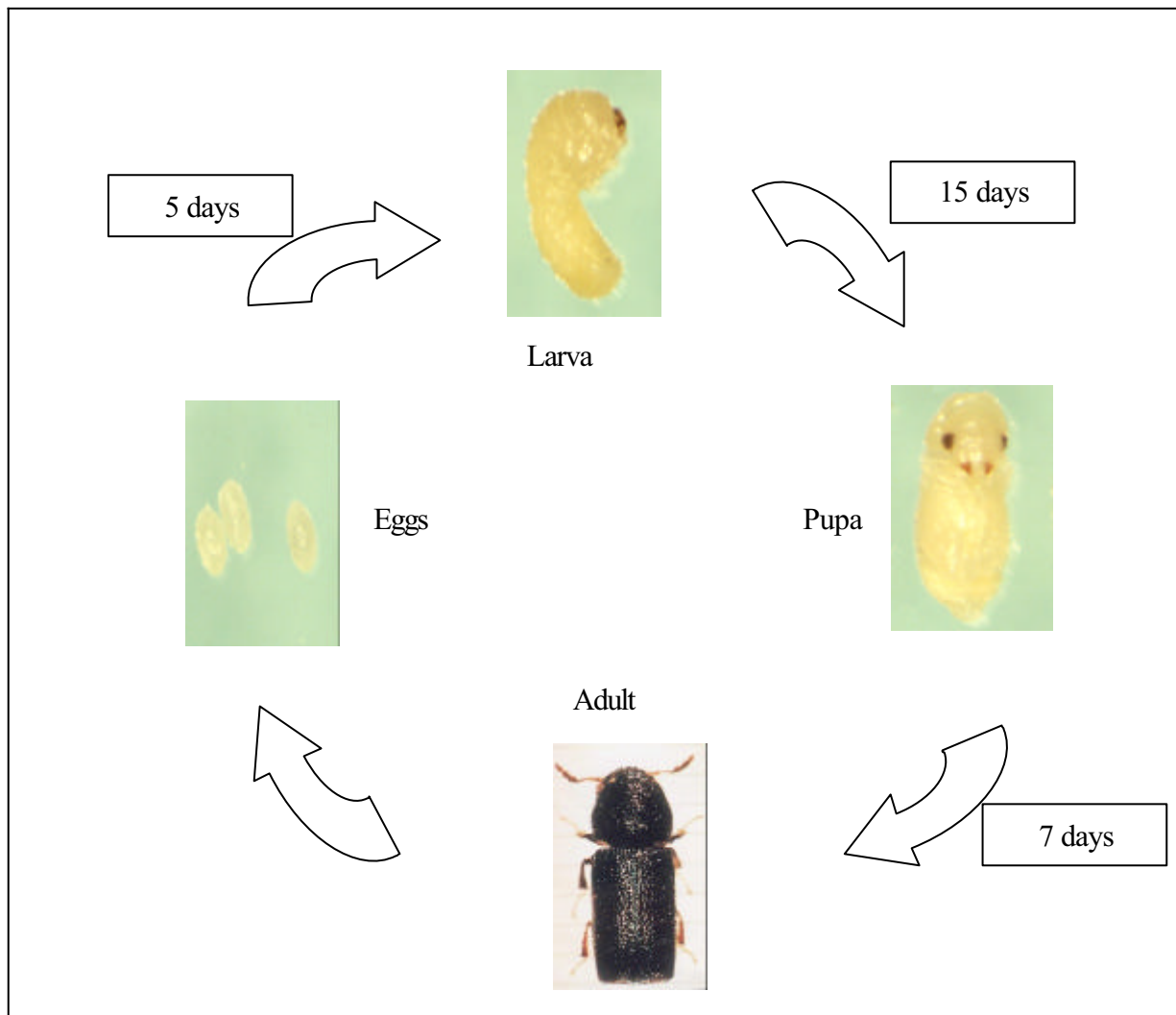
**Figure 2.5: Life cycle of *P. truncatus* under optimum conditions at 30°C and 70%r.h.
(Photos: Eggs, larva & pupa courtesy of Cereal Research Centre of Canada, AAFC)**

P. truncatus has been reported to produce a male aggregation pheromone (Cork *et al.*, 1991), to which both males and females respond. The function of the pheromone is linked to the reproductive biology since it has been demonstrated that the males shut down signalling temporarily in response to a non-volatile chemical signal produced by adult females (Smith *et al.*, 1996), and that the sex ratio of beetles caught when males are used in the traps is normally female biased (Scholz *et al.*, 1997), indicating the search for males by females. The pheromone production is also influenced by the suitability of a host for breeding and feeding. This implies that an unsuitable food source will both discourage aggregation and infestation. Scholz *et al.* (1998) also reported that maize cultures at high densities of infestation elicited no response.

P. truncatus is mainly a pest of maize, infesting both stored and standing crop (Hodges *et al.*, 1983; Giles and Leone, 1975), although the pest has also been shown to thrive on dried cassava (Hodges *et al.*, 1985). The beetle has been reported to breed on wheat (Shires, 1977), chickpea (Hodges, 1985), sorghum (Verma and Lal, 1987) and dried sweet potato (Mushi, 1984). Other products that the pest seems to bore into without feeding include cowpea, cocoa, haricot, coffee and rice. It also bores into many solid substrates regardless of their nutritive quality such as wood, Perspex and polythene (Hodges *et al.*, 1983; Ramirez Martinez and Silver, 1983; also in this study).

The infestation of maize grain starts in the field and much of the damage caused even as late as eight months after harvest is associated with this initial infestation (Borgemeister *et al.*, 1998). It may start when the moisture content is still as high as 40-50% (Giles and Leone, 1975). The adults bore into maize kernels where females lay most of the eggs in blind ending chambers bored at right angles to main tunnels (Howard, 1983; Hodges, 1982). There are three larval instars, which bore and feed while developing (Bell and Watters, 1982). Both the adults and larvae seem to feed indiscriminately within the grain (Howard, 1983; Ramirez Martinez and Silver, 1983). The pest's preference of maize that is still attached to cobs was first reported by Chittenden (1911) and has since been confirmed by Cowley *et al.* (1980), Howard (1983) and Golob *et al.* (1985). The reasons for this preference vary but there seems to be a greater agreement with the suggestion by Bell and Watters (1982) that the pest establishes itself by extension of the tunnels along the rows of tightly packed seeds, a condition that is difficult to attain in a discontinuous medium like loose grain.

Economic importance of *P. truncatus*

Losses caused by *P. truncatus* are normally much higher than those by the conventional pests that Kenyan farmers are used to. In maize, very high weight losses have been reported: 40% in Kenya (Anon., 1994) and Nicaragua (Giles and Leone, 1975) after six months, 60% in Tanzania after nine months (Keil, 1988), and 34% in Tanzania after three to six months of storage (Hodges *et al*., 1983). In cassava, weight losses may be as high as 70% (Anon., 1994; Hodges, 1985). In practice, this may amount to up to 100% loss in food value since such heavily damaged products have no market value (Compton *et al.*, 1998). Comparison with other pests clearly demonstrates the seriousness of *P. truncatus* as a storage pest. For example, in a comparative study, whereas maize infestation by *Sitophilus zeamais* and *Sitotroga cerealella* resulted in a weight loss of 1.6 and 1.3%, respectively, within nine months of storage, *P. truncatus* caused mean overall losses of 9% within six months under the same conditions (De Lima, 1979; Hodges *et al.*, 1983). In another experiment, *P. truncatus* caused an average loss of 88.7% compared to 22.4% caused by *S. zeamais* after six months under artificial infestation of maize cobs by the two (Keil, 1988).

Despite its recent introduction, *P. truncatus* is now regarded as the most important pest of maize in Kenya. Pierce and Schmidt (1992) estimated losses due to *P. truncatus* in Kenya at 1.2 billion Kenyan shillings (15 million US dollars) per year. It is important to note that this was when the pest was confined to only few districts. The current value must be much higher than that considering the recent spread. It is frightening to note that *P. truncatus* has already been reported in major maize growing areas of Kenya. With such high rates of loss, Kenya stands to suffer substantial economic losses because of *P. truncatus* infestation.
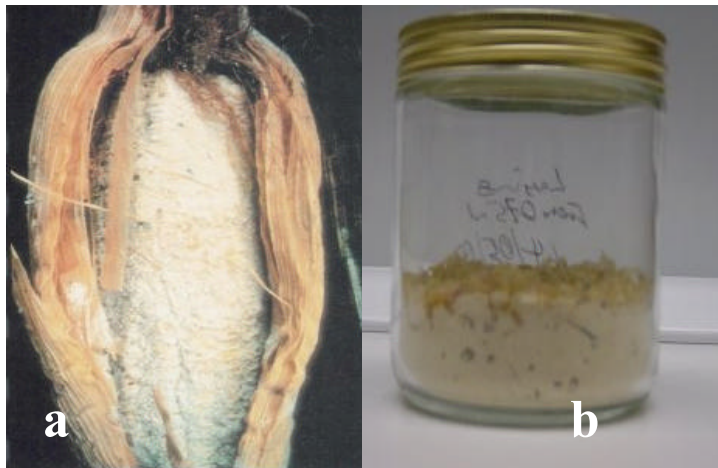
Figure 2.6:
Damage caused by *P. truncatus*:
(a) on cobs
(b) on shelled grains

Current control strategies

When *P. truncatus* was first detected in Kenya in Taita Taveta district in 1983, there were no defined control measures in place. The government embarked on legislation to stop the spread of the pest, but this was not successful since the pest spread soon after its introduction to neighbouring districts and had reached many districts by 1990, because the measures employed could not be effectively applied on small-scale trade and non-trade movements of maize. The leading storage pest chemical at that time, pirimiphos-methyl dust, a contact insecticide that effectively controlled all the common storage pests in Kenya, was found to be ineffective in the control of *P. truncatus*. Golob *et al.* (1985) suggested that this ineffective-ness might be related to rapid loss of active ingredient from the small quantities of maize treated, especially under well-ventilated conditions in the field. Pests such as *Sitophilus spp.*, *Tribolium spp.* and *Sitotroga cerealella* were much more readily controlled by organo-phosphates than by synthetic pyrethroids. Serious research and legislation has led to the approval of new dusts for use against *P. truncatus*. These are mainly a combination of organophosphates and pyrethroids such as Actellic Super® which contains 0.3% pyrethrin and 1.6% pirimiphos-methyl (Golob, 1988; Biliwa *et al.*, 1987). Used at the recommended rate of 50g/90kg maize (standard weight for a bag of maize in Kenya), they achieve acceptable control of the pest.

This chemical control method is not short of limitations, which are normally associated with the use of such synthetic insecticides especially by small-scale farmers, and which are more socio-economic than technical. These limitations include insufficient capital and unavail-ability of the chemicals to the rural farmers (Mück and Bell, 1997; Meikle *et al.*, 1996).

Stabrawa (1993) reported that only 42% of farmers in a survey in South Eastern Kenya used insecticides. There have recently been increased reports of counterfeit products; the vice is estimated to cause 60% of the post-harvest losses in Kenya (Anon. 1999). These problems lead to increased efforts towards finding alternatives to the control of *P. truncatus* with synthetic chemicals.

Natural products have also been tested in the control of *P. truncatus* and reported to affect the pest in various ways. Neem, *Ocimum spp* and *C. odorata* are some of the plant species that have been applied in the control of *P. truncatus* (Obeng-Ofori *et al.*, 1997, Belmain *et al.*, 1999). There have also been indications that plant genetic resistance to *P. truncatus* could be exploited. Maize varieties have been reported to vary in their susceptibility to *P. truncatus*. In an experiment to assess the loss in eight different varieties in Tanzania, Keil (1988) showed clear differences in susceptibility of the varieties, the highest loss by weight being 60% and the lowest 0.5%. Meikle *et al.* (1998) reported that resistance in some varieties of maize to *P. truncatus* tended to be associated with the husk cover and recommended that an ideal maize-breeding programme should include development of maize varieties resistant to insect attack for a long time in the store. Host plant resistance has also been reported in 19 landraces in Mexico (Kumar, 2002) while Arnason *et al.* (1997) reported a negative correlation between weight loss and percentage damage of kernels due to *P. truncatus* and plant chemical compounds. The effectiveness of inert dusts against *P. truncatus* has also been demonstrated by Golob (1997) and Stathers *et al.* (2002). These are among the many options that may be exploited in the future.
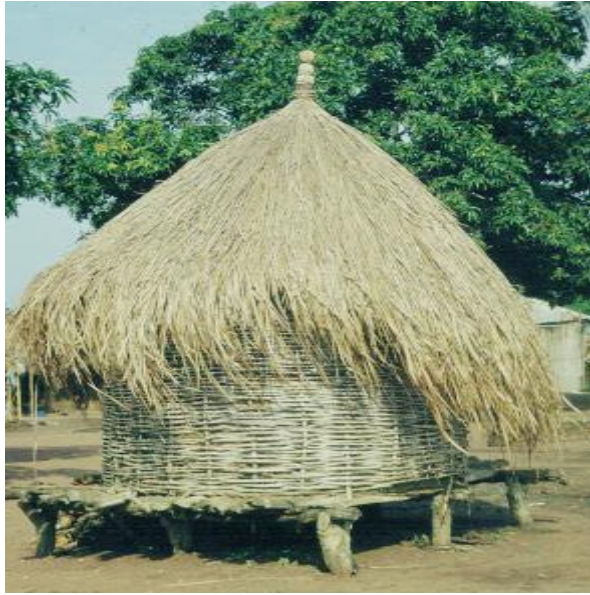
2.3   *Teretrius nigrescens*

Description, origin and distribution

Teretrius nigrescens belongs to the same order as *P. truncatus*, Coleoptera, and to the family Histeridae. It was first described by Lewis (1906), who proposed the name *Teretriosoma nigrescens,* and was initially found in association with *P. truncatus* infesting maize in traditional farmers' stores and with other Coleoptera in Mexico and Honduras (Rees, 1985). Mazur (1997) proposed a revision of the name *Teretriosoma* to *Teretrius* because of limited morphological differences within the genus. *T. nigrescens* was first released in Africa in Togo in 1991 (Biliwa *et al*., 1992; Helbig, 1995). In the East African region, the predator was released in Kenya in 1992 (Giles *et al*., 1996).

(a)   (b)   (c)

Figure 2.8: *Teretrius nigrescens***: (a) adult; (b) larva; (c) larva mandibles. Photos (a) and (b) courtesy of Pöschko, BBA collection.**

Biology and Ecology

Adult beetles are about 3mm long and shiny black in colour. They lay relatively large eggs (1.1 x 0.5mm) singly within the produce. The larvae hatch from the eggs in approximately seven days at 27°C (Rees, 1985), are 2-3mm long and possess a flat head bearing large and strong sickle-shaped mandibles (Figure 2.8c). There are two larval instars each lasting about ten days. A fully-grown *T. nigrescens* larva is relatively large, 1-1.2cm long and slender enough to conveniently predate on *P. truncatus* larvae by following them through their tunnels in the grains. The pupal stage lasts about three weeks, bringing the whole development period to about eight weeks. The predator larvae and adults feed on the larvae of *P. truncatus*. Rees (1985) reported that larvae fed on twice as many *P. truncatus* larvae as did the adults. The predator is able to survive and remain effective in maize grains with as low moisture content as 9%, making it an effective predator for *P. truncatus*, which also tolerates such low moisture content (Hodges *et al.*, 1983). *T. nigrescens* has also been reported to respond to the male aggregation pheromone released by *P. truncatus* (Böye *et al.*, 1992), making it possible for it to follow these to the location of its prey.

Predation on *P. truncatus*

Initial laboratory and field studies showed that *T. nigrescens* was an effective predator of *P. truncatus* (Rees, 1985, 1987, 1990) leading to its eventual release into Africa. The pest was reported to have successfully established in West Africa and to have considerably reduced the populations of P. *truncatus* (Borgemeister *et al.*, 1997). Richter *et al.*, (1997) reported a reduction in losses due to *T. nigrescens* of up to 8l.2% and that the population of *P. truncatus* decreased by 56.4% in the first year of release. In Kenya, test releases were conducted at two sites in 1992. At Makueni, on the hot, semi-arid plains, 2250 *T. nigrescens* were released. The predator established readily and some impact on pest populations in experimental stores was reported. At Wundanyi, a cooler, upland site in the Taita Hills on the other hand, establishment was much slower and no conclusive impact could be reported (Giles *et al.*, 1996).

Nevertheless, as much as the biological control of *P. truncatus* by the use of *T. nigrescens* may be counted as a success, the problem of *P. truncatus* as a pest of stored products cannot be said to have been conclusively resolved. There continues to be a need for complementary

strategies to be employed together with the biological control strategies. This was clearly shown by Meikle *et al*. (1999) who reported that despite the decline in infestation by *P. truncatus*, farmers who used field pesticides increased. Even in the original home of *T. nigrescens,* significant loses could still be reported (Wright, 1984; Giles and Leone, 1975). Recent reports have shown that *P. truncatus* infested 54% of the stores in Benin although *T. nigrescens* was well established in the region (Meikle *et al*., 2002).

Like all other methods of biological control, the use of *T. nigrescens* is slow and very demanding. In the monitoring of the spread of *P. truncatus* in Kenya, pheromone traps are used (Anon., 1994). These traps have always detected *P. truncatus* long before they detect *T. nigrescens*, implying that the rate of spread of *P. truncatus* is much higher than that of the introduced enemy. Borgemeister *et al*. (1997a) have also reported that the flight activity of *P. truncatus* is less associated with weather characteristics than that of *T. nigrescens* implying that the pest may establish better than the predator in the event of adverse weather. In cooler areas, the predator is likely to develop much more slowly as it is more limited by cold than its prey (Tigar *et al*., 1994; Giles *et al*., 1996). There is also a limitation of the predator's application in areas where the pest is mainly controlled by synthetic insecticides since Golob *et al*. (1990) have demonstrated that it is more susceptible to insecticides than the prey.

2.4 Description of the neem tree

2.4.1 Taxonomy, distribution and ecology

Taxonomy

The taxonomic position of neem as described by Adrien Henri Laurent de Jussieu (Schmutterer, 1995) is as follows:

Order	Rutales
Suborder	Rutineae
Family	Meliaceae
Sub-family	Melioideae
Tribe	Melieae
Genus	*Azadirachta*
Species	*indica*

Azadirachta indica A. Juss. is often confused with *M. azedarach* L., the Persian lilac or chinaberry (National Research Council, 1992) due to the taxonomic similarities of the two, but the most important distinguishing characteristic used by farmers is the pinnate leaves of the former as opposed to the bi-pinnate of the latter. Neem has very many common names, many of which are region specific and have various meanings in respective regions. In India, the tree has more than 100 common names. It is also known as Kohomba in Sri Lanka, Nimmi in Pakistan, Nim in Spain, Babo Yaro in Nigeria, Indian Lilac in England, and Indischer Flieder in Germany. The East African name, Muarubaini means "a tree that heals 40 diseases".
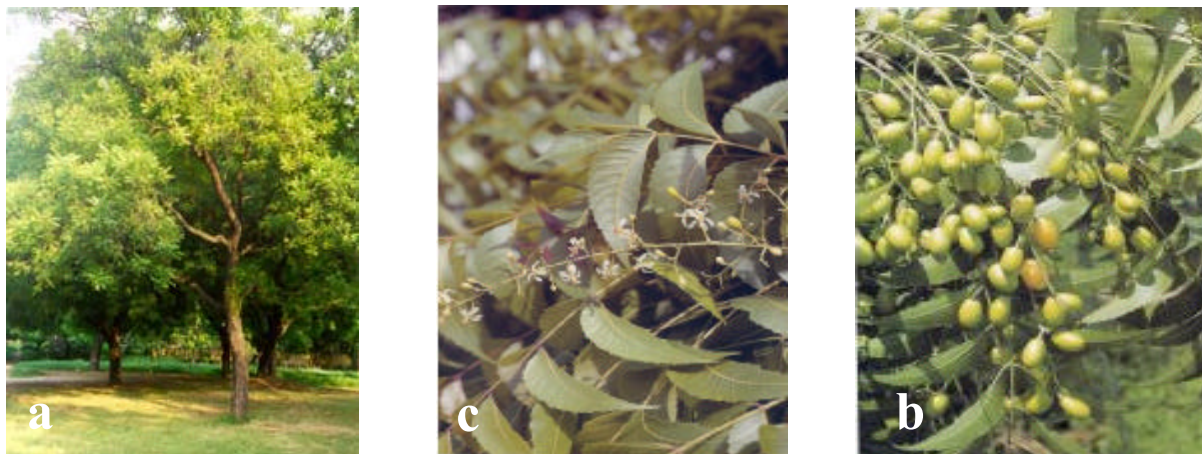
Figure 2.9: **The neem tree: (a) middle age tree, (b) fruits, (c) leaves and flowers (Photos courtesy of Schmutterer, 1995)**

Neem is a fast growing evergreen tree that normally reaches a height of 15-20 metres but under favourable conditions can grow up to 30m in height and 2.5m in girth (National Research Council, 1992). The branches form a beautiful round crown. The trunk is relatively short and straight and has a hard, scaly, whitish grey or reddish brown bark. The leaves are green when mature, and pinnate with short petioles (Schmutterer, 1995). The tree produces white fragrant flowers borne on branching inflorescences with five petals. It is largely self-pollinating although some solitary growing trees seem not to be able to self-pollinate. The fruit is an oval drupe, which is green when young and yellowish-green to yellow when mature. It is made of a thin exocarp, a bittersweet mesocarp and a hard endocarp, which encloses the seed kernels having a brown testa.

Geographic distribution

Neem is thought to have originated in India and Myanmar (Burma) although the exact origin is not known (Troup, 1921; National Research Council, 1992). The tree is now widely distributed by introduction in the tropical and sub-tropical zones of Asia, Africa, America, Australia and the South Pacific islands. In East Africa, the tree is thought to have been introduced by immigrants from the Indian sub-continent. It is widely distributed on the continent generally up to 17° North and can be found in Kenya, Uganda, Tanzania, Ethiopia, Sudan, Egypt, Mozambique, Malawi, Senegal, Nigeria, Chad, Cameroon, and many other African countries.

In Kenya, the tree was first confined to the coastal region where it was first introduced. Until recently, local knowledge of the tree was limited to these areas, but with the increased awareness creation by the national agricultural research and extension services together with such organisations as UNEP, ICIPE and GTZ, the cultivation and utilization of the tree has spread to almost all parts of the country. The tree is currently found in Lamu, Taita Taveta, Kilifi, Mombasa, Malindi, Wajir, Mandera (Förster *et al.*, 2000), Homa Bay, Busia, Kakamega and many other districts. There is daily introduction into new areas.

Ecology

The description of the areas where neem can grow as "almost anywhere in the lowland" (National Research Council, 1992) shows how wide the tree is adapted. It can grow well in areas with annual rainfall of 400-1200mm and at elevations from sea level to 1000m. At higher elevations (1000-1500), growth may be slow. The best soils are deep, well drained and sandy (Schmutterer, 1995) although the range of soils on which it can grow is also wide. According to Fishwick (1970), soil water availability appears to be the most critical factor. Neem can also grow on both alkaline and saline soils, a soil pH of between 6.2 and 7.0 is best but 5.9 and 10.0 may also be tolerated. Optimum temperatures range between 21-32°C. The tree can tolerate temperatures as high as 50°C, but low temperatures below 4°C are unfavourable. In Kenya, based on Geographic Information Systems analysis with rainfall, altitude and soil characteristics, it is estimated that over 25% of the land area is suitable for growing neem (Förster *et al.*, 2000), more than the 8% considered total arable land (Anon., 2002).

2.4.2   Chemical composition of neem products

Neem products have been used for many years, especially in India and neighbouring countries, in the control of insect pests. In these areas, the beneficial properties of neem have been appreciated despite the limited scientific investigations. Most of the scientific work was first conducted after Heinrich Schmutterer's important observation of locusts that did not feed on neem trees in the Sudan in 1959 (Schmutterer, 1995). Since then, many efforts have been made to study the properties of the compounds of neem and their modes of action.   The bioactivity of neem has been found to be as a result of four major compounds in association with about 20 other minor ones. These compounds belong to a general class of natural products called the triterpenes, more specifically, limonoids. Azadirachtin, salannin, meliantriol and nimbin are so far the most significant of all the limonoids discovered.

Azadirachtin (Figure 2.10) was first isolated by Butterworth and Morgan (1968) from ethanolic neem seed extracts. It has now been confirmed to be the major bioactive compound of neem seeds (Mordue and Blackwell, 1993) and the major cause of bioactivity of neem products (Ermel *et al*., 1987; Singh, 1987; Rembold, 1995). The first correct structure was published in 1985 (Kraus *et al*., 1985; Broughton *et al*., 1986). Azadirachtin is a highly oxidised limonoid whose biosynthesis involves a series of oxidation and rearrangement reactions (Mordue and Blackwell, 1993).
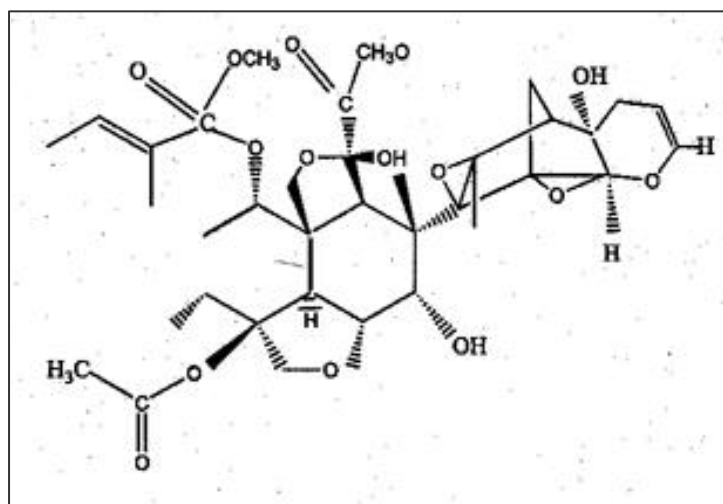
Figure 2.10:
The basic structure of azadirachtin

Neem oil contains most of the azadirachtin found in neem products and is often the leading starting material for the manufacture of insecticides or extraction of azadirachtin. The

bioactivity of neem oil is usually highly correlated to the content of azadirachtin (Isman *et al*, 1990). Important properties of the oil are given below:

Table 2.3: Important physical and chemical properties of neem oil

Specific gravity (g cm^{-1}, 25°C)	0.8300-0.9800
Refractive index	1.4560 (40°C)-1.4675(27°C)
Saponification value (mg KOH g^{-1})	175-200
Iodine value (%)	33.8-192.0
Acid value	2.6-49.0
Fatty acid composition (% by weight)	
-palmitic acid	13.6-25.0
-stearic acid	13.4-27.5
-oleic acid	48.6-69
-linoleic acid	ND-4.8
-arachidic acid	ND-2.0
Azadirachtin content (ppm)	ND-4026

Adapted from Kurmar and Parmar (1997, 1998)

2.4.3   Advantages and disadvantages of utilization of neem products

Several points make neem an important product in the control of *P. truncatus* and insect pests of stored products in general. These include:

Low mammalian toxicity

Neem generally has limited or no toxicity to humans. Many reports have returned neem as a safe product to human beings. Its long-term direct use such as by mixing with food during storage, consumption in medicinal concoctions and utilization of stems as toothbrush is a clear demonstration of this. Neem leaves have also been chewed for a long time without any adverse effects. In safety tests published by National Research Council (1992), neem products, particularly Margosan-O, were found to be safe to mallard ducks, bobwhite quail, rainbow trout, bluegill fish, rats and rabbits. The acute oral LD$_{50}$ of Margosan-O$^{®}$ was in excess of 16ml/kg body weight to mallard ducks and 5ml/kg to rats. This makes neem useful in the control of storage pests since it may be mixed with food and safely consumed.

24

Considering that there are very many cases of chemical poisoning in the world, the use of neem products would reduce cases that occur both during application of chemicals and consumption of treated food.

Safety to beneficial and non-target species

One of the major problems with synthetic insecticides is their toxicity to non-target species. Many synthetic chemicals have well been known to kill beneficial species in some cases leading to emergence of more difficult pest problems. Neem on the other hand has proved to be fairly safe to beneficial species. For example, in their report, Sontakke and Dash (1996) noted that the number of the beneficial pollinator bees, *Apis florea*, in mustard was normal after use of neem insecticides. Neem products have also been shown to be safe against various predaceous spiders and mites (Mansour *et al.,* 1987, 1993, 1997). Schmutterer (1997) concluded that neem products are, despite the effect on numerous pest insects, safe to spiders, adults of many beneficial insects and eggs of predators. Neem could be dangerous to insect predators and parasitoids; this will be considered in this study.

Pesticide resistance

The problem of resistance has increased considerably over the last few decades and has become a major obstacle to increased food production. Champ (1986) reviewed pesticide resistance among stored-product pests and listed 31 storage pests resistant to residual insecticides. The problem of multiple resistance poses even more danger to increased agricultural production. Interestingly, neem has been demonstrated to control agricultural insect pests showing far developed resistance such as *Spodoptera littoralis* (Behera and Satapathy, 1996) and *Bemesia tabaci* (Dimetry *et al.,* 1996). This is partly attributed to the many complex compounds found in neem products (National Research Council, 1992) and the unique mode of action related to growth regulation only in insects. So far, no insect has shown complete resistance to neem products although some cases have been reported, mostly involving refined neem products. Kao ChingHua and Cheng (2001) reported resistance of *Plutella xylostella* against azadirachtin while Vollinger (1987) reported lack of resistance to neem seed kernel extract in the same insect. Sarupa *et al.* (1999) demonstrated resistance of the plant hopper *Nilapartava lugens*.

Availability

Many of the small-scale farmers in developing countries are unable to purchase chemicals for the control of storage pests. In some cases, the chemicals are only available in major towns. Since neem trees can be grown by farmers on their own farms, they would increase the availability and affordability of protective products for stored products. Once farmers have the information on how to use neem products to manage their storage pest problems, they would apply them directly and cheaply.

Wide adaptation

The neem tree is very widely adapted to different growth conditions including poor soils and low moisture. It is adapted to a wide range of climate, from hot weather with shade temperatures of 49°C to as low as 0°C on altitudes up to 1500m (Hedge, 1993). It can also favourably withstand drought and saline conditions. The tree can therefore be grown in most parts of Kenya.

Wide use

Neem has found application in a wide range of areas. This is particularly useful to its acceptability. Neem products are useful for pest control in crop production, medicinals, industrial products, public health, reforestation, birth control, provision of fuel wood, provision of timber and soil fertility improvement among other uses (National Research Council, 1992).

Some constraints in the use of neem products

The utilization of neem products is not without constraints and limitations. It is important to view these limitations and constraints as challenges whose solving could create new opportunities in the utilization of neem products. Lack of standardization of neem products poses one of the greatest limitations in their use. It is difficult to recommend specific dosages since the products differ considerably in their contents. Related to this standardization problem is safety and regulation concerns. The complex mixture of chemical substances makes safe use recommendation very difficult (Weaver and Sumbramanyam, 2000). The registration of neem products is hence implemented with great caution. Several products have been registered especially in USA, Germany, Australia and India, although mostly not for

direct use on food products. Handling and application of crude neem products is also difficult since bulky quantities are involved. Hence, the use of such products in large-scale agriculture may not be practical in the near future. Some neem products have also been reported to be unstable, degrading fast under the sun's ultraviolet rays (National Research Council, 1992). Neem products are also slow acting, and occasionally result in incomplete mortality, compared to conventional synthetic insecticides and may hence not be readily acceptable to farmers.

2.4.4 Effects of neem products on insects

The effects of neem products on insects are best discussed specifically as the effects of azadirachtin and generally as the physical effects and effects of other compounds. They fall mainly into three categories:

Antifeedancy

An antifeedant was described by Munakata (1977) as a chemical that inhibits feeding but does not kill the insect directly. The insect may remain near the food material and die of starvation. Through elaborate bioassays, antifeedants can further be categorised into three groups (Warthen and Morgen, 1990): (1) repellents -repel insects away from the material, (2) suppressants -inhibit the initiation of feeding, (3) deterrents -deter the continuation of feeding. Repellents may be either olfactory, stimulating olfactory receptors in the vapour phase, or gustatory, acting upon receptors that are not normally receptive to vapours but are sensitive to food stimuli (Warthen and Morgen, 1990). The antifeedant effect of azadirachtin was first demonstrated by Butterworth and Morgan (1968) and since then, the effects of neem and azadirachtin have been reviewed by several authors (Mordue and Blackwell, 1993; Blaney and Simmonds, 1995; Mordue, 1998). It has been reported to affect feeding through chemo-reception and through a reduction in food intake due to toxic effects if consumed (Mordue and Blackwell, 1993). From the wide variety of products tested, from pure azadirachtin to crude azadirachtin containing products, it is difficult to make direct comparisons of the susceptibility of different insect species. Comparable protocols however show that Lepidoptera are the most sensitive (effective antifeedancy range from <1-50ppm), the Coleoptera, Hemiptera and Homoptera are less sensitive (100-600ppm) and Orthoptera show a very wide range of sensitivity (<1-1000ppm) (Mordue and Blackwell, 1993).

Insect growth regulation (IGR)

Neem products have been reported to cause growth inhibition, malformation and mortality especially when applied to the larval stages of many insects. Typical IGR effects include slowed growth, delayed moulting, moult abnormalities, inability to complete moulting, insects remaining as "over-aged" larvae for a greatly extended period of time, and mortality (Mordue and Blackwell, 1993; Mordue, 1998). Generally, IGR effects are more consistent between species than antifeedancy effects.

Reproduction

Neem and azadirachtin have shown several adverse effects on ovarian development, fecundity, and fertility of various insects as reviewed by Karnavar (1987). Azadirachtin has been shown to inhibit oogenesis and ovarian ecdysteroid synthesis in *Locusta migratoria* (Rembold and Sieber, 1981). Reduced fecundity was demonstrated in *Spodoptera exempta* (Tanzubil and McCaffrey, 1990), *Oncopeltus fasciatus* (Dorn *et al*., 1987), *Cordyceps capitata* and *Liriomyza trifolii* (Parkman and Pienkowski, 1990) among many other insects. Otto (1997) also demonstrated that neem ingredients suppressed vitellogenin and sex pheromone production in the Colorado potato beetle.

Physical effects

Physical effects are generally not very specific and are usually reported on whole neem products such as oils and powders. Again, only through elaborate bioassays is it possible to distinguish between physiological and physical effects. Such effects as oviposition deterrence, settling deterrence, interruption of hatching and even feeding inhibition may be associated with unfavourable physical conditions of the target product.

2.4.5   Mode of action of neem products

Antifeedancy

The instinct to secure the right type of food depends in most insects on the chemical senses, which are normally governed by the responses of the insect's taste (gustatory) and olfactory sensilla. Feeding behaviour depends upon neural input from contact receptors located on especially the tarsi, mouthparts and oral cavity. Specific elements of feeding behaviour are

only triggered when chemo-receptors detect a particular configuration of chemical stimuli (Schoonhoven, 1990). Much of the detailed electrophysiological studies have only been undertaken on locusts and Lepidopterans, but may serve to demonstrate the general mode of action of azadirachtin and neem products on insects.

Azadirachtin has been shown to stimulate a deterrent neurone, one that will normally respond to antifeedant compounds like azadirachtin, in two species of locust, *Locusta migratoria* and *Schistocerca gregaria* (Haskell and Schoonhoven, 1969; Blaney, 1981) and in many other insects (Blaney and Simmonds, 1988, 1990; Schoonhoven, 1982; Simmonds and Blaney, 1985). Because other neurones have also responded to other phagostimulants like sucrose (Schoonhoven, 1982), the magnitude of responsiveness of deterrent neurones must be influenced by a host of phagostimulant and deterrent neural inputs. Hence, antifeedancy will also depend on the product on which the deterrent is applied. This was demonstrated by Raffa (1987) who showed that the efficacy of azadirachtin varied depending on the crop on which it was prayed. The conflict between deterrent and phagostimulant neural inputs is handled differently between insects giving a labelled line response, in which deterrent input takes precedence over phagostimulatory input, and an across-fibre response where the relative abundance of each type of input is important (Blaney, 1981). Generally, polyphagous insects tend to exhibit the latter form of response in which increased deterrence is related to an overall increase in the rate of change of firing of chemo-receptors. In oligophagous insects, there is a "gating" mechanism whereby feeding is not initiated if sensory input is below a certain threshold (Mordue and Blackwell, 1993). This difference is important since *P. truncatus* exhibits some polyphagous behaviour.

Insect growth regulation

To understand the mode of action of azadirachtin with respect to growth and metamorphosis, it is important to have a general understanding of the hormonal control of growth and development in insects. Hormones are responsible for the regulation of growth and development. The most important are the moulting hormones (ecdysteroids) and the juvenile hormones, which are synthesized in the prothoracic glands. The synthesis of ecdysone is triggered by the prothoracicotropic hormone (PTTH), which in turn is synthesized in the lateral neurosecretory cells and released through the *corpus cardiacum* and *corpus allatum*. During the feeding period of the larva, juvenile hormone inhibits ecdysone synthesis

(Chapman, 1998). The juvenile hormone balance is achieved by two types of neuropeptides, the allatotropin, which activates the synthesis of the hormone, and the allatoinhibin, which is its antagonist (Rembold, 1995).

A major action of azadirachtin seems to be the modification of the haemolymph ecdysteroid titres. Contrary to earlier expectations, it has been shown that azadirachtin does not act directly on prothoracic glands and it also does not bind to ecdysteroid receptors (Bidmon, 1984; Koul *et al.,* 1987; Koolman *et al.*, 1988). Azadirachtin depresses the synthesis of neurohormones from the brain as well as their release from the *corpus cardiacum*. IGR effects of azadirachtin manifest as developmental aberrations in immature insects and are both dose and time dependent; can cause death before and during the moult, or delay of the moult (Rembold, 1995). Azadirachtin also inhibits the synthesis and release of juvenile hormone possibly by affecting the release of allatotropins into the *corpus allatum*. Many of the manifestations of azadirachtin effect on moulting may therefore be linked to the balance between both the presence and absence of ecdysone and juvenile hormone. These include delay in moulting (Nicol and Schmutterer, 1991; Langewald and Schmutterer, 1992), lack of differentiation of tissues (Schlüter, 1985, 1987), black spots (Hori *et al*., 1984; Malczewska *et al*., 1988) and supernumerary moulting (Freisewinkel and Schmutterer, 1991).

Other effects

Apart from antifeedancy and IGR, which are the most prominent and most widely reported effects of neem and azadirachtin on insects, there are several other manifestations of the mode of action, both conclusive and inconclusive.

Reproduction: Several modes of action can lead to reproductive effects in insects. Starvation has been shown to induce effects that may suggest disturbance of the neuro endocrine system and hence affect hormonal control of fecundity. The change in hormonal titters may delay vitellogenin synthesis and hence affect egg production (Sayah, *et al*., 1996; Otto, 1997).

Mitosis: Schlüter (1987) demonstrated that azadirachtin affected mitosis directly leading to complete degeneration of wing discs. Inhibition of cell proliferation and RNA synthesis has also been reported (Fritzsche and Cleffmann, 1987).

Effect on muscles: Insect muscle has been shown to be affected by azadirachtin. In the mid-gut of *L. migratoria* and *S. gregaria*, the muscles become swollen and disrupted in a dose and time dependent manner after azadirachtin treatment (Cottee, 1984). The mitochondria appear swollen and often burst and the passage of food through the gut is affected. The guts of treated insects lack tone, the mid- to hind-gut junction is flaccid and co-ordinated peristalsis may be lacking. Azadirachtin-treated adult locusts have also been reported to be sluggish and to show reduced locomotion and flight activity (Mordue and Blackwell, 1993). Azadirachtin is thought to exert its effect by reducing muscle tone probably by affecting the firing patterns of motor neurones.

2.4.6   Neem and pests of stored products

General effects

The use of neem products to control pests of stored products has been practiced since early years, especially in India and the neighbouring countries. Early records from India show that neem leaves were spread in 5-7 inch thick layers in grains for pest control (Saxena, 1995). The traditional use of neem for control of pests of stored products may differ with the region or cultural background of the farmers involved (Saxena, 1995). The effect of neem products on pests of stored products has been reviewed by several authors (Jotwani and Srivastava, 1981; Schmutterer, 1988; Saxena *et al.*, 1989; Singh, 1993; Saxena, 1995). Almost all pest species on which neem products have been tested have been found to be susceptible, what differs is their individual sensitivity (Saxena, 1995) depending on the species of the insect, the neem product used and the stored product on which it is applied. Table 2.4 gives a summary of the important pests of stored products in Kenya that have been reported to be susceptible to different neem products.

Effects on *P. truncatus*

From the information presented in Table 2.4, it is clear that there is very little information on the effect of neem products on *P. truncatus*, at least compared to other insects. The first report seems to have been the one by Maredia *et al.* (1992) who reported a non-profound effect by neem oil at 5-10ml/kg. Niber *et al.* (1992) also reported that *P. truncatus* showed high endurance to neem leaf and seed extracts and that the death rate did not increase with time. Further work by Niber (1994, 1995) showed that neem oil, seed powder and slurries

significantly reduced insect population increase and reduced grain weight loss. The powders were also reported to cause mortality. More recently, Belmain *et al.* (1999) reported that *P. truncatus* was repelled more than *R. dominica*, *S. zeamais* and *C. maculatus* by stored maize treated with neem plant materials. This seems to be just about all that has been reported and not much deductions can be conclusively made from it. What is the source of the contradiction in the reports? What really happens to *P. truncatus* that leads to the reduction in population that has been reported? How do neem products give the protection that has been reported? What is the effect of neem products on other stages other than adults?

This study was intended to address these issues and provide information on issues that were not clearly addressed by previous studies and available literature. The methodologies of the study were designed to ensure maximum capture of missing information. The standardization of neem products used with respect to azadirachtin content was unique to this study and was intended to ease the comparison of different but related results. Special attention was paid to the larval stage of *P. truncatus* since no information was available on the effects of neem products on this stage yet it played a significant role in determining the rate of insect population increase. The study was also designed to provide very specific evaluation of both lethal and repellent volatile effects of neem oil since the information available was insufficient and contradictory. The distinction between contact and volatile repellence was also unique to this study. Another unique aspect of the study was the intended provision of fast-hand information on the effect of neem oil on predation on *P. truncatus* by *T. nigrescens*. This information would be very useful in possible future application of neem products for the control of *P. truncatus* in areas where *T. nigrescens* is established. Other areas in which the study was intended to provide new information involved effects of NeemAzal® products, persistence of the effects of neem products, micro-calorimetric observations and antifeedant and repellent effects.

Table 2.4: Reported effects of neem products on stored-product pests common in Kenya

Insect	Host/Food	Neem Product	Effect					Reference
			AF	GM	RE	MO	OT	
Acanthoscelides obtectus	Bean	A, O, SP	✔	✔	✔		✔	Diezmann, 1986
Callosobruchus analis	Gram	O	-	-	✔	✔	-	Yadav, 1985
	x	LE, SE	-	-	-	✔	-	Naqvi, 1987
Callsobruchus chinensis	Gram	O	-	-	✔	-	-	Sangappa, 1977
	Chickpea	O	-	-	✔	✔	-	Das, 1986, 1987
	Gram	O	-	-	✔	-	-	Babu *et al.*, 1989
	Gram	LE, SE	-	✔	✔	-	-	Islam, 1987
	Gram	LE	-	-	✔	-	-	Ambika *et al.*, 1981
	Cowpea	LP	✔	-	-	-	-	Yadava and Bhatnagar, 1987
	Gram	O	-	-	-	✔	-	Jacob and Sheila, 1990
	Gram	O	-	-	✔	✔	-	Ali *et al.*, 1983
	Gram	LE	✔	-	✔	-	-	Pandey *et al.*, 1986
	Gram	O	-	-	✔	✔	-	Yadav, 1985
Callosobruchus maculatus	Cowpea	SP	-	✔	✔	-	-	Ivbijaro, 1987
	Cowpea/Gram	O	-	-	✔	-	-	Ketkar, 1987
	Cowpea/Maize	SE	-	-	✔	-	-	Makanjuola, 1989
	Cowpea	SP	-	✔	✔	-	-	Ivbijaro, 1983
	Cowpea	O	✔	-	-	✔	-	Zehrer, 1984
	Gram	O	-	-	✔	✔	-	Yadav, 1985
	Cowpea/Gram/Bean	SP	✔	-	-	-	-	Jotwani and Sirkar, 1967
Cryptolestes ferrugineus	Maize	LP, SP	-	-	-	✔	-	Pereira and Wohlgemuth, 1982
	Flour-disc	M-O	✔	✔	✔	✔	-	Xie *et al.*, 1996
	x	A	✔	-	-	✔	-	Xie *et al.*, 1995
Oryzaephilus surinamensis	Rice	x	✔	-	-	-	-	Prakash and Rao, 1986
	Rice	LP	-	-	-	-	✔	Mishra *et al.*, 2000
Prostephanus truncatus	Maize	x	✔	-	-	✔	-	Belmain *et al.*, 1999
	Maize	O, SS	-	-	-	-	✔	Niber, 1995
	Maize	LP, SP, SS	-	-	-	✔	✔	Niber, 1994
Rhyzopertha dominica	Wheat	LE	-	-	-	✔	-	Jaipal, 1984
	Maize	LP, SP	-	-	-	✔	-	Pereira and Wohlgemuth, 1982
	x	LP	✔	-	-	-	-	Singh, 1993
	Wheat	LE, SE	✔	-	-	-	-	Shams *et al*, 2002
	Wheat	LE	✔	-	-	✔	✔	Amin *et al.*, 2000
	Wheat	A	✔	-	✔	-	-	Muda and Cribb, 1999
	Wheat	x	-	✔	✔	-	-	Singh *et al.*, 1996
Sitophilus granarius	x	LP	✔	-	-	-	-	Singh, 1993
Sitophilus oryzae	wheat	LE, LP, SE, O	-	-	-	✔	-	Mukherjee *et al.*, 1990
	Maize	LP, SP	-	✔	✔	-	-	Perreira and Wohlgemuth, 1982
	Rice	LE	✔	-	-	✔	-	Owusu, 2001
	wheat	LP	✔	-	-	-	-	Ahmed *et al.*, 1980
	Flour-disc	M-O	✔	✔	✔	✔	-	Xie *et al.*, 1996

Insect	Host/Food	Neem Product	AF	GM	RE	MO	OT	Reference
Sitophilus oryzae	x	A	✔	-	-	✔	-	Xie *et al.*, 1995
	Maize	x	-	-	✔	-	✔	Singh, 1993
	Maize	SP	-	-	✔	✔	✔	Ivbijaro, 1983
	Maize	LP, SP, O	-	-	-	✔	✔	Sharma, 1999
	Paddy	LS	-	-	-	✔	-	Prasantha, 2002
	Rice	SP	-	-	-	✔	-	Rao and Sarangi, 1998
	Rice	CF	✔	-	-	-	-	Harish *et al.*, 2000
Sitophilus zeamais	Maize	O	✔	-	✔	-	-	Cobbinah, and Appiah-Kwarteng, 1989
	Maize	x	-	-	✔	-	✔	Singh, 1993
	Maize	O, SP	✔	-	-	✔	-	Akou-Edi, 1984
	Maize	LP, SP	-	✔	✔	-	-	Pereira and Wohlgemuth, 1982
Sitotroga cerealella	Wheat	O	-	-	✔	-	-	Verma *et al.*, 1985
	Maize	O	-	-	-	✔	✔	Sharma, 1999
	Maize, Paddy	SP, LP	-	-	-	-	✔	Yadu, *et al.*, 2000
Tribolium castaneum	Milled products	SE, SP	-	-	✔	✔	-	Singh, 1993
	Wheat flour	A	-	✔	-	-	-	Mukherjee *et al.*, 1993
	Flour-disc	M-O	✔	✔	✔	✔	-	Xie *et al.*, 1996
	x	A	✔	-	-	✔	-	Xie *et al.*, 1995
	x	O	✔	-	-	-	-	Mohiuddin *et al.*, 1987
	Rice	O	✔	-	-	-	-	Jilani *et al.*, 1988
	Wheat flour	M-O	-	✔	-	-	-	Jilani *et al.*, 1988
	x	A	-	✔	-	-	-	Marco *et al.*, 1990
	Wheat flour	FE, LE, SE	✔	-	✔	-	-	Jilani and Malik, 1973
	x	SV	-	-	✔	-	-	Maheswaran and Ganesalingam, 1988
	x	A	✔	-	-	-	-	Malik *et al.*, 1984
	Jowar	x	-	-	✔	-	-	Yogita, *et al.*, 2001
	Rice	x	✔	-	-	-	-	Prakash and Rao, 1986
	Rice	LE	✔	-	-	✔	-	Owusu, 2001
Tribolium confusum	Maize	O, SP	-	-	-	✔	-	Akou-Edi, 1984
	Wheat	O, SE, SP	✔	-	-	-	-	Maurer, 1985

A	= azadirachtin	MO	= mortality	RE	= reproduction
AF	= antifeedancy	OT	= other effects	SP	= seed powder
CP	= commercial product	O	= oil	SV	= seed volatiles
FE	= Flower extract	RE	= reproduction	x	= information missing
GM	= growth and	SE	= seed extract	✔	= observed effect
	metamorphosis	SS	= seed slurry	-	= effect not tested
L	= leaves	MO	= mortality		
LE	= leaf extract	OT	= other effects		

3 Materials and methods

3.1 The neem products

Type and source

The neem products used in this study were neem seed cake powder, neem leaf powder and neem oil, obtained from the International Centre for Insect Physiology and Ecology (ICIPE), Nairobi, Kenya, and NeemAzal® PC KG 01 and NeemAzal® KG 05, provided by Dr. Hubert Kleeberg of Trifolio GmbH, Lahnau, Germany. Neem oil and neem seed cake powder were prepared in Kenya by pressing neem seed kernels of the Kenyan neem tree, collected from the coastal and north eastern regions in 2000, using a manual oil expeller. The expeller removed oil from the kernels by a simple squeezing technique. This process resulted in neem oil and neem seed cake. The latter was then ground into neem seed cake powder. The method of preparation of the products is described in detail by Förster *et al.* (2000). Neem leaves were harvested and dried under shade and then ground into powder. The products were stored under refrigerated conditions at 5°C before the experiments and while they were not being used to avoid degradation of the active ingredients.

Determination of azadirachtin content

The azadirachtin content in the Kenyan neem products was determined at Trifolio GmbH by High Performance Liquid Chromatography (HPLC). The results are shown in Table 3.1.

Table 3.1: Content of azadirachtin in various neem products

Neem Product	Azadirachtin content in mg/g
Neem leaf	0.03 ± 0.005
Neem seed cake powder	0.07 ± 0.003
NeemAzal® PC 05	5.00
NeemAzal® PC KG 01	1.00
Neem seed oil	0.29 ± 0.0009

The content of the German products was as per manufacturer's specifications.

Preparation of dosages

Unless stated otherwise, three dosages of all the products, i.e. low, medium and high, were used in the experiments. 10ml/kg neem oil in maize was taken as the medium dosage. The amount of azadirachtin in this dosage was therefore taken as the medium content for all the products used while the low dosage was half and the high dosage was double this amount. This was intended to provide the same amount of azadirachtin at each of the levels used. Table 2 gives a summary of the calculation of the dosages. Neem leaf powder was discarded due to its poor performance in the preliminary experiments where treatment with 100g/kg resulted in 4.2% adult mortality, which was not significantly different from the control.

Table 3.2: Dosages of the four neem products on maize samples

Product	Amount per kg maize					
	Low		Medium		High	
	aza (mg)	Product	aza (mg)	Product	aza (mg)	Product
Neem seed cake powder	1.5	25.0g	3	50.0g	6	100.0g
NeemAzal®PC05	1.5	0.3g	3	0.6g	6	1.2g
NeemAzal®PCKG01	1.5	1.5g	3	3.0g	6	6.0g
Neem oil	1.5	5.0ml	3	10.0ml	6	20.0ml

aza - azadirachtin

3.2   Insect cultures

Source of insect cultures

Both *P. truncatus* and *T. nigrescens* used in this study were obtained from cultures maintained at the Institute for Stored Product Protection of the Federal Biological Research Centre for Agriculture and Forestry, Berlin, Germany. They were reared for more than ten years on shelled yellow maize at 25°C and 60-65%r.h. in two-litre glass jars, starting with 500 adult insects of mixed age on 500g of maize in each jar. The jars were covered using plastic stoppers reinforced on the inside with a 0.5mm gauze to prevent the insects from chewing through them.

Preparation of experimental cultures

The adults of ages between one and seven days to be used in the experiments were obtained by setting 500 unsexed adults on 500g of maize in two-litre glass jars as described above and allowing them to lay eggs for 3-5 days (Detmers, 1993). Because of the continuous laying and hatching exhibited, it was very difficult to remove all the adults from the grains to ensure that all the beetles used were of known age. Hashem (1990) reported that the insects could be removed by exposing the grains to 75°C in an oven for three minutes, but this method was found not to be suitable since at these temperatures not all the insects got out of the grains. If temperature was increased, a significant number of insects died within the grain. The beetles were therefore removed by repeated gentle sieving of the grains through a 3mm mesh sieve to retain the grains and then through a 1mm sieve to separate the beetles from frass. Those that did not come out during this sieving were forced out by probing with a plastic fibre. After all the beetles were removed, the grains and the frass were returned into the glass jars and kept at 30°C and 70% r.h. until the adults emerged after 25-32 days. These were also obtained by sieving as described above.

Sex determination in *P. truncatus* adults

Where a definite distinction between males and females was necessary, sexing was carried out using the procedure and characters described by Shires and McCarthy (1976). Live adults were held using fine forceps, ventral surface uppermost, and their tubercles observed at 40x magnification. The female insects' tubercles are generally more prominent and widely separated than those of males (Figure 3.1). The procedure has an accuracy of 86-97% (Shires and McCarthy 1976).

3.3   Maize varieties and preparation

The varieties of maize used in this study were the yellow Guyana EU variety and the while Kenyan hybrid KH626. The maize was kept at −15ºC for two weeks to kill any living insects from previous infestation. After this period, the grains were removed and kept at room temperature and were normally kept under experimental temperature and humidity conditions for one week before being used in the experiments.

Female tubercles:
large and widely
spaced

Male tubercles:
small and
closely spaced
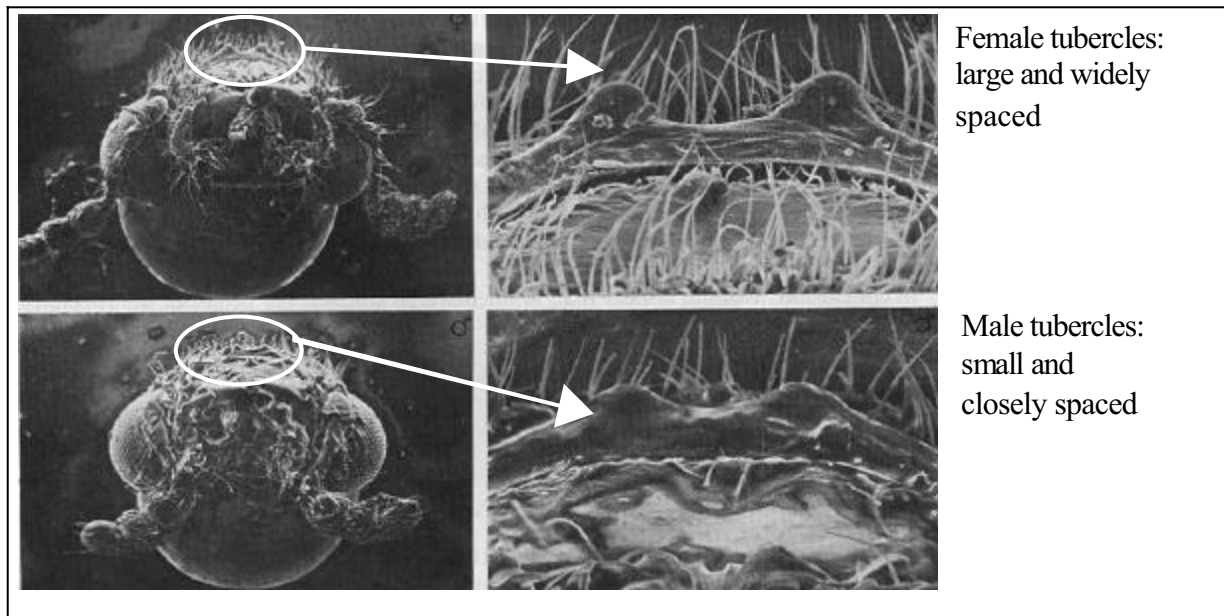
Figure 3.1: **Sex determination using tubercles on the head of** *P. truncatus*
(Source: Shires and McCarthy, 1976)

3.4   Experimental conditions

The experiments and the cultures were maintained in controlled temperature and relative humidity rooms at 30°C (± 1) and 70%(± 5) under darkness. Temperature was monitored both by minimum-maximum thermometer and over a control panel. Separate rooms were used for culturing of the insects and for experiments. The relative humidity was recorded by a hygrometer. The jars were kept on metallic stands that stood in a 2mm-deep layer of paraffin oil to guard against mites, lice and ants.

3.5   Determination of the effect of neem products on *P. truncatus*

Determination of the effect on mortality

100g maize grains were weighed into 250ml glass jars, 7cm diameter and 10cm height, (Figure 3.2) and the three dosages of the neem products added as shown in Table 3.2 above. The samples were thoroughly mixed by shaking, first by hand followed by rolling using an electric horizontal roller (Figure 3.3) at 120rpm for ten minutes to ensure uniform distribution and maximum adhesion of the test materials onto the grains. Although previous reports showed that stabilizing the grain by use of simulated cobs or by weighing them down with glass beads increased damage by the pest (Cowley *et al*., 1980; Howard, 1983), preliminary

investigations did not show any significant difference in terms of damage or population increase between the weighed down and loose grains under the conditions employed (Table 3.3). The experiments were therefore conducted without weighing down the grains with glass beads.
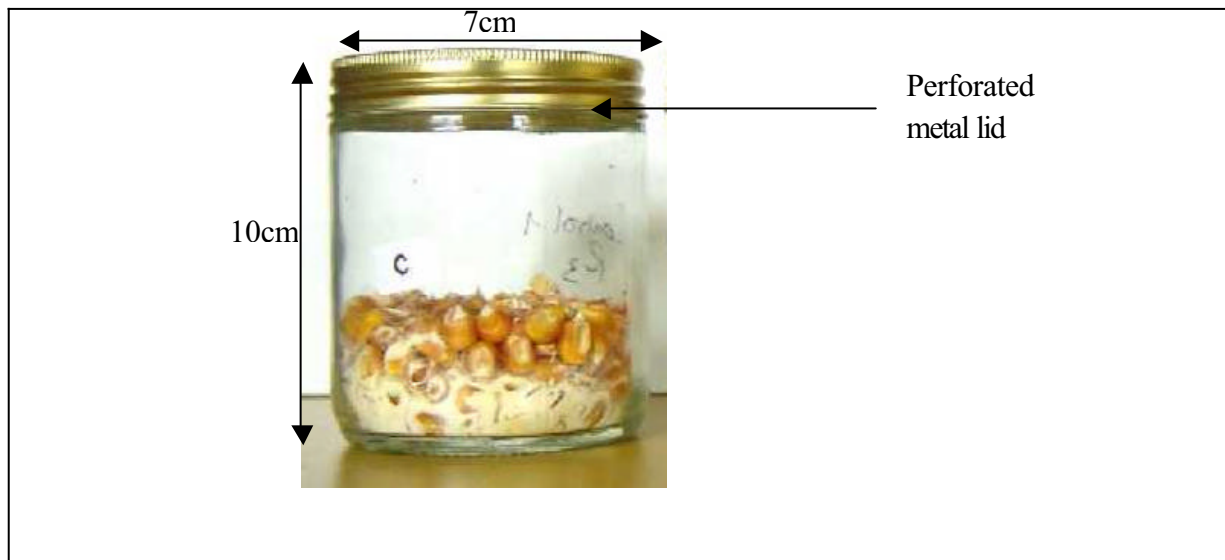
Figure 3.2: Perforated metal lid glass jar containing 100g maize
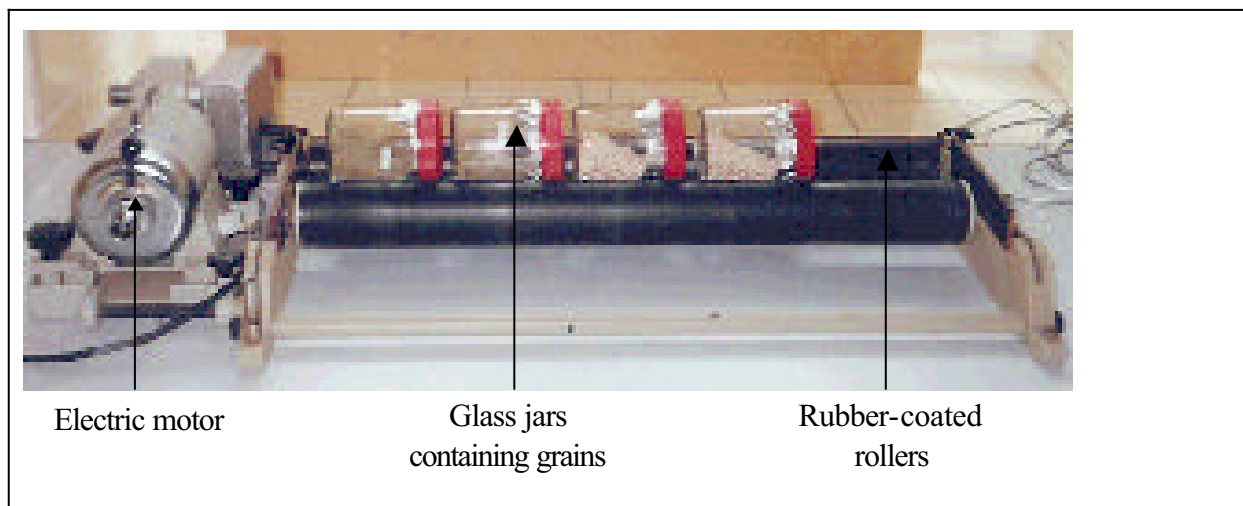
Figure 3.3: The horizontal roller used to mix the samples

Table 3.3: **Effect of weighing down maize grains using glass beads on performance by** *P. truncatus*

	Weight loss (g)	Population (No.) after 35 days
Loose grain	12.3 ± 0.4^a	446.7 ± 60.8^a
Weighed down grain	12.1 ± 0.2^a	461.0 ± 45.6^a

Data are means of five replicates. Values with the same letter down the columns are not significantly different (t-test, p > 0.05).

50 unsexed adult beetles of ages between one and seven days were placed into each of the treated samples and kept at 30°C and 70% r.h. for one month, after which they were sieved out as earlier described. The beetles that did not show any movement on probing with a pinhead were counted and recorded as dead. Preliminary experiments had shown that all dead beetles lie within the frass outside the grains.

Determination of grain weight loss

The samples in 3.5.1 above were sieved through a 3mm sieve. This conforms to the standard procedure employed by farmers to clean the grains before utilisation. None that goes through the sieve is normally utilised for human food. The resultant grains were weighed using an electric balance (Sartorius, model 1213MP, accuracy = 0.01g) and weight loss calculated as the difference between the weight of the grains before and after sieving.

Determination of insect population increase

The frass sieved out in section 3.5.2 above was returned into the jars together with the grain and the live insects and kept at the same conditions for another 35 days after the first experiment. The total number of beetles present was determined after a total exposure period of 63 days. 50 unsexed adults of ages between one and seven days were also added into maize samples prepared as described in 3.5.1 above but containing sub-lethal dosages of neem oil of 2.5, 5 and 7.5ml/kg and 0.1, 0.3 and 0.3g/kg of NeemAzal® PC KG 01, respectively, and kept at the same conditions for 63 days. The sub-lethal dosages were determined using mortality values obtained in section 3.5.1, which were used to predict the 25% lethal dosages. For NeemAzal®, the 25% lethal dosage was 0.3g/kg while for neem oil it was 7.5ml/kg (Figure 3.4).
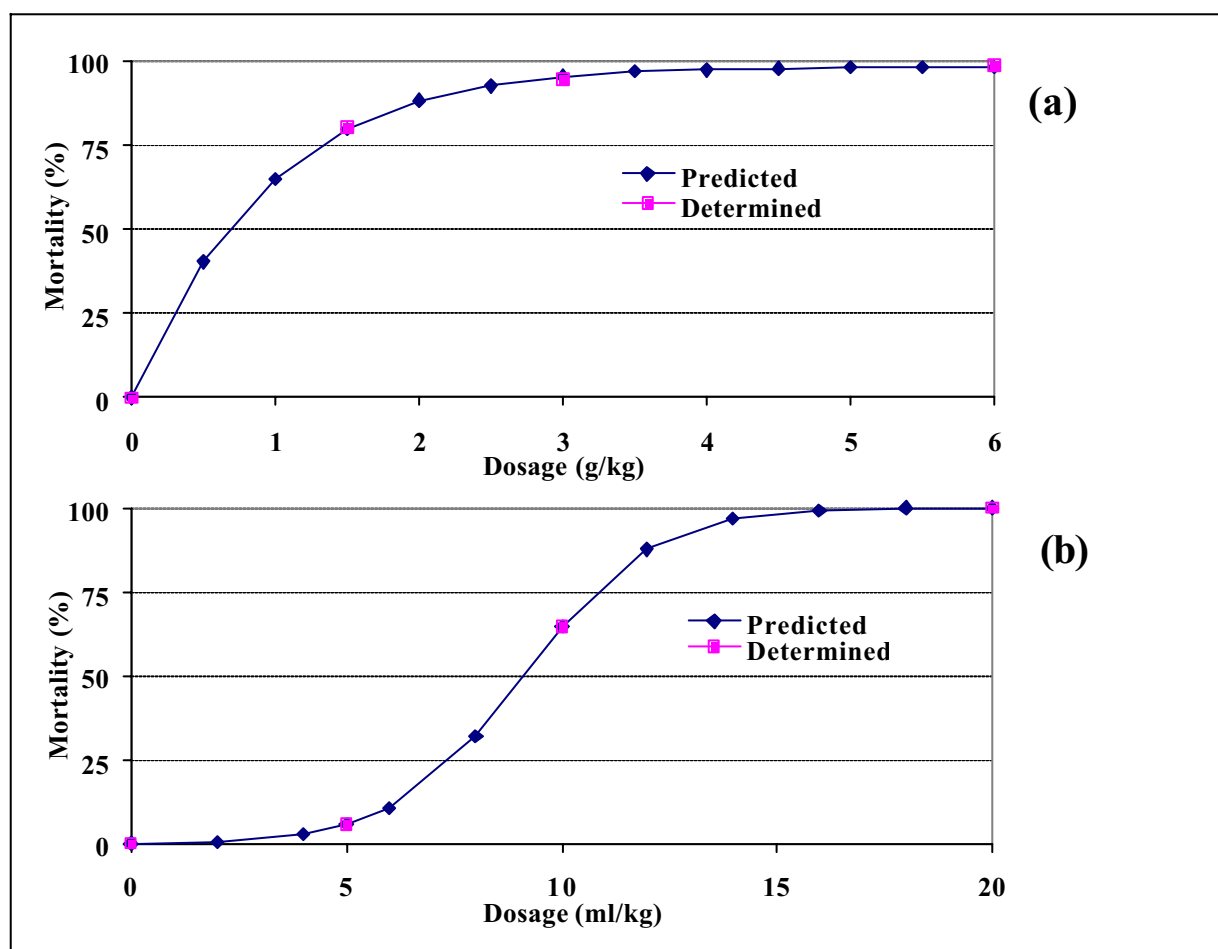
Figure 3.4: Determination of the sub-lethal dosages by fitting prediction curves using Table Curve®-2D (1996) programme for;
(a) NeemAzal® PC KG 01: mortality (%) = 100 /(1+exp (-x+1.3)/0.8)
(b) neem oil: mortality (%) = 100+0.2/(1+exp (-x+9.1)/1.5)).

Determination of effect of neem products on oviposition

50 unsexed adults of ages between one and seven days were added into maize samples prepared as described in section 3.4.1 but containing sub-lethal dosages of 2.5, 5 and 7.5ml/kg and 0.1, 0.2 and 0.3g/kg for neem oil and NeemAzal®, respectively, and kept at 30°C and 70%r.h. for seven days. The samples were then sieved, first through a 3mm sieve to retain the grains, then through a 1mm sieve to retain the beetles and finally through a 0.2mm sieve to hold the eggs (Figure 3.5). The eggs retained by the last sieving were counted and the size (length and width) of some of them selected at random was determined under a stereo-microscope at a magnification of 20x. The reduction in the number of eggs laid was determined by the following formula:

% egg reduction = [1-(TLE/TEE)] x 100

where   TLE = number of eggs laid in the treated sample

TEE = expected number of eggs in the treated sample

= CLE(TS/CS)

where CLE = number of eggs laid in the control sample

TS   = number of surviving insects in the treated sample

CS   = number of surviving insects in the control
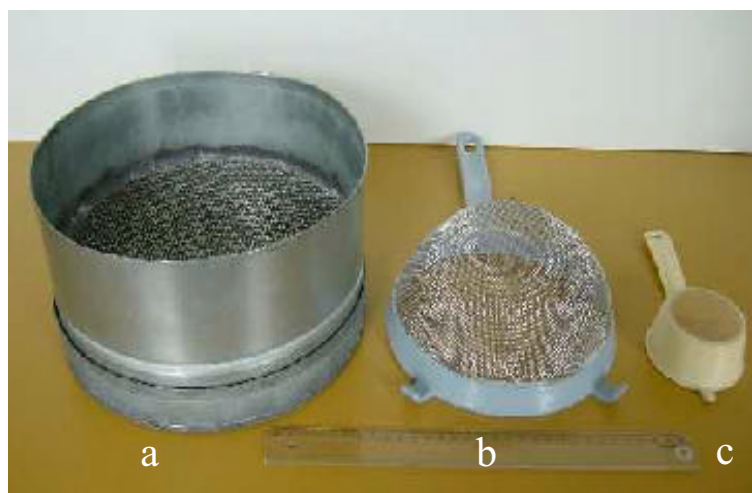
Figure 3.5:
The sieves used to retain
(a) grains (3mm)
(b) beetles (1mm) and
(c) eggs (0.2mm)
Ruler is 30cm long

Effect of post-oviposition treatment of the grains

To determine the effect of neem products on the eggs and larvae, the samples were treated after the eggs were laid. Fifty *P. truncatus* were kept on 100g samples of untreated grains and allowed to lay eggs for five days. All the adults were then removed by probing. The resultant grains together with frass were returned into the jars and treated with the highest dosages of neem oil and NeemAzal® given in Table 3.2 and gently mixed by rotating to avoid causing damage to the eggs. The treated samples were then kept at 30°C and 70%r.h. for 35 days after which the total number of larvae and both live and dead adults was determined.

Determination of the effect of volatiles

An experiment was designed to determine the effect of the volatiles from neem oil on reproduction and feeding of *P. truncatus*. Fine gauze cages, 5cm long and 1.5cm diameter (Figure 3.6), were filled with untreated maize grains and ten insects, five males and five females were introduced. Maize in 250ml glass jars was treated with neem oil as in section 3.5.1. The highest dosage of 20ml/kg was used. The cages were then placed into the treated grain such that they were completely covered by the grains and the samples were kept at 30°C and 70%r.h. Two separate sets of cages were used; one set kept for seven days for determination of egg number and another kept for 35 days for determination of weight loss and total number of insects.
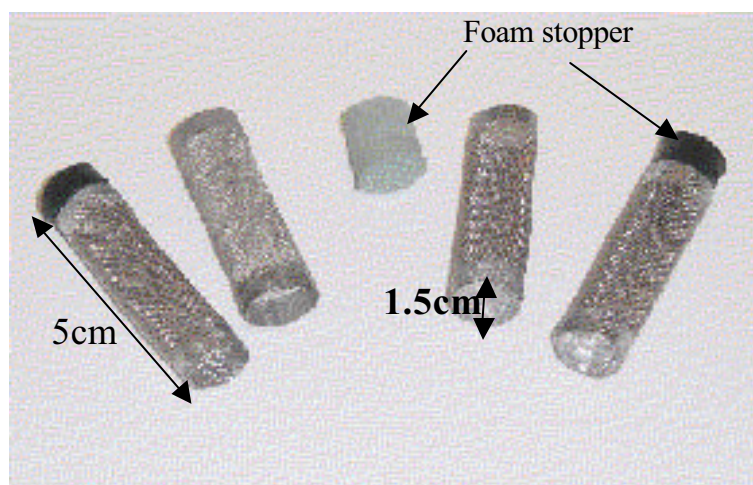
Figure 3.6:
The fine gauze cages with foam stoppers

3.6   Determination of the effect of neem oil on *Teretrius nigrescens*

Lethal effect on treated maize grains

50 unsexed adults of *P. truncatus* and *T. nigrescens* were introduced into 100g maize in 250ml glass jars treated with 1, 2.5, 5, 10, and 20ml of neem oil as described in 3.5.1. They were kept at 30°C and 70%r.h. and the total number of dead beetles determined after seven days.

Lethal effect on treated glass beads

50 unsexed adults of *P. truncatus* and *T. nigrescens* were introduced into an amount of glass beads (6mm diameter) (Figure 3.7) equivalent by volume to 100g maize (maize volume equivalent, MVE) in 250ml glass jars treated with 1, 2.5, 5, 10, and 20ml of neem oil as in 3.5.1. The MVE was determined by measuring the volume occupied by 100g of maize grains in the glass jars. They were kept at 30°C and 70%r.h. and the total number of dead beetles determined after seven days.

Figure 3.7:
The glass beads (6mm diameter) used to test the effect of neem oil on *T. nigrescens*

Determination of the effect of neem oil on predatory ability

Experimental samples were prepared by adding 0.25, 0.5, and 0.75ml neem oil to 100g maize grains and mixing as in section 3.5.1. Fifty unsexed adult *P. truncatus* were then added into the samples and kept for one week, after which ten adult *T. nigrescens* were introduced. In one set of the samples, 20 larvae were added every day as food material for the predator. These were obtained from cultures raised starting with 100g maize grains in 250ml glass jars and 50 adult *P. truncatus*. The samples were then kept at 30ºC and 70%rh for eight weeks. After this period, the total number of adults of both insects and their larvae, weight loss and grain damage were determined. Grain damage was determined by counting all the grains into which any degree of boring had been made by *P. truncatus*.

Determination of insect population increase

At the end of the experiment in 3.6.3, the samples were sieved through a 1mm and 0.5mm sieves to obtain *T. nigrescens*. The predator insects were counted and the total number of both the adults and the larvae recorded. The insects were also observed under a microscope for any deformities or abnormalities.

3.7 Persistence of neem products on maize grains

An experiment was designed to determine how long the effects of neem oil and NeemAzal® would last after application onto the grains. Maize samples were treated with the high dosage of neem oil and NeemAzal® following the procedure in section 3.5.1. Each set of samples represented one of the periods of 1, 2, 3, 4, 5, and 6 months from treatment. For each period, two sets of samples were prepared, each having five replicates. All the treatments were performed at the same time. After treatment, the samples were kept at 30º C and 70%r.h. until the appropriate time had elapsed. After the required time, 50 adult unsexed *P. truncatus* were introduced into both sets of the samples. One set was kept for seven days and the relevant parameters were determined. Frass activity was determined by weighing the grain before and after sieving. The frass was then sieved through a 0.2mm sieve to retain the eggs, which were counted under a stereo-microscope. The number of both the living and dead beetles was also recorded. The second set of samples was kept for 63 days to determine the effect of the products on insect population increase. In this set, the total number of insects was determined.

3.8 Absorption and evaporation of neem oil

A series of measurements were taken to determine the fate of neem oil that is applied onto maize grains. Loss of neem oil weight due to evaporation was first determined by repeated weighing of neem oil over a period of one week on filter paper and glass beads and two weeks on maize grains. Glass beads were treated with neem oil at a dosage of 20ml/kg maize volume equivalent. One hundred of these beads were placed on Petri dishes and kept both at 25 and 70%r.h. Their weights were determined first at one-hour intervals on the first day and then at one-day intervals for one week. In a supplementary experiment, 500µl of neem oil were added onto a filter paper (11cm diameter), which was weighed at similar intervals as the glass beads. A standard electric balance (Sartorius, model 1213MP, accuracy = 1mg) was used. The decrease in weight was taken as weight loss due to evaporation of the volatile components of

neem oil. Similarly, maize grains were treated with neem oil at 20ml/kg dosage and 100 of them were removed and kept on Petri dishes at both 25 and 70%r.h. and their weights determined at regular intervals using the same electric balance. Another set of samples was kept under the same conditions without any oil treatment to serve as a control.

To determine the rate of loss of the oil from the surface of the grains, the following procedure was followed. One hundred maize grains were selected from the treated sample and placed on a Petri dish. After their weight was determined, all the oil on the surface was wiped off by use of a soft tissue paper (Kimwipes® Lite 200). The weight of the paper was determined before and after the wiping, and the weight of neem oil on the surface of the grain was taken as the difference in weight of the paper before and after wiping the grains. Adjustments were made for unavoidable weight loss by subtracting the weight loss from non-treated grains wiped in the same manner. The procedure was repeated at various intervals for a period of two weeks and was performed on five replicates of two varieties of maize.

3.9   Repellent effect of neem products

Experiments in this section were designed to investigate the repellent effect of neem oil on *P. truncatus*. Because of possible interaction between contact and volatile repellent effects, two separate sets of experiments were conducted for the two types of effects.

Volatile repellent effect

An olfactometer (Figure 3.8) similar to that one described by Steidle and Schöller (1997) was used. It was made of acrylic glass, consisting of a cylinder (4cm high and 21cm diameter) divided by a vertical plate of the same material and height into two equal chambers. The test materials were placed into Petri dishes (5cm diameter) and these were introduced into the two chambers. The control chamber contained empty Petri dishes. A removable insect walking arena (1cm high, 21cm diameter) was placed on top of the cylinder. It consisted of plastic gauze (0.5mm) with a rim of acrylic glass (0.9cm high) that fitted exactly into a dent between the dividing plate and the cylinder. Another glass was used to cover the arena to prevent the insects from escaping.

Maize grains were placed on a Petri dish and then introduced into the arena and the top was covered. The insects to be tested were starved for 24 hours before the experiments. Initial experiments were conducted using a single insect at a time. The insect was placed at the centre of the test arena and allowed to move about. The location of the insect was recorded using a special computer program, The Observer 3.0 (Noldus, 1988). In preliminary experiments, *P. truncatus* showed very slow and irregular movements with frequent long stops that no clear pattern was observed. It was therefore decided to use a group of insects to be observed over a long period. For each treatment therefore, 50 adult *P. truncatus* were released onto the top cover and observed for a period of three hours. In the case of *T. nigrescens*, a single insect was used for each trial since they were generally more active and faster than *P. truncatus*. The walking area was cleaned after each run by wiping with a soft tissue soaked in ethanol. Each insect or group of insects was used only once.
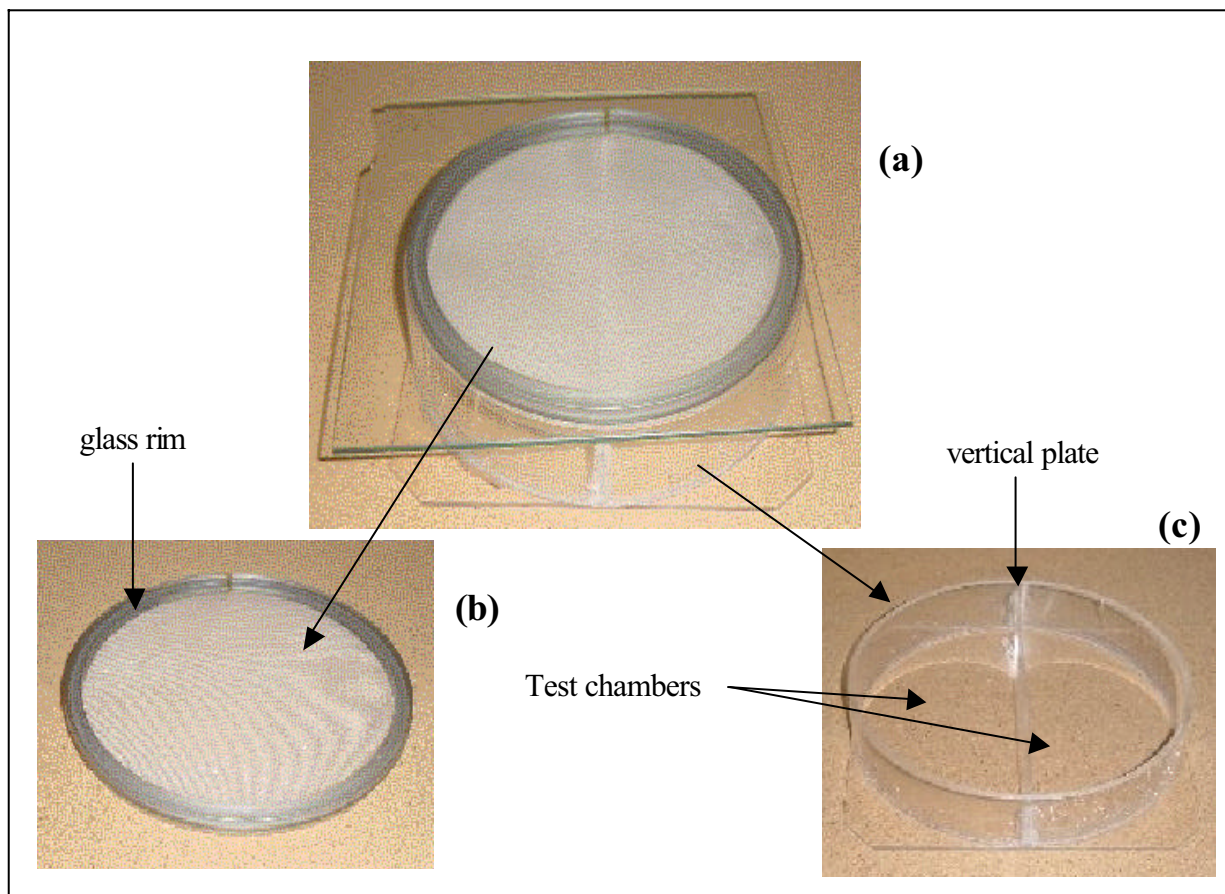
Figure 3.8: **The olfactometer apparatus: (a) the complete set-up (b) the walking arena (c) the chambers**

Contact repellent effect

The contact repellent effect of neem oil was determined using a food preference apparatus made from a 3mm wire gauze and consisting of eight sections of equal size (Figure 3.9). The sections were filled with treated and non-treated maize in alternative order and a piece of filter paper (7cm diameter) was placed at the centre over the grains to serve as an insect release platform. A total of 50 adult *P. truncatus* were released onto the filter paper platform and allowed to freely infest the grains. After 24 hours (observed in preliminary experiments to be adequate), the distribution of the insects between the sections was determined by counting the beetles in the grain. The repellence of the insects was calculated as the response index according to Nawrot (1973) using the formula below:

RI = NP - NK/NP + NK,

where

       RI = response index

       NP = number of insects in the compartments with treated material

       NK = number of insects in the compartment with untreated material

The RI values may range from 0 to +1 for attractive material and from 0 to −1 for repellent material.
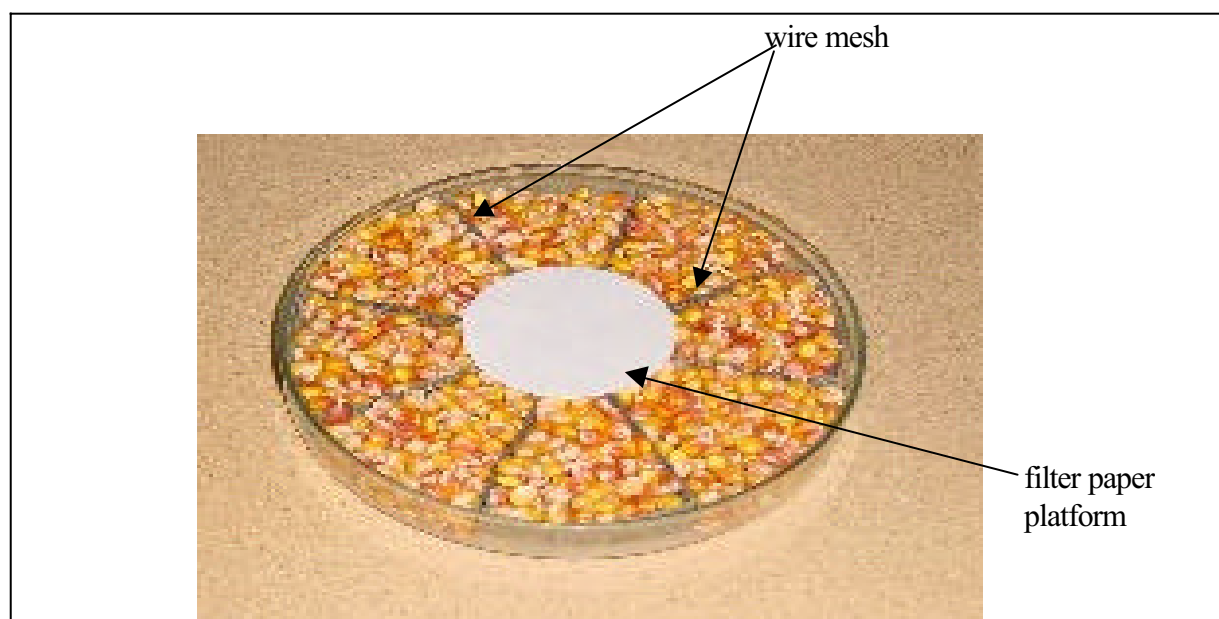
Figure 3.9: **Food preference apparatus used in the contact repellence experiment**

In another set of experiments, intended to determine whether the insects preferred staying within treated grains or outside, only four of the eight compartments of the apparatus were used. Separate apparatus were used for treated and non-treated grain (Figure 3.10). These were filled with either treated or non-treated grains at a time. The remaining four compartments were left empty. Forty insects were introduced into each compartment and left to settle in preferred areas. The beetles that moved out of the grain and settled in empty compartments were counted including those that were found on the outer side of the side gauze of the apparatus.
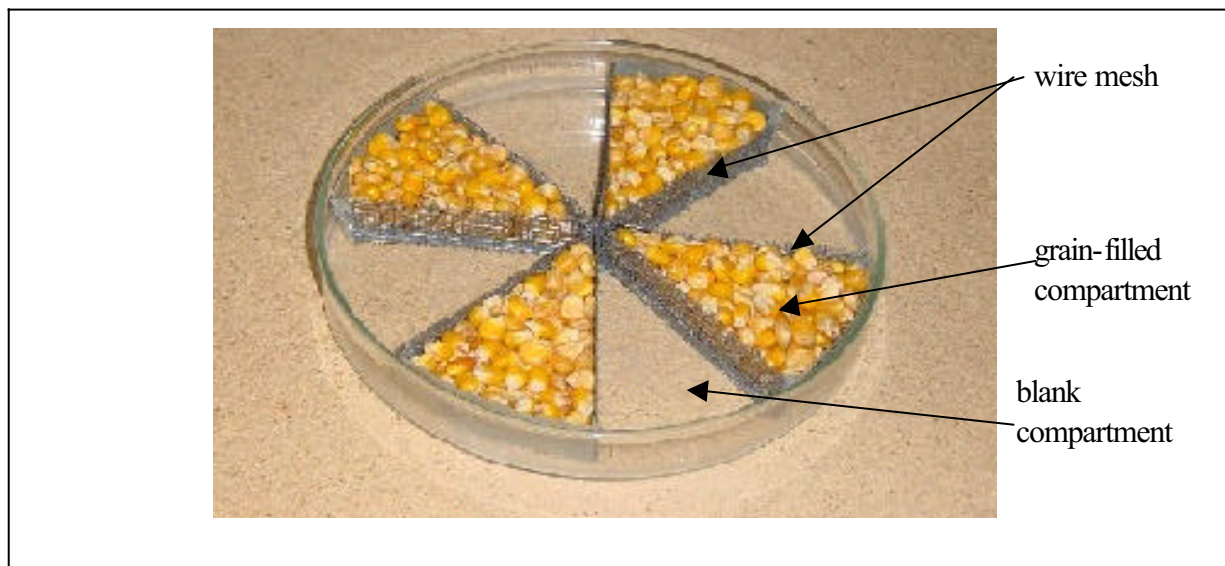
Figure 3.10: The food preference apparatus as used in the escape experiment

3.10   Feeding deterrent effect

Larval feeding deterrence

Ground maize grains were used to investigate the feeding deterrent effect of neem oil on larvae of *P. truncatus*. Maize grains were ground using a hammer mill and treated with neem oil at the rates of 0.5, 1, 2, 3, and 4% (v/w). Mixing was carried out in 100ml glass jars where the flour was stirred continuously until the oil was uniformly distributed. The treated flour was then divided into 10g portions and placed in glass Petri dishes.

Larvae were sieved out of cultures and sorted according to age. The young and delicate larvae were carefully picked using a fine-hair brush. The sorting utilized both the time from hatching and estimation of the size of the head capsules according to procedures used by Bell and

Watters (1982) and Detmers (1993) to separate the larvae into three larval stages as shown in Table 3.4.

Table 3.4: Relationship between age and head capsule size of larvae of *P. truncatus* on maize grains at 30°C and 70%r.h.

Head capsule width (mm)	Larval age (days)	Larval stage
0.3-0.4	0-4	1st instar
0.5-0.6	5-12	2nd instar
0.7-0.9	13-21	3rd instar

The weight of the larvae was determined using an analytical balance before they were introduced into the treated flour. Shallow furrows were made into the flour and the larvae were placed in them and covered with a small amount of the same flour. Generally, the development of larvae under such conditions is not optimal, however, for the purpose of determining weight gains and losses using the same larvae, this approach provided the best available alternative. The samples were kept at 30ºC and 70%r.h. The larvae were removed seven days later from the flour using a fine brush and their weights determined again. They were returned into the treated flour and kept for another seven days. The weighing was repeated after a total of 14 days.

Adult feeding deterrence

Maize flour

Ground maize flour used in 3.10.1 was used to prepare "cakes" for rearing adult *P. truncatus*. The flour was mixed with Mondamin® commercial starch at a ratio of 3:1. Neem oil was then added into the mixture before a small amount of water was added. These resulted into a paste that was then pressed into cells (1.5cm x 1.5cm x 1.5cm) made from plastic (Adder, personal communication). The "cakes" were dried in open air for 24 hours before being removed from the cells and placed in Petri dishes and 20 insects introduced in each dish. The "cakes" in which neem oil was not added served as control. Another control was set with *P. truncatus* in a Petri dish without any food. The insects were kept at 30ºC and 70%r.h. for a total of 28 days. At this time, the cakes were broken and the number of both living and dead insects in each treatment was determined. Preliminary experiments had shown that the insects could survive without food for a maximum of 33 days. This period could however not be attained in

real experiments because F_l adults would emerge and confusion between them and the parents would arise.

Treated maize grains

This experiment was conducted using a maize variety that had been noticed to rapidly lose the treated neem oil from its surface, most probably by absorption or evaporation, to such an extent that the mortality of the beetles declined rapidly to negligible levels. It was intended to determine feeding deterrence without the direct lethal effect caused by the presence of oil. The samples were treated as in 3.5.1 with neem oil dosages of 5, 10, 20, 30 and 40ml/kg and kept at 30°C and 70%r.h. for one month, when all the oil was absorbed or simply lost from the surface of the grains. At this stage, 50 unsexed adult *P. truncatus* of the same age were introduced into the samples and allowed to feed for seven days, after which they were removed and the amount of frass produced was determined by sieving the samples and weighing the resultant frass. The insects were returned into the samples and kept for a total of 28 days. The survival of the beetles to the end of the period was determined by counting the number of live beetles at the end of the experiment.

3.11   Micro-calorimetric observation of larval activity

To determine the activity of *P. truncatus* larvae on treated maize flour, a conductive type differential scanning calorimeter (Micro calorimeter type: MBC Thermanalyse) was used. It contained two cells, the reference cell and the test cell. The apparatus was well thermally insulated and the temperature difference between the measuring and the reference cell was measured as heat output signal at a sensitivity of $65\mu VmW^{-1}$. The output signal was amplified 1000 times by a micro volt amplifier (Type: Mikrovolt Verstärker MA170, Kisch Messgeräte GmbH), and recorded by a PC using a signal analysis program (5-Kanal-Multimeter, H. & C. Bentert Hard und Software, 1994). Larvae were weighed before and after the experiments. They were introduced into the micro calorimeter under different conditions, on non-treated maize flour, on treated maize flour, without flour and in the presence of a neem oil-treated filter paper, and their heat production at 303°K was determined for 60-120 hours. The recorded output (mV) was converted into heat production (mW/insect).

3.12   Data analysis, presentation and statistical procedures

Data were analysed using various analysis procedures depending on the design of the experiment and the nature of the results obtained. Both SAS (1990) and SPSS (2001) analysis tools were used. The Levene test (Levene, 1960) was used to determine homogeneity of variance. Where data did not conform to the normal distribution, transformations were performed. Where transformed values still did not conform, non-parametric procedures were employed. ANOVA procedures (Steel and Torrie, 1980) were used for multiple comparisons with normal distribution. Prediction curves were generated using Table Curve-2D software (1996). Means were generally separated using Duncan's Multiple Range Test (Lorenz, 1988) or modified LSD (Bonferroni) (Seneta, 1993) where more than four means were involved, or by non-parametric procedures for non-parametric data. For two-sample tests, Student t-test was used for normal distribution data with variance homogeneity and Mann-Whitney U-test (Lorenz, 1988) and Wilcoxon test (Lorenz, 1988) for independent and dependent non-parametric data, respectively.

Unless otherwise stated, five replicates were used for the purpose of minimizing unavoidable errors. Efforts were made to minimize variations in temperature and relative humidity to a maximum of $\pm$ 1°C and $\pm$ 5%, respectively. Data were presented mostly in form of bar and line graphs and tables. Where graphs were used, standard errors were shown as line bars at the top of the data bars in bar graphs and at the data points in line graphs. Occasionally, the standard error was too small for the indicators to be noticed. Significance was denoted using letters, all treatments that fell in the same group and hence were not significantly different were denoted by the same letter. Where two factors were involved, comparison between the two of them at a specific treatment (level) was indicated with small letters while comparison between different treatments was shown by capital letters. In all cases, explanation as to which letter was used for which factor is given in the legends.

For a general clarification, the description of Figure 4.3 is as follows: two factors were involved, namely azadirachtin (four levels) and neem products (four levels). Comparison between the four neem products at the 1.5 dosage level is indicated by small letters, only values for NO and NAPCKG01 were significantly different from the rest hence the letters b and c, respectively. Comparison between different dosages of NAPC05 is indicated by capital letters, only the value for 6mg/kg was significantly different, hence the letter B.

4   Results

4.1   Effect of neem products on *P. truncatus*

Effect on mortality and grain weight loss after one month

Mortality

Neem seed cake powder (NSCP): the lethal effect of neem seed cake powder used in this study on *P. truncatus* was significant (ANOVA, $p < 0.05$). NSCP at 25, 50 and 100g/kg caused 5.2, 7.2 and 12.7% mortality, respectively, compared to 4% in the control (Figure 4.1, Table 4.1.). The lethal effect at the 25g/kg dosage was not significantly different from that of both the control and 50g/kg dosage (Duncan's Multiple Range Test, DMRT, $p = 0.05$), but was significantly lower than that at 100g/kg. The treatment with 100g/kg dosage caused significantly higher mortality than all the other dosages. The effect of NSCP generally increased with increase in dosage.
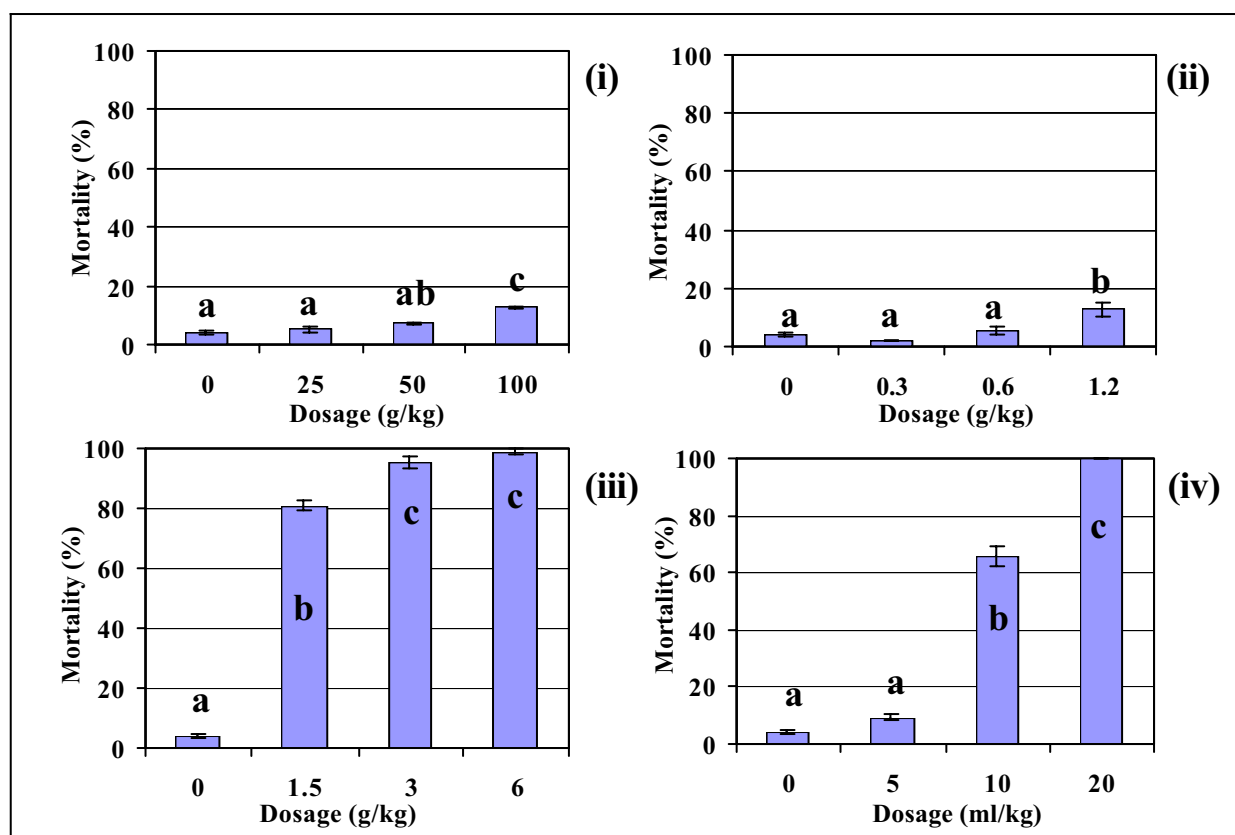
Figure 4.1: Lethal effect of (i) neem seed cake powder (ii) NeemAzal® PC 05 (iii) NeemAzal® PC KG 01 (iv) neem oil on *P. truncatus* adults after one month at 30°C and 70%r.h. Bars, means of five replicates, with the same letter are not significantly different according to DMRT ($p = 0.05$).

NeemAzal® PC 05: the lethal effect of NeemAzal® PC 05 on *P. truncatus* adults was significant (ANOVA, $p < 0.05$). The mortality figures were 4, 2, 5.2 and 12.7 for the control and for 0.3, 0.6 and 1.2g/kg, respectively. Only the treatment with 1.2g/kg dosage however caused a significantly different effect from the control and all other dosages (DMRT, p=0.05). The effect of the product generally increased with increase in dosage.

NeemAzal ® PC KG 01: treatment with NeemAzal ® PC KG 01 resulted in a significant lethal effect on *P. truncatus* adults (ANOVA, $p < 0.001$). The mortality figures were 4, 81, 95.2 and 99% for the control, 1.5, 3 and 6g/kg, respectively. All the dosage levels resulted in a significantly higher effect on *P. truncatus* than in the control (DMRT, $p = 0.05$). The effect of the treatment with 1.5g/kg dosage was significantly lower than that of both the 3 and 6g/kg dosage levels while the difference between the 3 and 6g/kg dosages was not significant. The effect of the product tended to increase at a decreasing rate with increase in dosage.

Neem oil: the lethal effect of neem oil on *P. truncatus* adults was significant (ANOVA, $p < 0.001$). The treatment of samples with 5, 10 and 20ml/kg dosage levels resulted in mortality figures of 9.2, 65.7 and 100% respectively, compared to the 4% in the control. The lethal effect in the 5ml/kg dosage samples was not significantly different from that in the control (DMRT, $p = 0.05$) but was significantly lower than that of the 10 and 20ml/kg dosage levels. Apart from the difference between the control and the 5ml/kg dosage treatment, all the other differences between dosages were significant.

Table 4.1: Lethal effects of four neem products on *P. truncatus* on maize grains with three dosage levels after a 28-day exposure period at 30°C and 70% r.h.

Treatment	% Mortality			
	Control	a	b	c
Neem seed cake powder	0.0 ± 0.0^a	1.4 ± 0.9^a	3.5 ± 0.4^a	9.0 ± 0.4^b
NeemAzal® PC 05	0.0 ± 0.0^a	-2.1 ± 0.0^a	1.4 ± 1.1^a	9.0 ± 1.5^b
NeemAzal® PC kg 01	$0\ 0\pm0.0^a$	80.1 ± 1.5^b	95.0 ± 1.6^c	98.0 ± 0.9^c
Neem oil	$0\ 0\pm0.0^a$	5.5 ± 0.9^a	64.4 ± 3.0^b	100.0 ± 0.0^c

Data are means of five replicates corrected according to Abbott's (1925) formula. Means followed by the same letter in the rows are not significantly different (DMRT, $p = 0.05$).

Weight loss

Neem seed cake powder: the activity effect of neem seed cake powder used in this experiment on maize weight loss by *P. truncatus* was significant (ANOVA, $p < 0.05$). The maize samples treated with seed cake powder at 25, 50, and 100g/kg dosage levels resulted in percentage weight losses of 7.9, 6.4 and 6.2, respectively, compared to 9.7 in the control. The weight losses from all the dosages were significantly lower than in the untreated control (DMRT, $p = 0.05$). The weight losses from the 50 and 100g/kg dosages were not significantly different from each other but the two were significantly lower than that from the 25g/kg dosage.

NeemAzal[®] PC 05: treatment of maize samples with NeemAzal[®] PC 05 resulted in a significant effect on maize weight loss by *P. truncatus* (ANOVA, $p < 0.05$). The losses were 9.7, 8.4, 6.4 and 5% for the control, 0.3, 0.6 and 1.2g/kg dosage levels, respectively. All the dosages resulted in significantly lower losses than the control (DMRT, $p = 0.05$) and were all significantly different from one another.

NeemAzal [®] PC KG 01: treatment of samples with NeemAzal [®] PC KG 01 resulted in a significant effect on maize weight loss by P. truncatus (ANOVA, $p < 0.001$). The losses from the 1.5, 3 and 6g/kg dosage levels were 1.1, 0.8 and 0.5% respectively. All the treatments resulted in significantly lower losses compared to the control (DMRT, $p = 0.05$). The differences between 1.5 and 3g/kg and between 3 and 6g/kg dosage levels were not significant, but the 1.5 g/kg dosage resulted in significantly higher loss than in the 6g/kg dosage.

Neem oil: maize grain samples treated with neem oil resulted in a significantly lower weight loss by *P. truncatus* than the control (ANOVA, $p < 0.001$; DMRT, $p = 0.05$). The pest caused losses of 4.2, 1.2 and 0.1% in the samples treated with 5, 10 and 20ml/kg neem oil, respectively, and were all significantly different from one another.
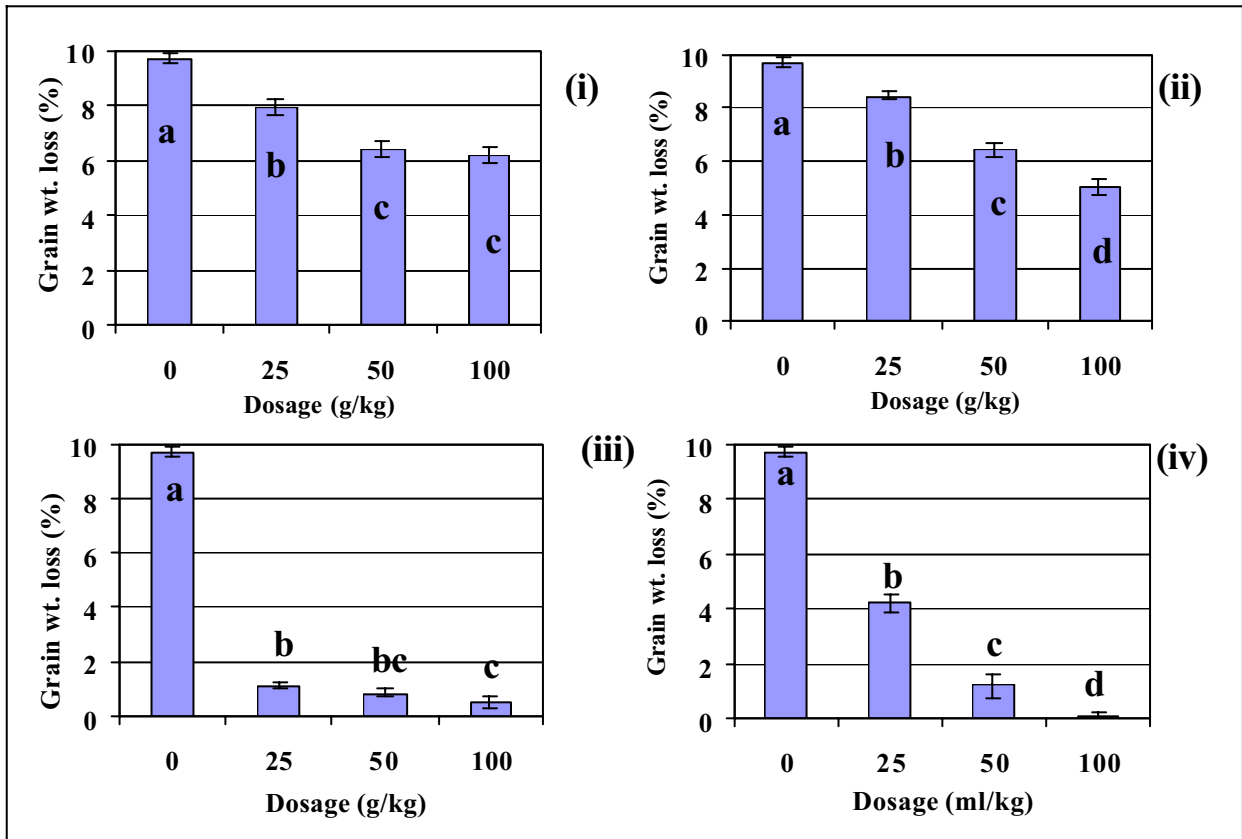
Figure 4.2: Effect of (i) neem seed cake powder (ii) NeemAzal® PC 05, (iii) NeemAzal ® PC KG 01 and (iv) neem oil on weight loss by *P. truncatus* after one month at 30°C and 70%r.h. Bars, means of five replicates, with the same letter are not significantly different according to DMRT (p = 0.05).

The mortality and weight loss values were also plotted against the azadirachtin dosage for the purpose of comparing its effect at similar dosages for the products used (Figure 4.3 and 4.4). Under these comparisons, both mortality and weight loss were clearly different for different products at the same dosage levels. NeemAzal® PC KG 01 resulted in the highest mortality at 1.5 and 3.0mg/kg dosage levels while neem oil gave the highest at 6mg/kg. The differences between NSCP and NeemAzal® PC 05 was not significant at all the dosage levels. NeemAzal® PC KG 01 resulted in the lowest weight loss at 1.5 and 3mg/kg dosage levels while neem oil gave the lowest at 6mg/kg. NeemAzal® PC 05 resulted in the highest weight loss at 1.5mg/kg while NSCP gave the highest at 6mg/kg.
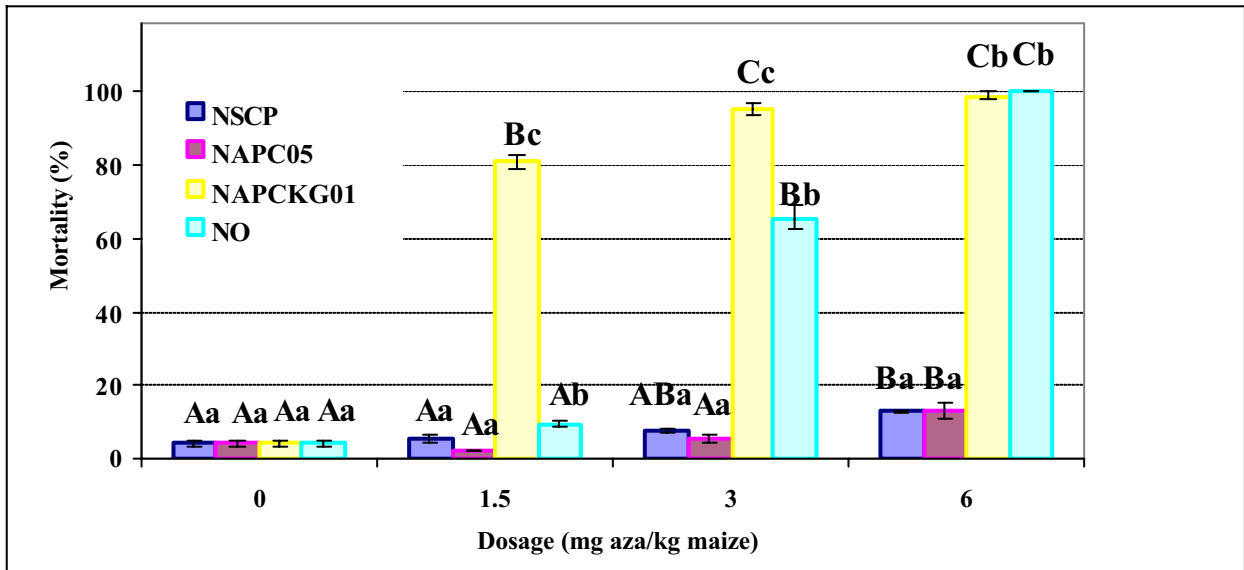
Figure 4.3: Lethal effect of neem products at dosage levels of 0-6mg azadirachtin/kg maize kept for one month at 30°C and 70%r.h. NSCP-neem seed cake powder, NAPC05-NeemAzal® PC 05, NAPCKG01-NeemAzal® PC KG 01, NO-neem oil. Bars are means of five replicates. Values followed by the same letter are not significantly different (DMRT, p = 0.05), capital and small for comparison within and between means, respectively.
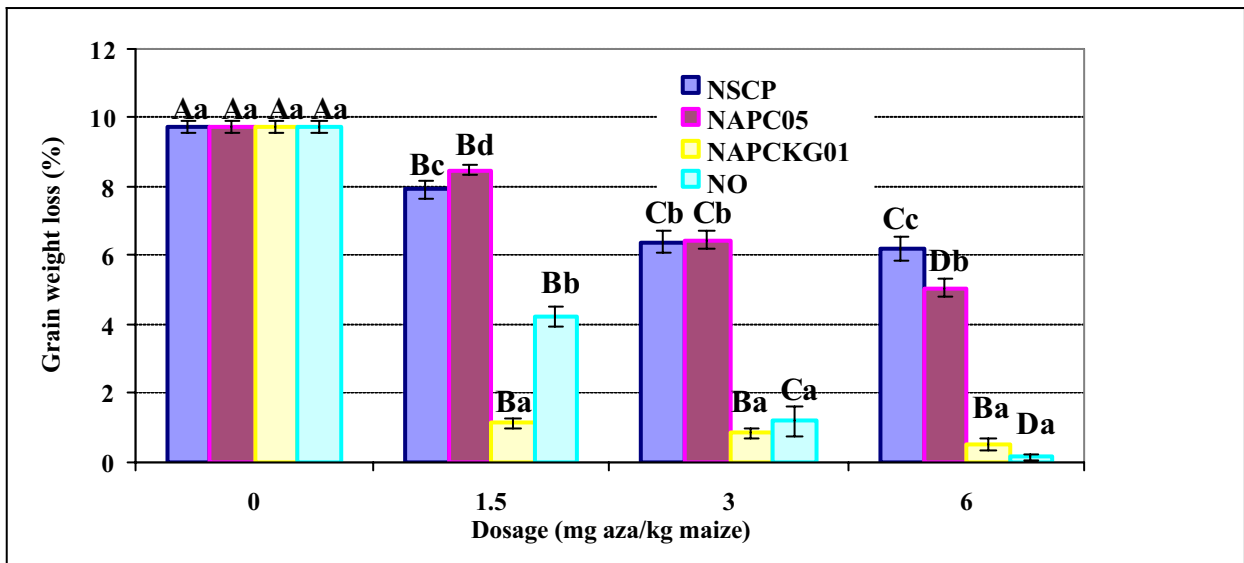
Figure 4.4: Effect of neem products at dosage levels of 0-6mg azadirachtin/kg maize kept for one month at 30°C and 70%r.h. on weight loss caused by 50 *P. truncatus*. NSCP-neem seed cake powder, NAPC05-NeemAzal® PC 05, NAPCKG01-NeemAzal® PC KG 01, NO-neem oil. Bars are means of five replicates. Values followed by the same letter are not significantly different (DMRT, p = 0.05), capital and small for comparison within and between means, respectively.

Effect on mortality after varied exposure periods

Effect of NeemAzal® PC KG 01

The ANOVA of the lethal effect of NeemAzal® PC KG 01 on *P. truncatus* is given in Table 4.2. The mortality effect of NeemAzal® PC KG 01 was significant ($p < 0.001$) and so was the effect of time. The interaction between the neem product dosages and the exposure time was also significant ($p < 0.001$). At any given dosage including the control, mortality increased with increase in time. The least change was in the control where mortality increased from 0 % at one day to 5.2% after 28 days. The only significant difference in means (LSD-Bonferroni, $p = 0.05$) was between day 1 and day 28 (Figure 4.5). At the 1.5g/kg dosage level, mortality increased from 12% at the first day to 90.4% after 28 days. The highest increase was between day 1 and day 7 (12 to 76%) while the change between day 7, 14, 21 and 28 were moderate and with a decreasing rate (76 to 83.2, 83.2 to 89.2 and 89.2 to 90.4, respectively). Mortality at day 1 was significantly less than at all the other four exposure periods. The difference between mortality at day 7 on the one hand, and day 21 and day 28 on the other, was also significant although that between day 21 and day 28 was not. For the 3g/kg dosage, the largest increase was between day 1 and day 7 (37.6 to 96.8%) while the increases between day 7, 14, and 21 (96.8 to 97.6% and 97.6 to 98.4%) were negligible. Only mortality at day 1 was significantly different from all the rest. Equal mortality was recorded at day 21 and 28. At the 6g/kg dosage level, the only significant difference was between day 1 and day 7 (49.2 to 98.8%). The increase between day 7 and day 14 (98.8 to 99.6%) was negligible while all the other times recorded the same mortality of 99.6%.

Table 4.2: Analysis of variance for the lethal effect of three dosages of NeemAzal® PC KG 01 after five different exposure times on *P. truncatus* on maize grains kept at 30°C and 70%r.h.

Source	d.f.	Sum of squares	Mean square	F value	p
NeemAzal PC KG 01 (NA)	3	123057.7	41019.2	2825	<0.001
Time (T)	4	34997.0	8749.2	602.6	<0.001
NA x T	12	11467.3	955.6	65.8	<0.001
Error	80	1161.6	14.5		
Total	**99**	**170683.6**			
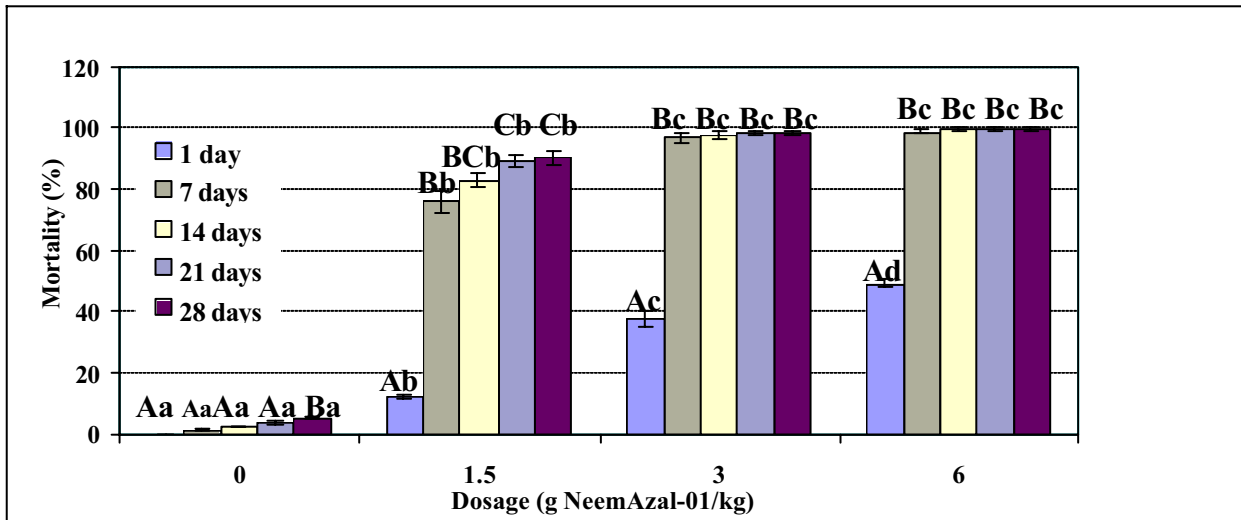
Figure 4.5: Lethal effect of NeemAzal® PC KG 01 on *P. truncatus* on maize with three dosage levels after five different exposure periods at 30°C and 70% r.h., means of five replicates each with 50 beetles. Bars with the same letter are not significantly different: capital letters for exposure periods (LSD-Bonferroni, p = 0.05) and small letters for dosage levels (DMRT, p = 0.05).

Effect of neem oil

The ANOVA of the lethal effect of neem oil on *P. truncatus* is given in Table 4.3. Both the effects of neem oil and time on mortality of *P. truncatus* were significant (ANOVA, p < 0.001). The interaction between neem oil and exposure time was also significant (p < 0.001). At the 5ml/kg dosage level, mortality increased steadily with exposure time from 0.4 to 8%, and the effect at day 14 was significantly higher than at day 1 (LSD-Bonferroni, p = 0.05; Figure 4.6) but not significantly different from that at day 7, 21 and 28. The lethal effect at day 7 was however significantly lower than at day 21. At the 10ml/kg dosage, the mortality effect at day 1 (12%) was significantly lower than at all the other exposure periods. The difference between the lethal effects at all other exposure period was not significant. The same trend was observed at the 20ml/kg dosage where the effect at day 1 (94.4%) was significantly lower than all the rest (100%).

Table 4.3: **Analysis of variance for the effect of three dosages of neem oil after five different exposure times on *P. truncatus* on maize grains kept at 30°C and 70%r.h.**

Source	d.f.	Sum of squares	Mean square	F value	p
Neem oil (NO)	3	151851.3	50617.1	7443.7	<0.001
Time (T)	4	1714.6	428.6	63.0	<0.001
NO x T	12	1434.5	119.5	17.6	<0.001
Error	80	544.0	6.8		
Total	**99**	**155544.4**			
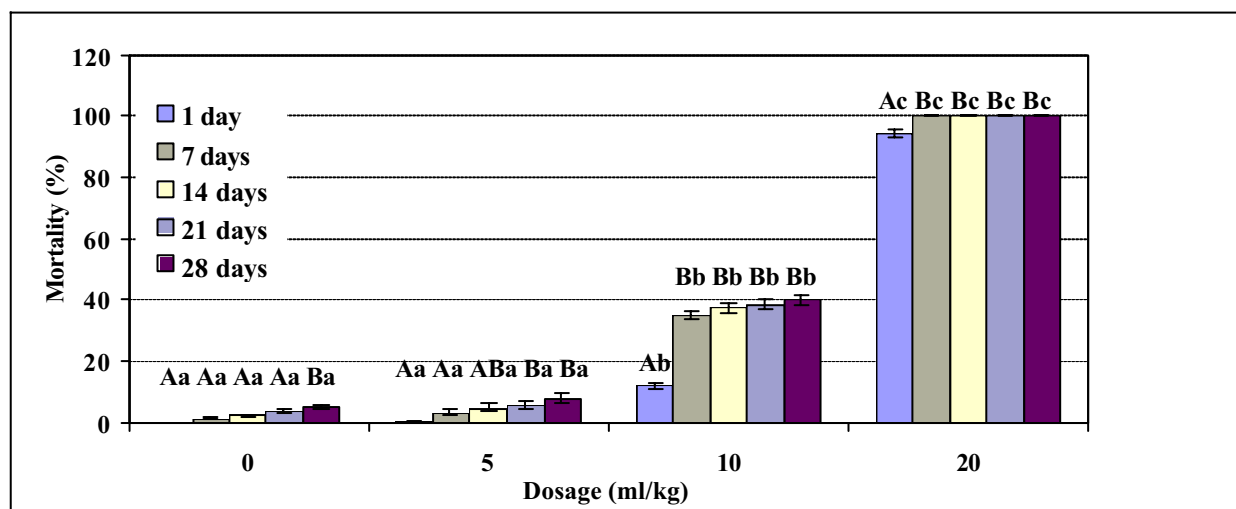
Figure 4.6: **Lethal effects of neem oil on *P. truncatus* on maize with three dosage levels after five different exposure periods at 30°C and 70% r.h., means of five replicates each with 50 beetles. Bars with the same letter are not significantly different: capital letters for exposure periods (LSD-Bonferroni, p = 0.05) and small letters for dosage levels (DMRT, p = 0.05).**

Time-dosage interactions

The significant interactions between exposure time and the dosage levels for both NeemAzal® and neem oil were also plotted as interaction graphs in Figure 4.7. For NeemAzal®, all the dosage levels showed interaction, which was most profound between day 1 and day 7. Between day 7 and day 28, only 3g/kg dosage showed a marked interaction. It was generally clear that the difference among treated samples was more evident at day 1 and day 7 than at the rest of the exposure periods. The difference between them and the control was however largest at day 28. For neem oil, there generally seemed to be no interaction between the treatment and exposure period, apart from for the period between day 1 and day 7, when the results from 10 and 20ml/kg dosage levels showed interaction. The two dosage levels were more effective at day 7 than at day 1. The 1.5g/kg dosage showed no interaction for the whole period.
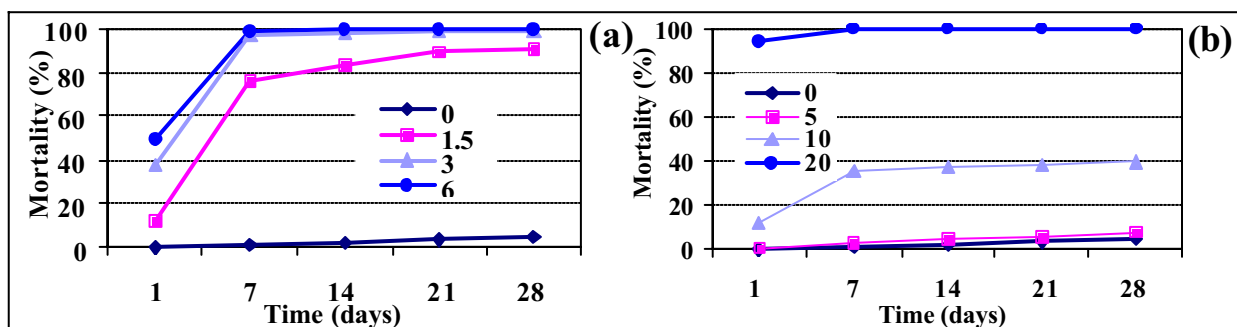
Figure 4.7: Interaction between the effects of (a) NeemAzal® PC KG 01 and (b) neem oil and exposure period on mortality of *P. truncatus*

Effect of neem products on reproduction of *P. truncatus*

Oviposition

The effect of neem products on oviposition of *P. truncatus* is given in Figure 4.8 and 4.9. Although the products affected the total number of eggs laid, the eggs seemed to be of normal size, and shape. The average length and breadth of the eggs from treated samples were not significantly different from those from the untreated control (t-test: $p > 0.05$) (Table 4.4). The mean number of eggs laid in samples treated with neem oil and NeemAzal® was 25.6 and 111, respectively, compared to 116.4 in the control. In terms of percentage reduction in egg production, neem oil effected the greatest reduction of 76.3% while NeemAzal® resulted in a non-significant reduction of only 0.6%.

Table 4.4: Effect of neem oil on the size of eggs laid by *P. truncatus*

	Length (n = 36)	Width (n = 36)
Untreated	1.32 (0.017)[a]	0.67 (0,016)[a]
Neem oil treated	1.30 (0.018)[a]	0.69 (0.019)[a]

Values are means of 36 measurements. () = Standard error of the mean. Values in the columns followed by the same letter are not significantly different (Student t-test, p > 0.05).
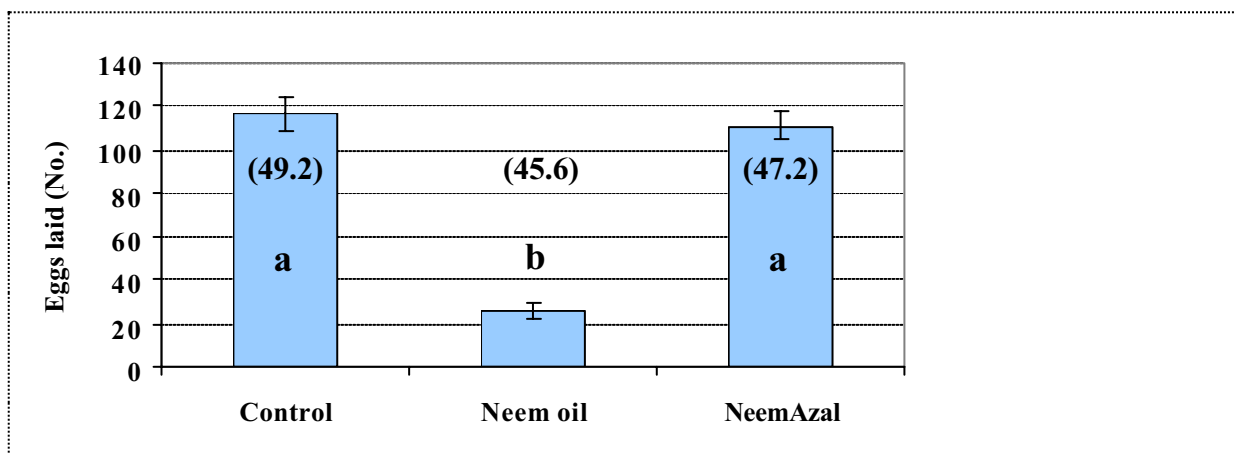
Figure 4.8: Effect of neem products on egg laying by *P. truncatus* adults on maize treated with NeemAzal® (0.3g/kg) and neem oil (7.5ml/kg) at 30°C and 70%r.h. The values in brackets are the numbers of surviving adults out of the original 50. Bars denoted by the same letter are not significantly different (t-test of transformed values: $p > 0.05$).
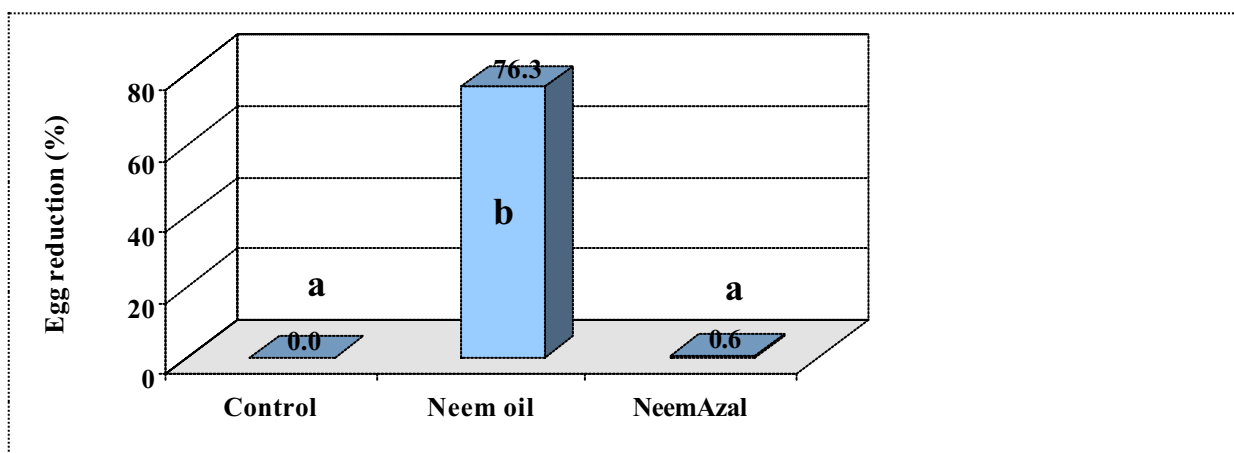
Figure 4.9: Reduction of egg laying by *P. truncatus* on maize treated with NeemAzal® (0.3g/kg) and neem oil (7.5ml/kg) at 30°C and 70%r.h. Values are means of five replicates. Bars denoted by the same letter are not significantly different (t-test, $p > 0.05$).

Insect population increase

Effect of pre-oviposition treatment: the total number of *P. truncatus* adults obtained from samples treated with different dosages of neem oil and NeemAzal® PC KG 01 are given in Figure 4.6. ANOVA was performed using transformed values ($y = \sqrt{x}$). All the treatments at all the dosage levels resulted in significantly less adults than the control with significant differences between some dosage levels. Treatment with NeemAzal® at 3 and 6g/kg and neem oil at 20ml/kg dosage levels resulted in nil insects. The samples treated with 5ml/kg dosage of neem oil resulted in significantly more progeny than those of 10ml/kg. Sieving of the frass at the end of the experiment revealed many larvae in the neem oil treated samples which, because

62

of their body size, seemed to have died fairly early in their development (Figure 4.16). They did not show any abnormal forms or characteristics and they appeared fairly uniform in body size and shape. The surviving adults also did not show any degree of morphological malformation. Fitting a typical dose response sigmoid curve for values for percentage reduction in the number of insects showed differences between total populations and progeny numbers (Figure 4.7, 4.8). For NeemAzal®, total population was reduced more than the progeny at lower treatment dosages while the reverse was true for neem oil.
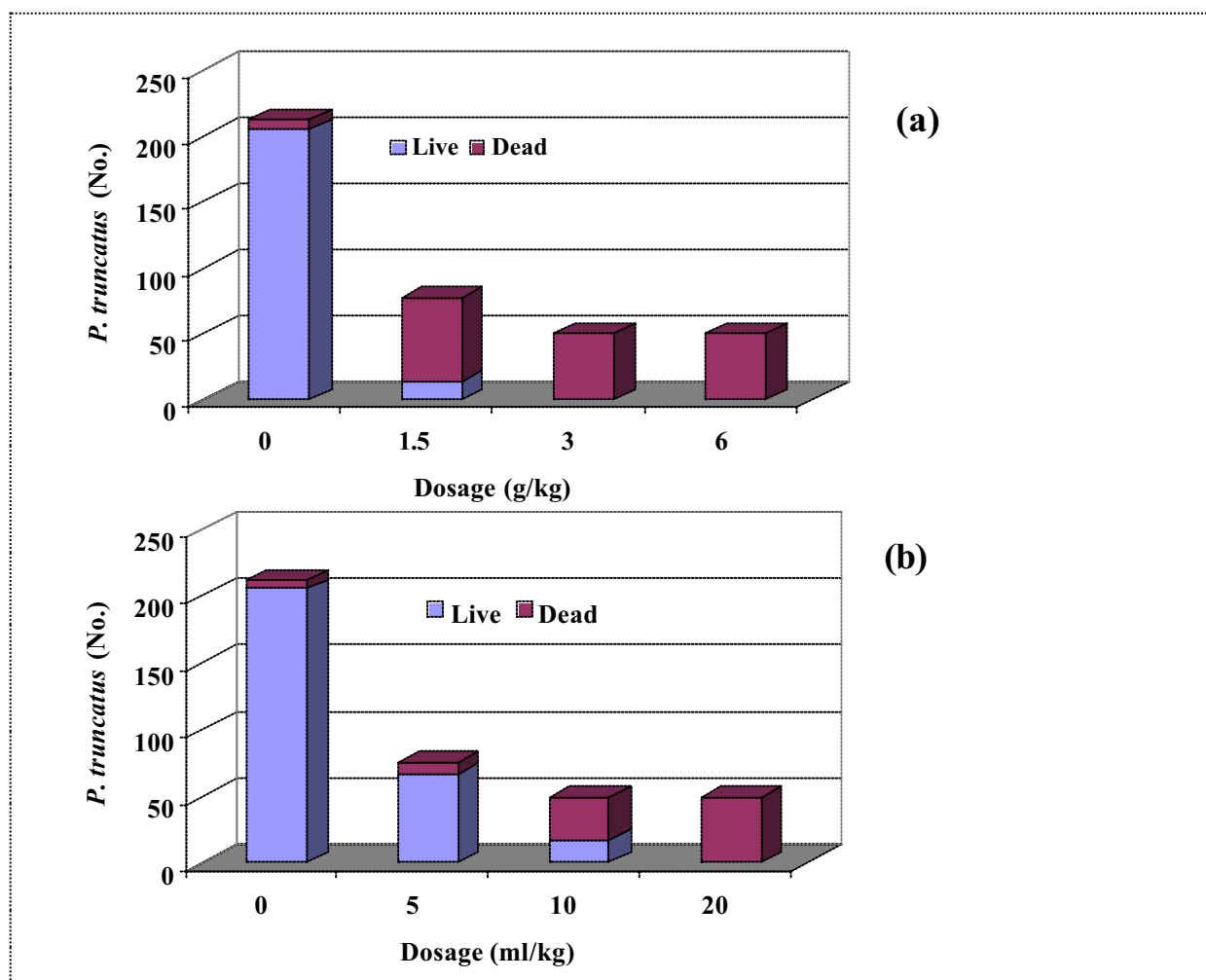
Figure 4.10: **Effect of (a) NeemAzal PC KG 01 and (b) neem oil on the population increase of 50 *P. truncatus* kept at 30°C and 70%r.h. for a period of 63 days**
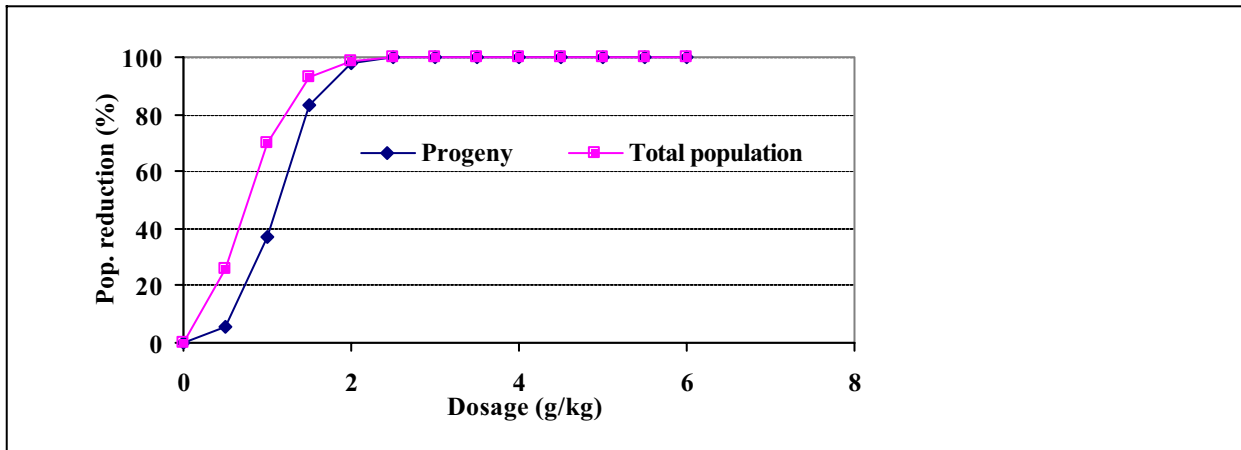
Figure 4.11: Insect population reduction by NeemAzal® PC KG 01:
Progeny reduction (%) = $100/(1+\exp(-x+1.12)/0.23)$; Adj $r^2 = 0.99$
Total population reduction (%) = $100/(1+\exp(-x+0.72)/0.29)$; Adj $r^2 = 0.99$
Curves fitted using Table Curve® 2D programme.
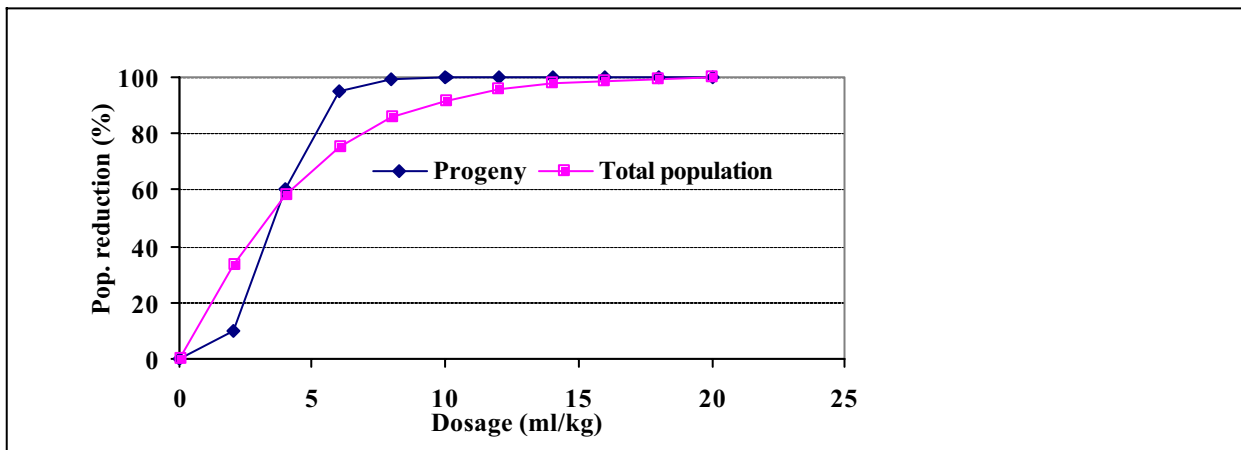
Figure 4.12: Insect population reduction by neem oil:
Progeny reduction (%) = $100/(1+\exp(-(x-3.66)/0.8))$; Adj $r^2 = 0.99$
Total population reduction (%) = $100/(1+\exp(-(x-2.1)/3.5))$; Adj $r^2 = 0.99$
Curves fitted using Table Curve® 2D programme.

Effect of post-oviposition treatment: the insect population of *P. truncatus* was significantly affected when grains containing eggs were treated with neem products. In the neem oil treated grains, no progeny was observed while the number of progeny observed in the NeemAzal® treated samples was significantly lower than that in the control. Adults formed the largest percentage of the insects observed in both the NeemAzal®-treated and control samples, but in the NeemAzal® samples, there were more dead than living insects (Figure 4.13). Sieving of the neem oil treated samples revealed tiny dead larvae similar to those observed in the pre-oviposition treatment samples (Figure 4.16).
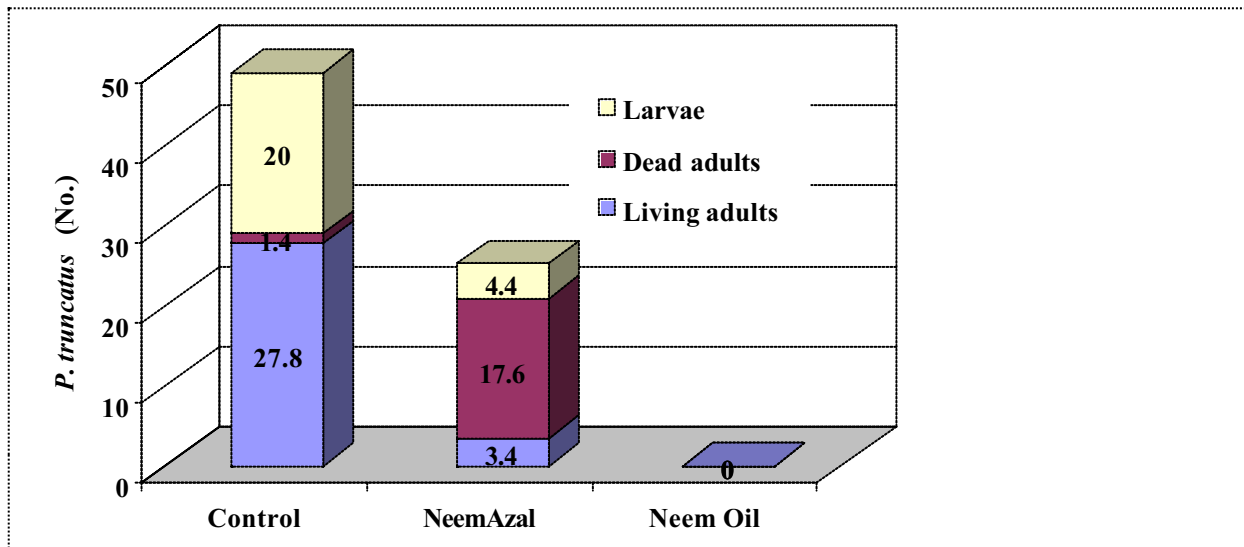
Figure 4.13: **Number of *P. truncatus* obtained from samples treated with 6g/kg NeemAzal® and 20ml/kg neem oil after egg laying by 50 *P. truncatus* for five days and keeping at 30°C and 70%r.h. for 35 days.**

Effect of sub-lethal dosages: the effect of all the three sub-lethal dosages of NeemAzal® PC KG 01 used was significant for the number of live and dead insects and for grain weight loss (ANOVA, $p < 0.001$). The number of insects decreased with increase in dosage levels and values for each dosage were significantly different from the control and from one another at the 5% probability level (Figure 4.14). Mean numbers of the total living insects were 519, 462.8 and 402.4 for 0.3, 0.2, and 0.3g/kg dosages, respectively, and 578 in the control. The grain weight loss values followed a similar pattern as the insect numbers, decreasing with increasing dosage values. All treatment means were significantly lower than the control although those of 0.1 and 0.2g/kg were not significantly different. The number of dead beetles increased with increasing dosage and were all significantly different from one another and from the control.

Treatment of samples with sub-lethal dosages of neem oil resulted in significantly different numbers of live and dead insects and amount of grain weight loss (ANOVA, $p < 0.001$). The number of insects decreased with increase in dosage levels and values for each dosage were significantly different from the control and from one another except for those of 5 and 7.5ml/kg dosages, which were not significantly different at the 5% probability level (Figure 4.15). The grain weight loss values followed a similar pattern as the insect numbers and exhibited a typical decreasing curve. All treatment means for weight loss were significantly lower than the control and significantly different from one another. The number of dead beetles decreased with increasing dosage up to 5ml/kg. The mean number of dead beetles for 5ml/kg was lower than for both 7.5ml/kg (9.6) and 2.5ml/kg (12.2) and significantly lower than for all the other

dosages (DMRT, p = 0.05). The difference between 2.5ml/kg dosage and the control was not significant.
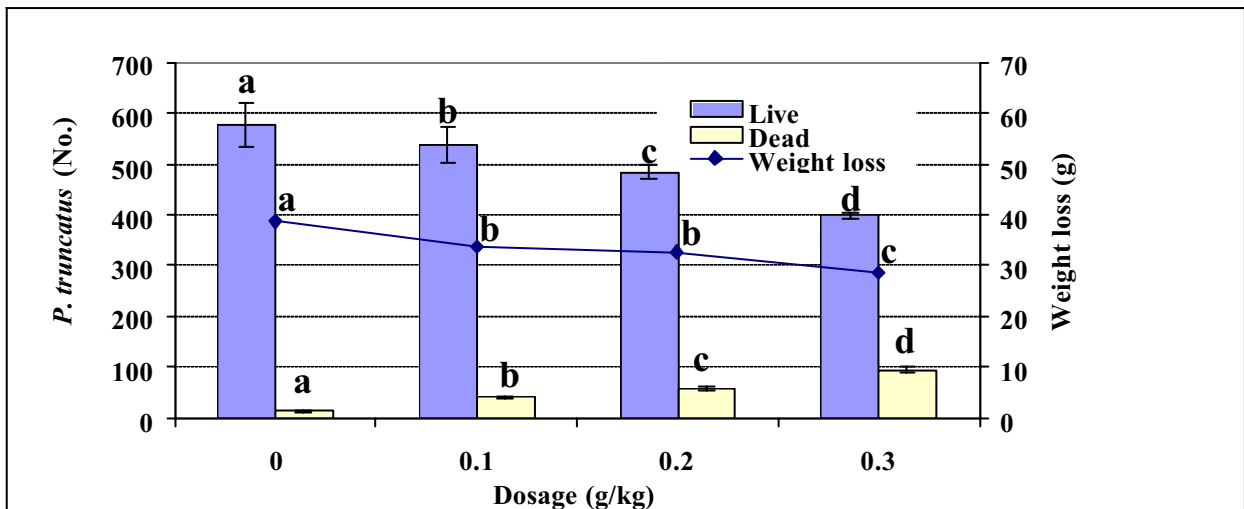
Figure 4.14: Effect of sub-lethal dosages of NeemAzal® PC KG 01 on the population increase of, and grain weight loss by 50 *P. truncatus* kept at 30°C and 70%r.h. for 63 days. Data are means of five replicates. Values denoted with the same letter are not significantly different (DMRT of transformed data, p = 0.05).
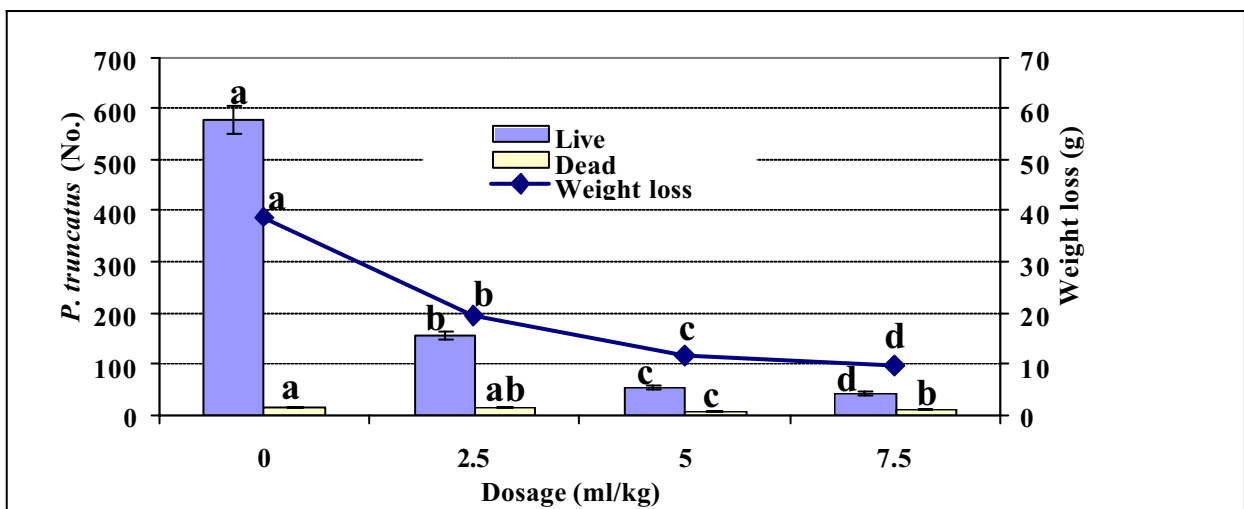
Figure 4.15: Effect of sub-lethal dosages of neem oil on the population increase of, and grain weight loss by 50 *P. truncatus* kept at 30°C and 70%r.h. for 63 days. Data are means of five replicates. Values denoted with the same letter are not significantly different (DMRT of transformed data, p = 0.05).

a) Neem seed cake powder settled at the bottom of the grains.
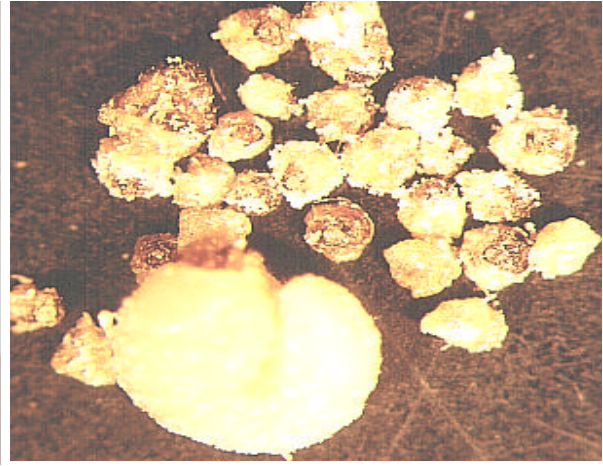
b) Dead *P. truncatus* larvae from neem oil-treated grains appeared uniformly shrunken. In the fore-ground is a single live larva.

Maize grain (50X)

c) NeemAzal® coating on a grain treated with the product
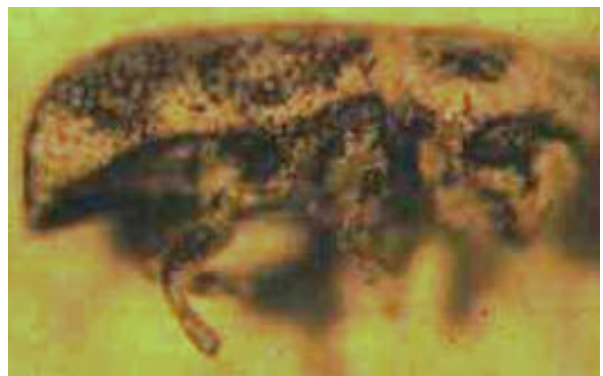
d) Dead *P. truncatus* adults from NeemAzal®-treated grains were coated with the product.

Figure 4.16: Treated maize grains and dead insects from various experiments in the study

Effect of neem oil volatiles on *P. truncatus*

The effect of neem oil volatiles on mortality, weight loss and reproduction of *P. truncatus* is given in Table 4.5. Generally, the volatiles did not have a significant effect on any of the three parameters. Both the parents and F_1 did not display any morphological defects and were as normal as those from the control.

Table 4.5: Effect of neem volatiles on various parameters of 10 *P. truncatus* kept in cages filled with maize grains and kept at 30°C and 70%r.h for 7 days (eggs) and 35 days (mortality, weight loss and total number of adults)

Measure	Control	Treatment	Difference
Weight loss (g)	0.54 (0.04)	0.51 (0.05)	ns[a]
Total adults (No.)	30.20 (4.49)	28.20 (6.62)	ns[b]
Eggs (No.)	12.20 (1.39)	9.20 (1.43)	ns[b]
Mortality (%)	3.20 (1.78)	3.60 (1.16)	ns[b]

ns-not significant; a- Student t-test; b- Mann-Whitney U-test; ()-std error

4.2   Effect of neem oil on *Teretrius nigrescens*

Lethal effect on maize grains and on glass beads

The lethal effect of neem oil on *T. nigrescens* on treated maize grains is given in Figure 4.17. Neem oil caused 10.8 and 57.6% mortality of *T. nigrescens* at the dosage levels of 10 and 20 ml/kg, respectively, while the predator managed to survive at the lower dosages. Compared to *P. truncatus*, *T. nigrescens* showed higher tolerance on maize grains, the difference between the two being significant at all the dosage levels tested (Student t-test, $p < 0.05$). On glass beads, neem oil caused mortality of *T. nigrescens* starting from 2.5ml/kg maize volume equivalent (Figure 4.18). The lethal effect of neem oil on *T. nigrescens* was significantly lower than that on *P. truncatus* (Student t-test) for all the dosages except at the highest dosage tested (20ml/kg) where both insects suffered 100% mortality.
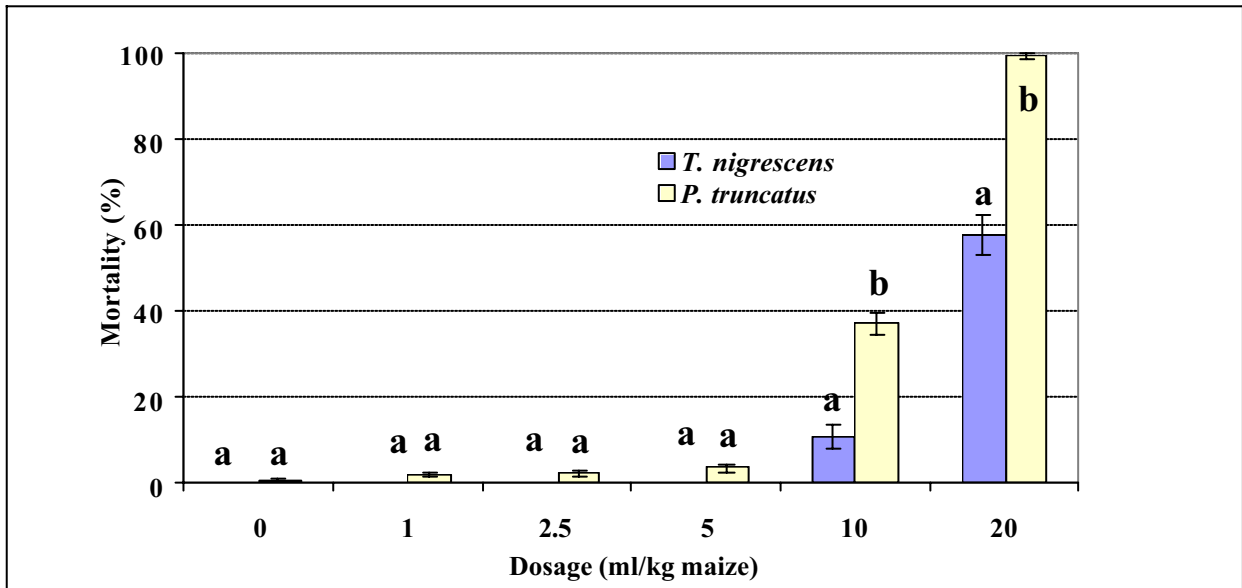
Figure 4.17: Lethal effect of neem oil on *P. truncatus* and *T. nigrescens* on treated maize grains kept at 30°C and 70%r.h. Data are means of five replicates. Bars within the same treatment dosage denoted with the same letter are not significantly different (Student t-test, p > 0.05).
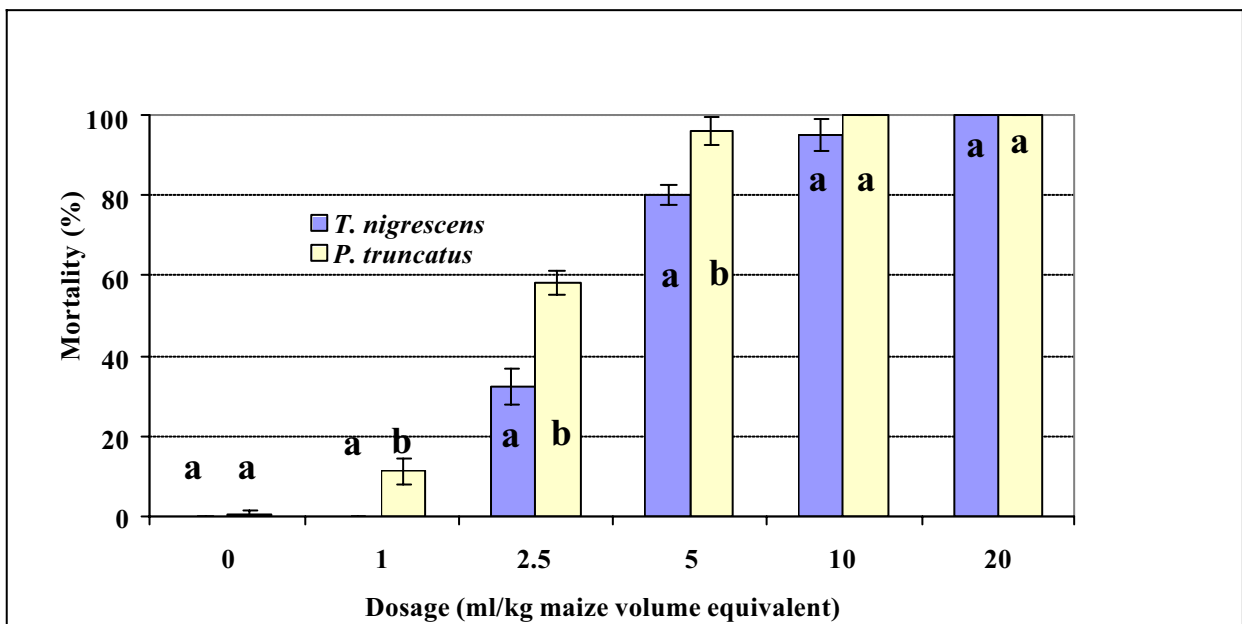
Figure 4.18: Lethal effect of neem oil on *P. truncatus* and *T. nigrescens* on treated glass beads kept at 30°C and 70%r.h.. Data are means of five replicates. Bars within the same treatment dosage denoted with the same letter are not significantly different (Student t-test, p > 0.05).

69

Effect on predatory activity

Grain weight loss and damage

Data for grain weight loss and number of grains bored by *P. truncatus* both in the presence and absence of *T. nigrescens* at different dosages of neem oil are given in Figure 4.19. Both weight loss and number of bored grains were significantly reduced by treatment with neem oil (Split-plot ANOVA, $p < 0.001$) (Table 4.6 and 4.7). Values for the number of bored grains were transformed using the function $y = \log (x + 1)$. For both weight loss and the number of grains bored, all the treated samples resulted in significantly lower values than the control (DMRT, $p = 0.05$). Both values showed a very similar pattern, decreasing with increase in dosage and all dosages were significantly different in their effects from one another. The interaction between neem oil and *T. nigrescens* was also significant ($p < 0.001$) for both weight loss and grain damage.

Table 4.6: Analysis of variance for grain weight loss from 100g maize caused by *P. truncatus* affected by different dosages of neem oil with and without *T. nigrescens* for eight weeks at 30°C and 70%r.h.

Source	d.f.	Sum of squares	Mean square	F value	p
Neem oil (NO)	3	1301.0	433.7	1016.39	<0.001
T. nigrescens (*T.n.*)	1	230.1	230.1	539.31	<0.001
NO x *T.n.*	3	539.7	180.0	421.75	<0.001
Block	4	1.8	0.4	1.08	0.400
Error (main)	12	1.8	0.2		
Error (sub)	16	7.0	0.4		
Total	**39**	**2081.4**			

Table 4.7: **Analysis of variance for the number of grains, out of 100g maize, bored by** *P. truncatus* **affected by different dosages of neem oil with and without** *T. nigrescens* **for eight weeks at 30°C and 70%r.h.**

Source	d.f.	Sum of squares	Mean square	F value	p
Neem oil (NO)	3	1.67	0.560	303.03	<0.001
T. Nigrescens (*T.n.*)	1	0.08	0.080	41.03	<0.001
NO x *T.n.*	3	0.15	0.150	26.89	<0.001
Block	4	0.01	0.002	1.23	0.337
Error (main)	12	0.02	0.001		
Error (sub)	16	7.00	0.002		
Total	**39**	**2081.40**			
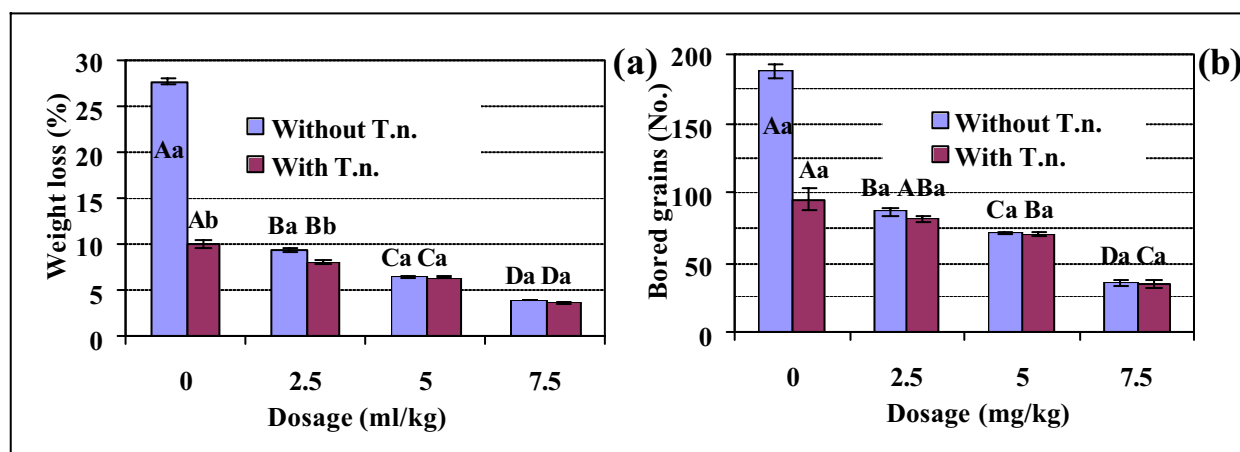
Figure 4.19: **Effect of neem oil on maize grain (a) weight loss and (b) boring by** *P. truncatus* **on 100g maize kept at 30°C and 70%r.h. with and without** *T. nigrescens*. **Data are means of five replicates. Bars denoted by the same letter are not significantly different, capital letters for comparison within (DMRT, p = 0.05), and small letters between treatments, respectively (Student t-test and Mann-Whitney U test, p < 0.05).**

P. truncatus population

The mean number of living and dead insects in samples treated with various dosages of neem oil and either containing or not containing *T. nigrescens* is given in Figure 4.20. The effects of both neem oil and presence of *T. nigrescens* were significant (Split-plot ANOVA, p < 0.001) (Table 4.8 and 4.9) for both live and dead insects. Values for number of insects were transformed using the function y = (log x + 1). For both live and dead insects, treated samples resulted in significantly lower values than the control (DMRT, p = 0.05). For live insects, there was no significant difference between the results at all the three dosage levels for both the samples with and without *T. nigrescens*. For dead insects, the highest figure for samples

71

containing *T. nigrescens* was recorded for 7.5g/kg dosage level followed by the control while for samples without *T. nigrescens* the values were in the order 2.5 < 0 < 5 < 7.5ml/kg, the difference between them being significant.

Table 4.8: Analysis of variance for the number of live *P. truncatus* from initial 50 adults in 100g maize samples treated with different dosages of neem oil with and without *T. nigrescens* and kept at 30°C and 70%r.h. for eight weeks

Source	d.f.	Sum of squares	Mean square	F value	p
Neem oil (NO)	3	3.15	1.050	240.55	<0.001
T. nigrescens (*T.n.*)	1	1.46	1.460	336.67	<0.001
NO x *T.n.*	3	2.30	0.770	176.12	<0.001
Block	4	0.01	0.002	0.63	0.650
Error (main)	12	0.04	0.003		
Error (sub)	16	0.06	0.004		
Total	**39**	**7.06**			

Table 4.9: Analysis of variance for dead *P. truncatus* from initial 50 adults in 100g maize samples treated with different dosages of neem oil with and without *T. nigrescens* and kept at 30°C and 70%r.h. for eight weeks

Source	d.f.	Sum of squares	Mean square	F value	*p*
Neem oil (NO)	3	0.57	0.19	25.1	<0.001
T. nigrescens (*T.n.*)	1	0.36	0.36	48.0	<0.001
NO x *T.n.*	3	0.31	0.10	13.5	<0.001
Block	4	0.04	0.01	1.2	0.351
Error (main)	12	0.17	0.01		
Error (sub)	16	0.12	0.01		
Total	**39**	**1.57**			
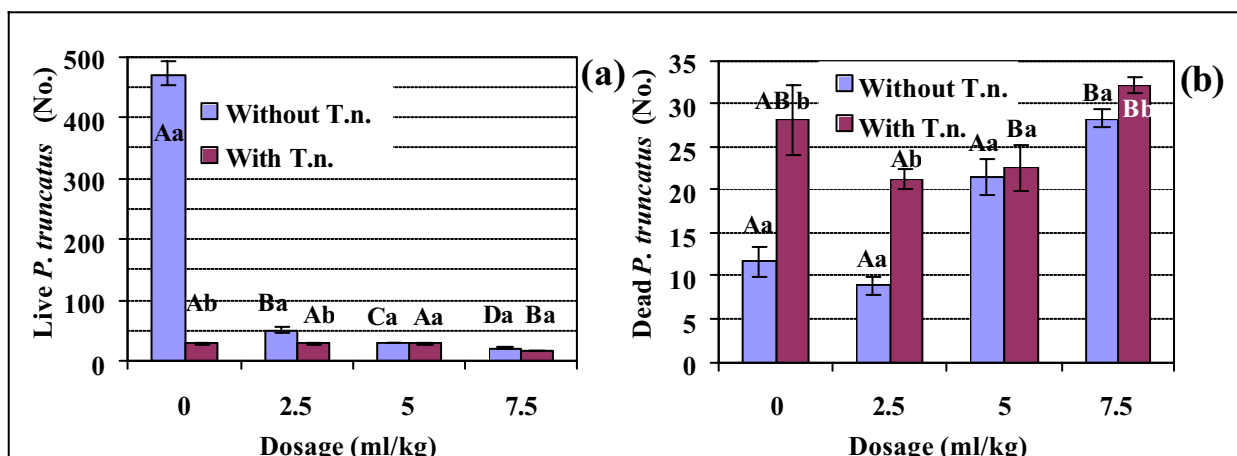
Figure 4.20: Numbers of (a) live and (b) dead *P. truncatus* in 100g maize treated with different dosages of neem oil and kept at 30°C and 70%r.h. with and without *T. nigrescens*. Data are means of five replicates. Bars denoted by the same letter are not significantly different; capital letter for comparison between dosages (DMRT, p = 0.05) and small letters for comparison within dosages (Student t-test and Mann-Whitney U test, p > 0.05).
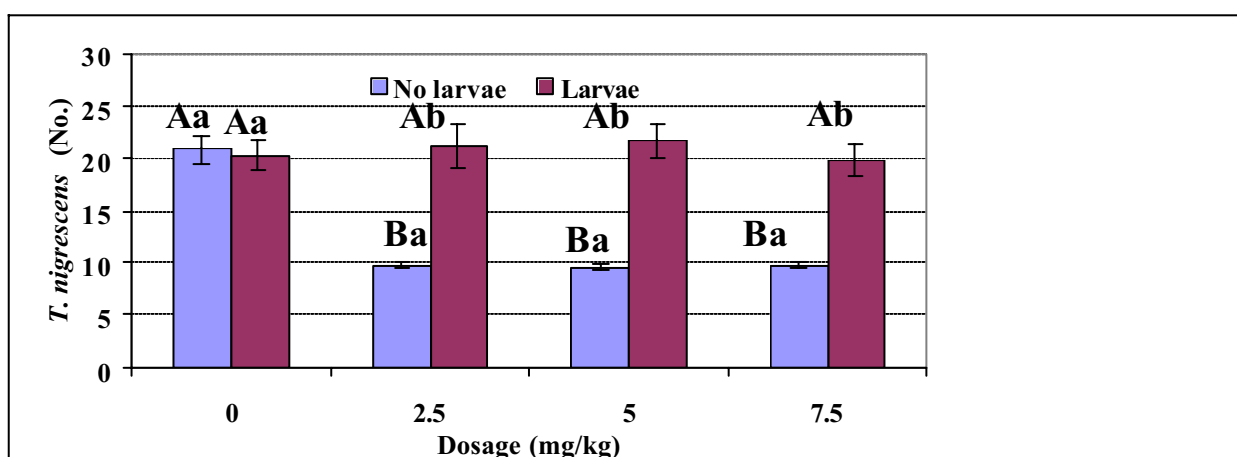
Figure 4.21: Numbers of *T. nigrescens* in samples treated with different dosages of neem oil with and without additional larvae after eight weeks at 30°C and 70%r.h. Data are means of five replicates. Bars denoted by the same letter are not significantly different; capital letters for comparison between dosages (DMRT p = 0.05) and small letters for comparison within dosages (Student t-test and Mann-Whitney U test, p > 0.05).

T. nigrescens population

The mean number of *T. nigrescens* was compared between samples in which larvae were added and those in which they were not. The effect of neem oil on the resulting number of *T. nigrescens* was significant (ANOVA, p < 0.001) for samples without additional larvae but not for those with additional larvae. For samples without additional larvae, there was no significant difference between the effect of dosage levels, which were all significantly lower than the control. Apart from the control, the difference within results from all dosages between presence and absence of additional larvae was significant (Mann-Whitney U-test,

p < 0.05) with the samples that received additional larvae resulting in higher numbers of *T. nigrescens* than those that did not (Figure 4.21).

4.3 Persistence of neem products

Mortality

The lethal effect of neem products on *P truncatus* over a period of six months is given in Figure 4.22. For both NeemAzal[®] and neem oil, mortality did not change significantly over the whole period of six months. Mean mortality values ranged between 0.8 and 1.6% in the control, 1.6 and 3.6% in neem oil-treated and 95.2 and 98% in NeemAzal[®]-treated samples. The lethal effect of neem oil treatment was not significantly different from the control while treatment with NeemAzal[®] resulted in significantly lower mortality than the control.

Weight loss

The effect of neem products on frass activity of *P. truncatus* determined as weight loss over a period of six months is given in Figure 4.23. For both NeemAzal[®] and neem oil, weight loss values were not significantly different at the different time period up to six months. Mean weight loss values ranged from 1.94% to 2.05% in the control, 1.38% to 1.49% in neem oil-treated and 0.39% to 0.47% in NeemAzal[®]-treated samples. At all the time periods, the weight loss resulting from both treatments was significantly lower than that from the control.
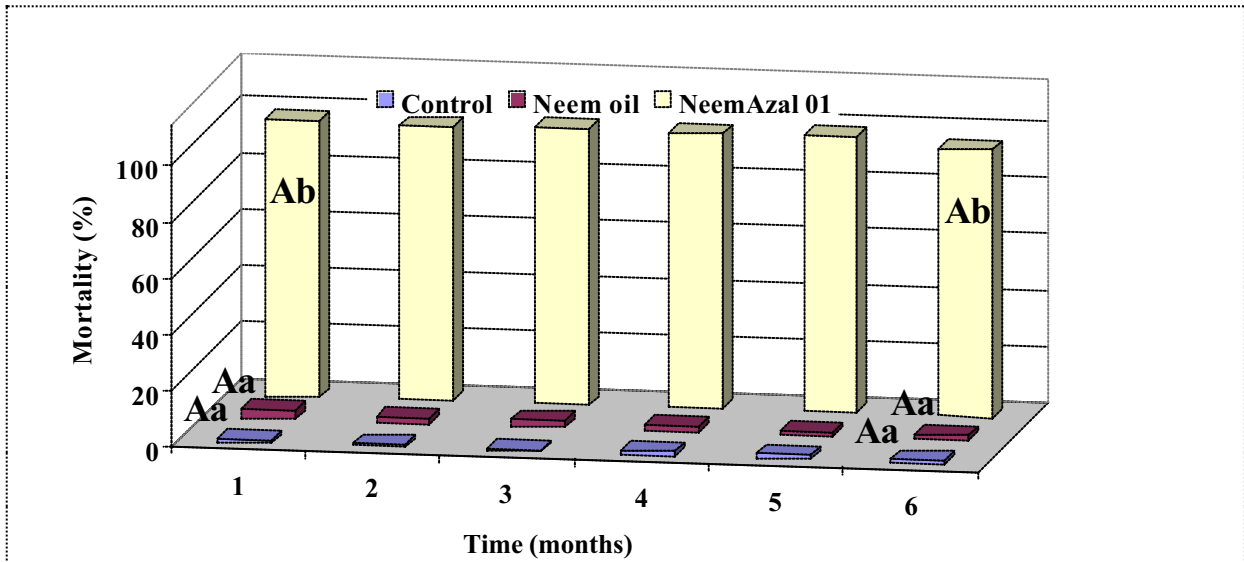
Figure 4.22: Persistence of lethal effect of NeemAzal® (6g/kg) and neem oil (20ml/kg) on 50 *P. truncatus* adults after various periods up to six months at 30°C and 70%r.h.. Bars denoted by the same letter are not significantly different, capital letters for comparison within treatments (DMRT, p = 0.05) and small letters between treatments and the control (Student t-test, p > 0.05). Differences between all other treatments were similar to those indicated.
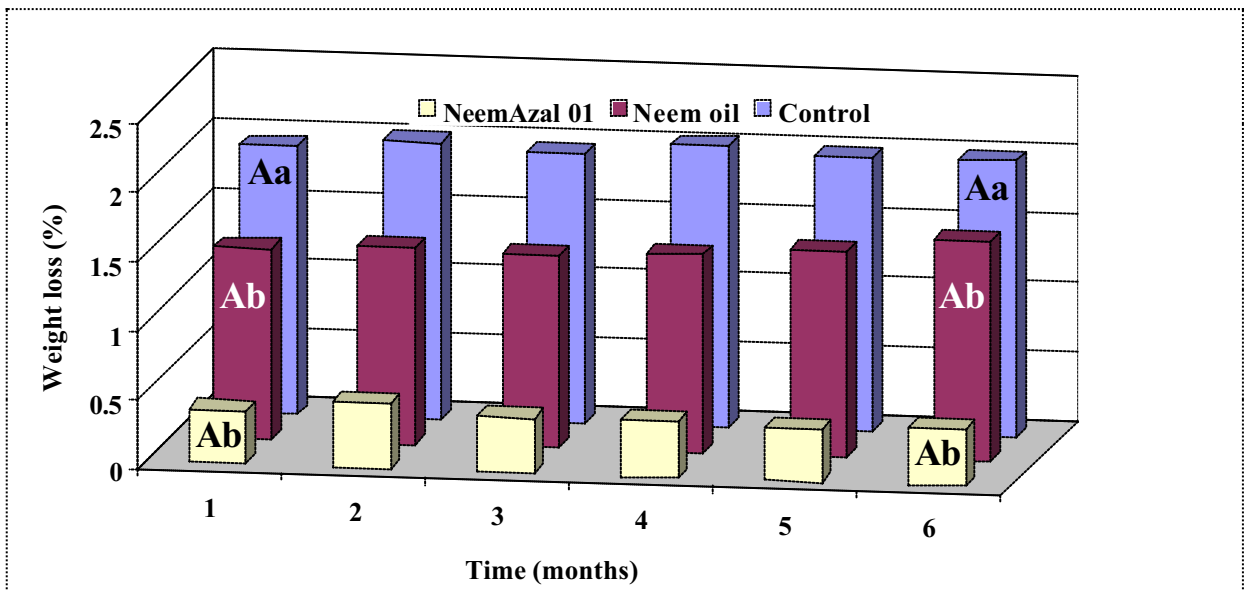
Figure 4.23: Persistence of effect of NeemAzal® (6g/kg) and neem oil (20ml/kg) on weight loss by 50 *P. truncatus* after various durations up to six months at 30°C and 70%r.h.. Bars denoted by the same letter are not significantly different, capital letters for comparison within treatments (DMRT, p = 0.05) and small letters between treatments and the control (Student t-test, p > 0.05). Differences between all other treatments were similar to those indicated.

Egg production

The number of eggs laid by *P. truncatus* on grains kept for various time durations up to six months is given in Figure 4.24. Treatment with both NeemAzal[®] and neem oil resulted in significantly fewer eggs than in the control at tested periods. The number of eggs laid increased slightly with time in the neem oil-treated samples but the difference was not statistically significant between any of the periods. The mean number of eggs laid ranged from 121.2 to 139 in the control, 13.8 to 21.2 in neem oil-treated and 10 to 15.8 in NeemAzal[®]-treated samples.

Insect population increase

The persistent effect of neem products on insect population increase of *P. truncatus* for a period of six months is illustrated in Figure 4.25. The effects of both NeemAzal[®] and neem oil on the total number of insects did not change significantly with time over this period. The results show that at each period tested, treatment of samples with both products resulted in significantly fewer total live insects than in the control. The means for different periods were not significantly different from one another (DMRT, $p = 0.05$). Mean numbers ranged from 505.2 to 535 in the control, 47.2 to 48.8 in neem oil and 1.4 and 2.8 in NeemAzal[®]-treated samples.

4.4   Repellent effect of neem oil

Volatile repellence

Volatile repellent effect on *P. truncatus*

The repellent effect of neem oil volatiles on *P. truncatus* is summarised in Table 4.10. *P. truncatus* were significantly more attracted to the chambers containing non-treated maize and non–treated maize with *P. truncatus* than to the blank chambers and to chambers containing treated maize (Wilcoxon two-sample test, $p < 0.05$). The differences in distribution of *P. truncatus* for all the other comparisons were not statistically significant. *P. truncatus* was not repelled by any of the treatments but showed attraction to maize grains.
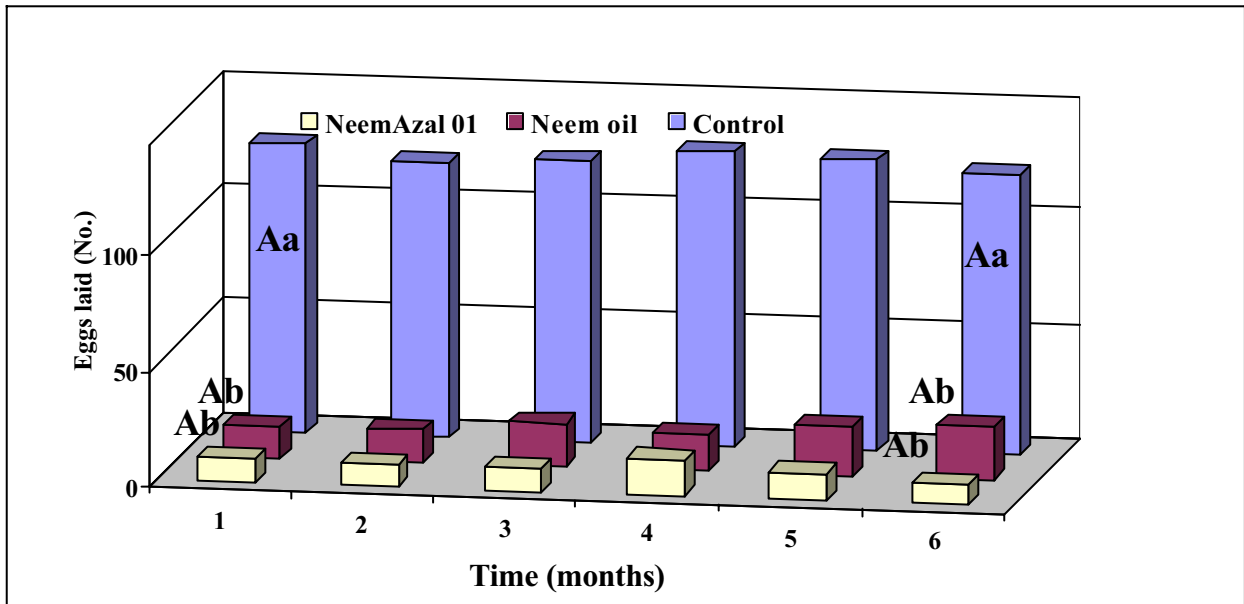
Figure 4.24: Persistence of effect of NeemAzal® (6g/kg) and neem oil (20ml/kg) on egg laying by 50 *P. truncatus* after various durations up to six months at 30°C and 70%r.h. Bars denoted by the same letter are not significantly different, capital letters for comparison within treatments (DMRT, p = 0.05) and small letters between treatments and the control (Student t-test, p > 0.05). Differences between all other treatments were similar to those indicated.
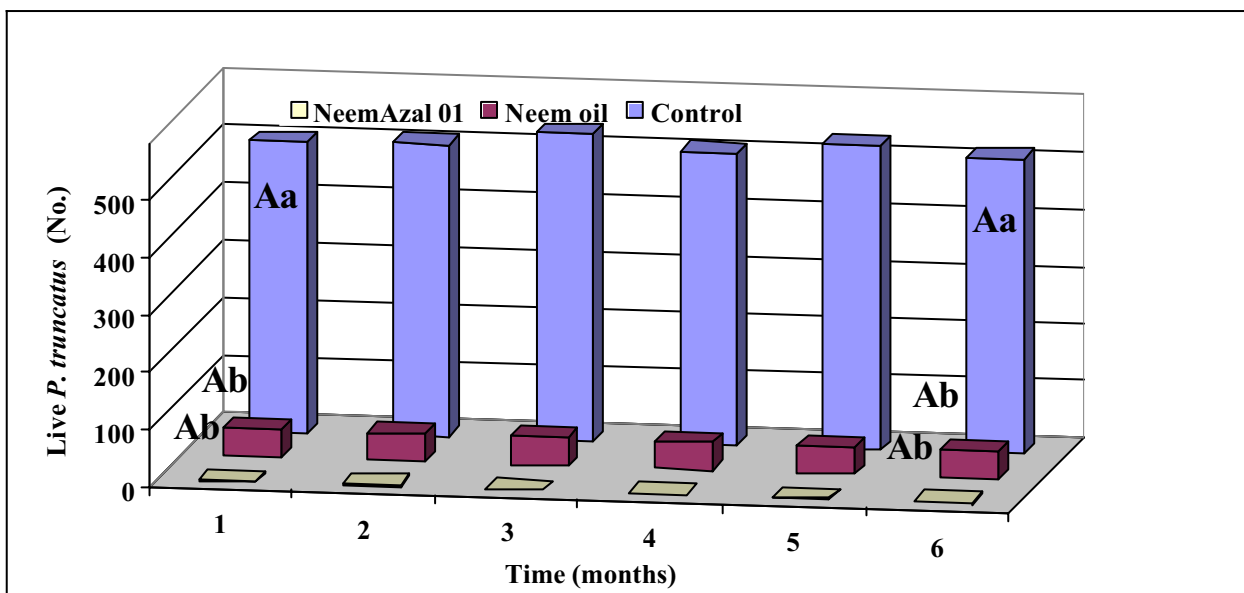
Figure 4.25: Persistence of effect of NeemAzal® (6g/kg) and neem oil (20ml/kg) on *P. truncatus* population increase from 50 adults after various durations at 30°C and 70%r.h. Bars denoted by the same letter are not significantly different, capital letters for comparison within treatments (DMRT, p = 0.05) and small letters between treatments and the control (Student t-test, p > 0.05). Differences between all other treatments were similar to those indicated.

Volatile repellent effect on *T. nigrescens*

Table 4.11 gives the repellent effect of neem oil volatiles on *T. nigrescens*. The predator responded equally to both neem oil-treated and non-treated samples. It showed attraction to maize and the response was not affected by the presence of neem oil. The mean walking time over both treated and non-treated maize both in the presence and absence of *P. truncatus* were significantly higher (Wilcoxon two-sample test, $p < 0.05$) than over the respective blanks. All comparisons between treated and non-treated maize grains did not show significant differences.

Table 4.10: Response of female *P. truncatus* to volatiles of neem oil and male *P. truncatus* on a two-chamber walking arena (see figure 3.8) at 30°C and 70%r.h.

Chamber 1		Chamber 2		
	Mean No. (%)		Mean No. (%)	p value*
Blank	35.5	NT maize	64.5(3.6)	0.007
Blank	29.3	NT maize + *P.t.*	70.7(2.1)	0.005
Blank	51.0	TR maize	49.0(2.5)	1.000
Blank	49.5	TR maize + *P.t.*	50.5(2.5)	1.000
Blank	51.5	(TR maize + *P.t.*)+NT maize	48.5(1.8)	0.374
Blank	49.0	(NT maize + *P.t.*)+TR maize	51.0(5.7)	0.333
TR maize	30.7	NT maize	69.3(3.5)	0.007
TR maize	32.5	NT maize + *P.t.*	67.5(2.4)	0.005

* p according to Wilcoxon-two-sample test. Values are percentages of original 50 insects found in respective chambers after a three-hour waiting period: Values in brackets = ±SE of the mean, NT = non-treated grains, TR = treated grains

Contact repellence

Food preference experiment

The distribution of *P. truncatus* between grains containing different dosages of neem oil is summarised in Table 4.11. Generally, the insects were significantly repelled from neem-treated grains more than from the control. The repellence was dose dependent and increased with increasing dosage, from RI = -0.23 in 2.5g/kg dosage to RI = -0.62 in 7.5ml/kg. The difference between repellence at 0.25 and 0.5ml/kg was significant while between repellence at 0.5 and 0.75ml/kg was not (Table 4.12). All the insects entered the grains and in all the tests, no insect was found on the release platform after a short while of about one hour.

Table 4.11: **Response of *T. nigrescens* to volatiles of neem oil in a two-chamber walking arena at 30°C and 70%r.h.**

	Chamber 1		Chamber 2	
	Mean walking time (seconds)		Mean walking time (seconds)	*P* value*
Blank	270.5	NT maize	329.5 (9.1)	0.004
Blank	277.8	NT maize + *P.t.*	322.2 (9.4)	0.028
Blank	276.7	TR maize	323.3 (10.3)	0.035
Blank	275.5	TR maize + *P.t.*	324.5 (9.5)	0.017
TR maize	285.0	TR maize + *P.t.*	315.0 (8.7)	0.101
NT maize	292.7	NT maize + P.t.	307.3 (9.4)	0.443
TR maize	308.3	NT maize	291.6 (11.5)	0.477

* p according to Wilcoxon-two-sample test. Values are the time spent by the insect above each chamber in seconds. Values in brackets = ±SE of the mean: NT = non-treated grains, TR = treated grains

Table 4.12: **Repellence of *P. truncatus* by maize grains treated with different dosages of neem oil after 24 hours in a food preference apparatus at 30°C and 70%r.h.**

Dosage ml/kg	Insects in control maize (%)	Insects in treated maize (%)	SE of mean (±)	RI	p value*
2.5	61.6	38.4	4.5	-0.23	0.037a
5.0	78.7	21.3	1.4	-0.57	0.009b
7.5	81.0	19.0	2.5	-0.62	0.007b

*p-value according to Wilcoxon-two-sample test. Values are the percentage of the original 50 insects. Values with the same letter down the p column are not significantly different (Mann-Whitney U-test, p > 0.05).

Escape experiment

Figure 4.26 gives the number of *P. truncatus* that escaped from the treated samples to the outside of the grains over time. It is important to note that the escape was not permanent since there was continuous movement of insects into and out of the grains, but generally the total number of escaping insects increased gradually with time for all the dosages apart from in the control in which the numbers fluctuated. After 24 hours from the start of the experiment, 20, 49.6 and 67.6% of the original numbers were located outside the grains treated with 2.5, 5 and 7.5ml/kg, respectively, and were significantly higher than the 8.8% value observed outside the grains in the control.
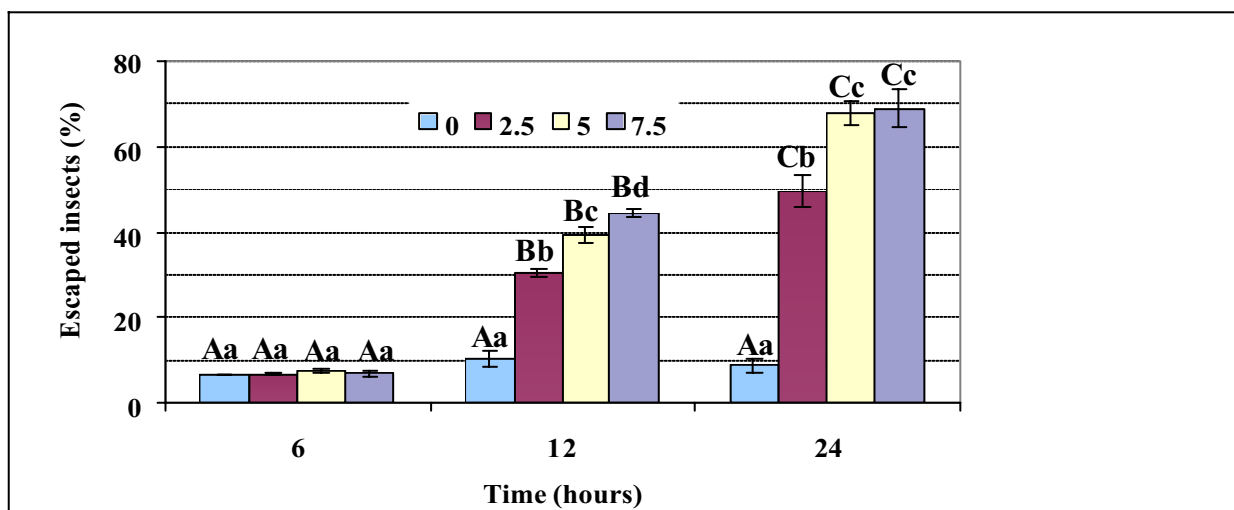
Figure 4.26: **The percentage number of 40 *P. truncatus* escaping from treated grains after various time intervals at 30°C and 70%r.h. Values are means of five replicates. Bars denoted with the same letter are not significantly different, capital letters for comparison between times and small letters within times (DMRT, p = 0.05).**

4.5   Feeding deterrence

Larval feeding deterrence

First instar larvae

The effect of neem oil on the development of the first instar larvae of *P. truncatus* on flour is shown in Figure 4.27. The effect of the oil was significant (ANOVA, p < 0.001); treatment of flour resulted in larvae with significantly lower bodyweight (LSD Bonferroni, p = 0.05) than non-treated flour, after an exposure period of seven days. The samples that contained no flour also resulted in significantly lower weight than in the control but were not significantly different from the treated flour samples. The difference in the effect of treatments with various dosages of neem oil was not significant. After a 14-day exposure period, only the control samples contained live larvae. All larvae in the neem-treated flour samples and in the samples without flour died in the 14-day period. The dead larvae did not show any obvious morphological malformation or abnormal colouration, they were shrunken and dry.

Second instar larvae

Figure 4.28 gives a summary of the effect of neem oil on the development of the second instar larvae of *P. truncatus* under various treatments for a period of 14 days. Treating the flour with neem oil caused a significant reduction (ANOVA, p < 0.001) in larval body weight. Larvae on

flour treated with neem oil at 20 and 40ml/kg weighed significantly less (LSD Bonferroni, p = 0.05) than those on non-treated flour in the control samples, after a seven-day exposure period. The treatment without any flour also resulted in significantly lower weight than the control but not significantly different from the treated flour samples. The effects of the 5 and 10ml/kg dosage level treatments were also not significantly different from the control. The larvae in the control and in 5 and 10ml/kg dosage samples gained weight while those in all the other treatments lost. After a 14-day exposure period, the 20 and 40ml/kg samples resulted in significantly lower larval weight than the other treatments and the control. Between seven and 14 days, body weight declined in all the samples. The larvae did not show any obvious morphological malformation or abnormal colouration.

Third instar larvae

In the third instar larvae, the change in weight could not be clearly related to the different treatments used most likely because of the decline in body weight at the onset of pupation. Because of this inconsistency, only weights after a seven-day exposure period were recorded (Figure 4.29). The effect of the oil treatment for this exposure period was however significant (ANOVA, p < 0.05). Only the weight of the larvae in 20ml/kg-treated flour among the neem oil-treated samples resulted in larvae with significantly lower bodyweight (LSD Bonferroni, p = 0.05) than in the control, which contained non-treated flour. The larvae from this dosage, together with those from the samples without flour weighed significantly less than those from control samples, but only those from 20ml/kg samples were significantly different from those from other dosages.
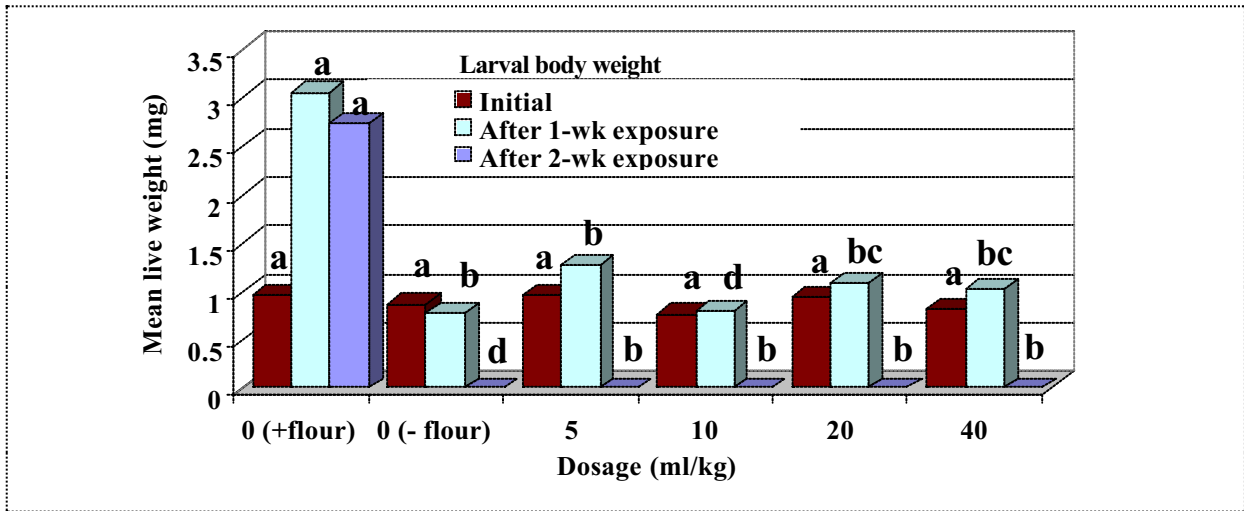
Figure 4.27: Effect of neem oil on first instar larvae of *P. truncatus* kept on maize flour for 14 days at 30°C and 70%r.h., (+flour)-sample contained non-treated flour, (-flour)-sample did not contain any flour. Values are means of fve replicates. Means denoted with the same letter in different treatments are not significantly different (LSD Bonferroni, p = 0.05).
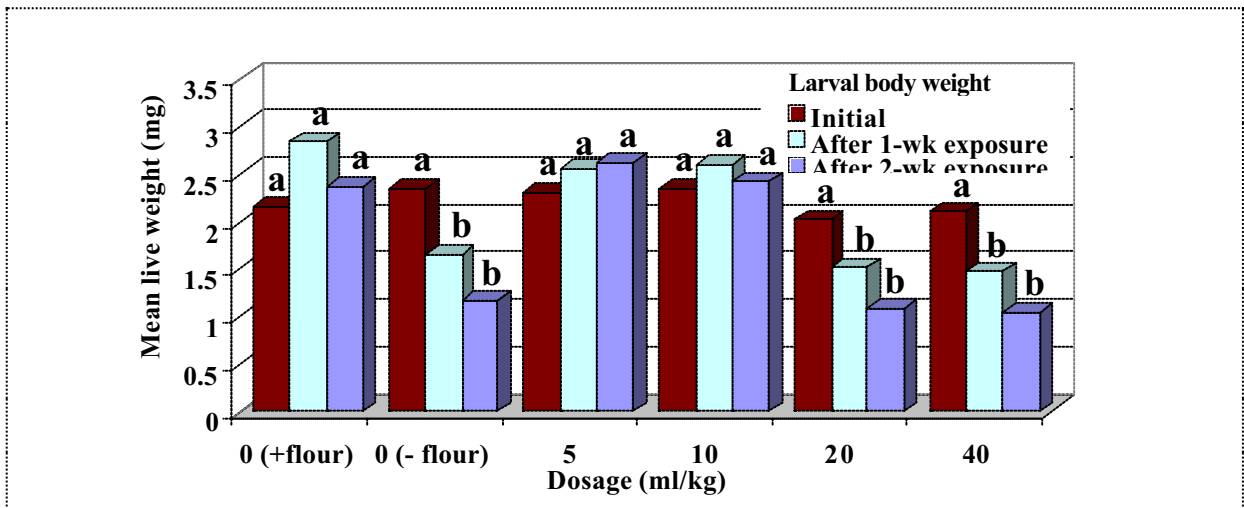
Figure 4.28: Effect of neem oil on second instar larvae of *P. truncatus* kept on maize flour for 14 days at 30°C and 70%r.h., (+flour)-sample contained non-treated flour, (-flour)-sample did not contain any flour. Values are means of five replicates. Means denoted with the same letter in different treatments are not significantly different (LSD Bonferroni, p = 0.05).

82
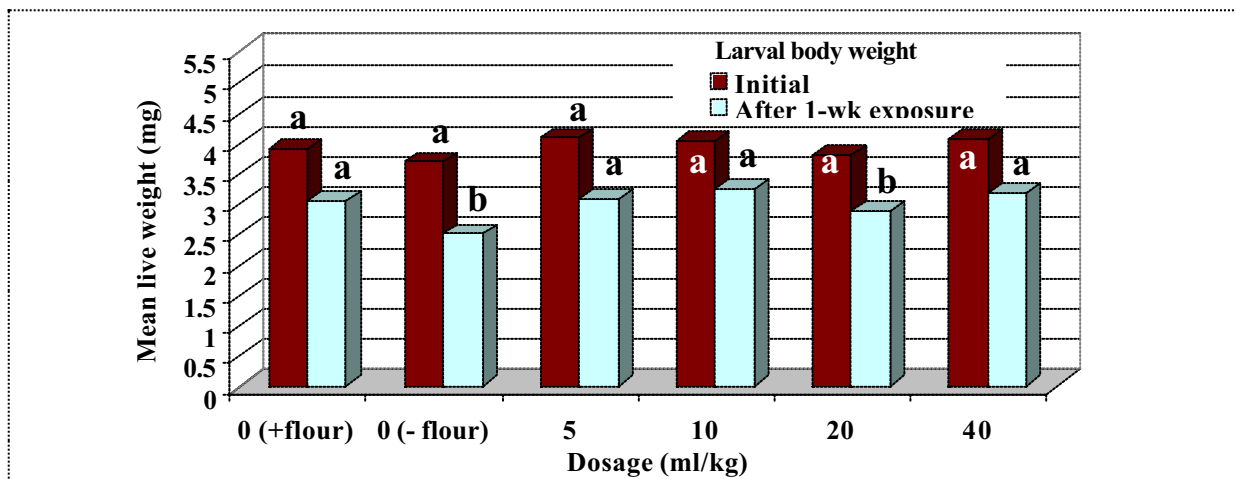
Figure 4.29: Effect of neem oil on third instar larvae of *P. truncatus* kept on maize flour for seven days at 30°C and 70%r.h., (+flour)-sample contained non-treated flour, (-flour)-sample did not contain any flour. Means denoted with the same letter in different treatments are not significantly different (LSD Bonferroni, p = 0.05).

Adult feeding deterrence

Feeding deterrence on maize flour

Treatment of flour with neem oil caused a significant effect on the survival of *P. truncatus* on maize (ANOVA, p < 0.001). However, only the effects of 30 and 40ml/kg treatments were significantly different (LSD Bonferroni, p = 0.05) from the control, resulting in lower percentage survival than in the control samples (Figure 4.30). The 40ml/kg dosage treatment also resulted in a significantly lower value than those of 5, 10 and 20ml/kg treatments but the difference between the effect of 30 and 40ml/kg dosage treatments was not significant. The effects of 10, 20 and 30ml/kg treatments were also not significantly different from one another.

Feeding deterrence on maize grains

The percentages of surviving adult insects introduced onto maize grains one month after treatment with 0-40ml/kg neem oil, after a 28-day exposure period, are given in Figure 4.30. The effect of neem oil on the survival of *P. truncatus* on maize grains was significant (ANOVA, p < 0.001). The percentage of surviving insects from samples treated with 5 and 10ml/kg dosages were not significantly different (LSD Bonferroni, p = 0.05) from that in the control. Treatment with all the other dosage levels resulted in lower percentage survival than in the control. Results from 20, 30 and 40ml/kg dosage levels were significantly different from one another with a very sharp decline in survival between 30 and 40ml/kg dosage levels. The results from 5 and 10ml/kg dosage levels were also significantly different from all the other dosages.

In the case of grain weight loss, the effect of neem oil was significant (ANOVA, p < 0.001), but only 40ml/kg dosage treatment values were significantly different from values for all the other treatments including the control (LSD Bonferroni, p = 0.05). The effects of the 20 and 30ml/kg dosage treatments were not significantly different but the latter was significantly different from those of the rest of the dosages including the control. The effects of 10 and 20ml/kg dosage levels were not significantly different but the latter was significantly different from that of both 5ml/kg and the control. The difference between treatments with 5 and 10ml/kg dosage and the control was not significant. Maize grain weight loss was highly positively correlated to survival (Pearsons' correlation coefficient = 0.9558, p < 0.001).
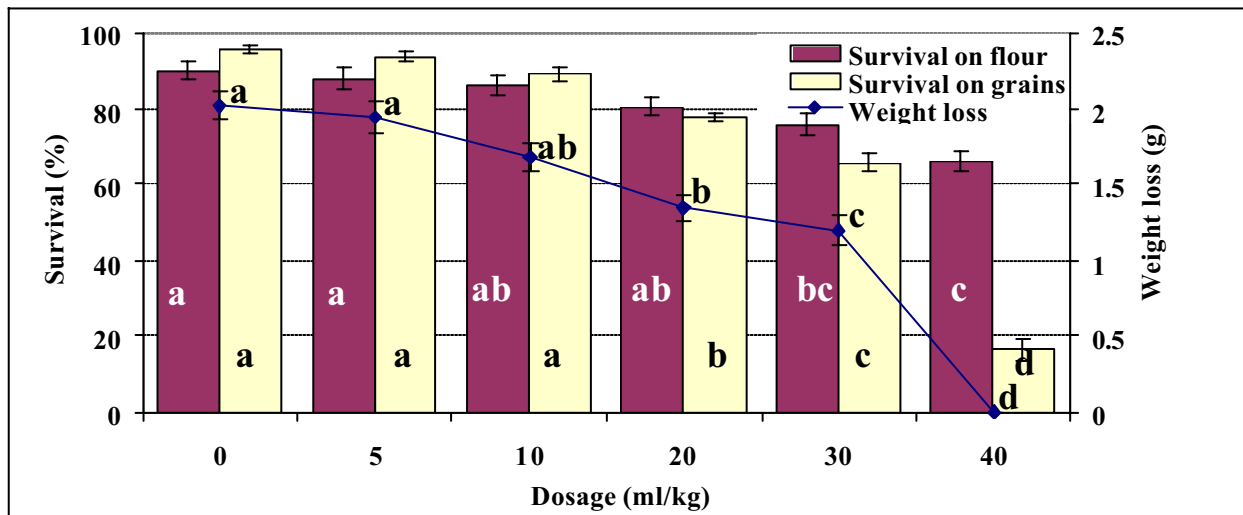
Figure 4.30: Survival of *P. truncatus* on maize grains and on flour, and grain weight loss by *P. truncatus,* after 28 days on samples treated with 0-40ml/kg neem oil and kept at 30°C an 70%r.h.for one month. Values denoted by the same letter at different dosages are not significantly different (LSD, Bonferroni, p = 0.05).

4.6   Fate of neem oil on maize grains

Evaporation of neem oil

The loss of neem oil by evaporation with time from both the filter paper and glass beads was generally not significant (ANOVA, $p > 0.05$). The weight of the two materials did not change significantly with time. (Figure 4.31). On maize grains, the cumulative moisture loss did not differ significantly between treated grains and the control. However, a non- significant difference (Student t-test, $p > 0.05$) was observed between the two varieties that were used, KH626 generally losing more weight than Guyana EU (Figure 4.32).

Absorption of neem oil

The weight of neem oil that was wiped off the grain surface of 100g of two varieties of maize, Guyana EU yellow variety (GE) and Kenya hybrid 626 white variety (KH626) at various time intervals after treatment is given in Figure 4.33. At all the determination times, the amount of oil wiped off GE variety was more than that from KH626 variety. The reduction in the amount of oil on the surface of GE variety was faster than for KH626 variety, which tended to remain constant after just about five hours. The total amount of oil which could be wiped off KH626 variety seemed to stabilize at 0.08g while that from GE variety continued to steadily reduce up to below 0.02g.
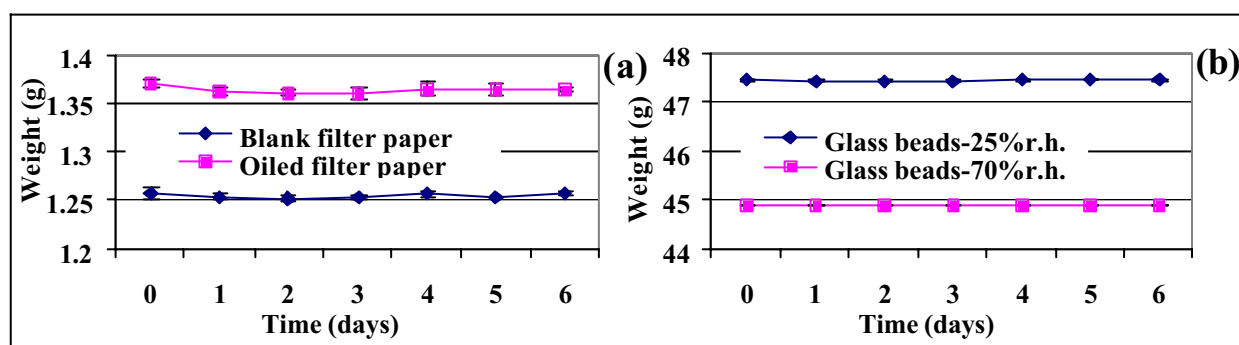
Figure 4.31: **Weight change due to evaporation of neem oil from (a) an 11cm-diameter Whatman filter paper and (b) 100 glass beads for a period of one week**
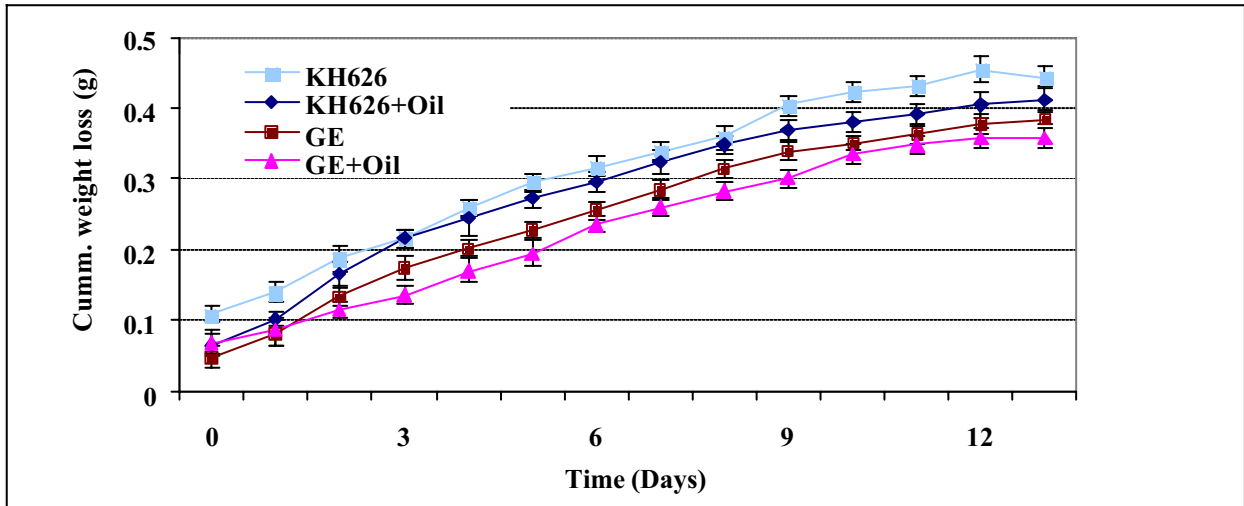
Figure 4.32: Cumulative weight loss of 100 maize grains kept for two weeks with or without treatment with neem oil at 20ml/kg, GE-Guyana-EU variety, KH626-Kenya hybrid 626 variety
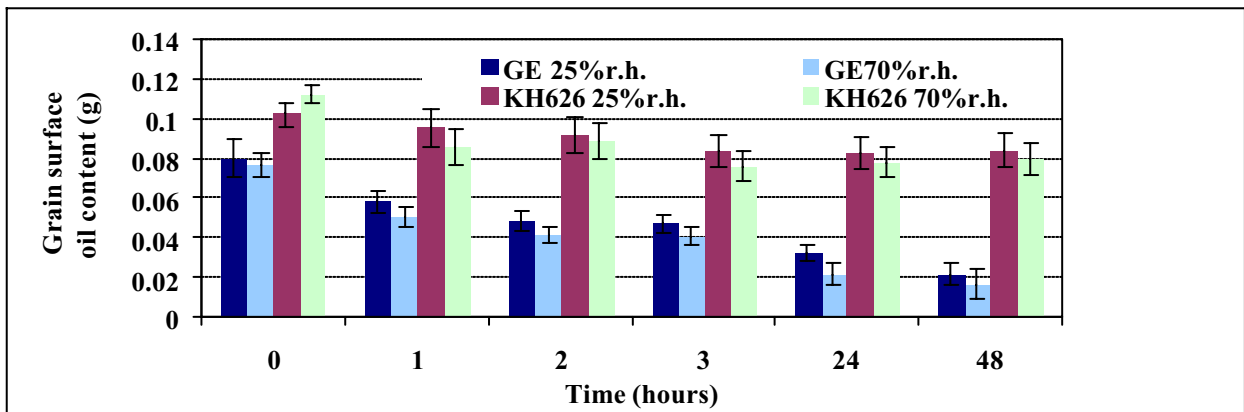
Figure 4.33: Amount of neem oil obtained from the surface of 100 grains of Guyana EU (GE) and Kenya Hybrid 626 (KH626) maize varieties after various time intervals up to 48 hours

4.7   Micro-calorimetric observation of insect activity

Metabolic heat production by larvae of *P. truncatus* under different conditions is shown in figures 4.34 - 4.37. A regression was performed by use of the Table Curve® 2D programme. In each case the best fit equation and the regression coefficient is given. Figure 4.34 gives the metabolic heat production of larvae kept on non-treated maize flour. Mean heat production was 0.01373mW/larva with a standard deviation of 0.0012 for the whole experimental period and 0.01354mW/larva with a standard deviation of 0.0010 for a ten hour period, respectively. Metabolic heat production varied from a minimum of 0.01317mW/larva to a maximum of 0.0143mW/larva and fluctuated around the mean. In Figure 4.35, metabolic heat production of larvae kept on flour treated with neem oil at the dosage of 2ml/kg was 0.01354mW/larva with a

standard deviation of 0.00183 for the whole experimental period and 0.01273mW/larva with a standard deviation of 0.00095 for a ten hour period, respectively. Metabolic heat production varied from a minimum of 0.01090mW/larva to a maximum of 0.01617mW/larva.

Figure 4.36 shows the metabolic heat production of larvae kept without any flour to feed on. Mean heat production was 0.01067mW/larva with a standard deviation of 0.00136 for the whole experimental period and 0.01318mW/larva with a standard deviation of 0.00034 for a ten hour period, respectively. Metabolic heat production varied from a minimum of 0.00842mW/larva to a maximum of 0.01292mW/larva. For larvae kept on filter paper treated with neem oil without any flour to feed on, mean heat production was 0.01191mW/larva with a standard deviation of 0.00174 for the whole experimental period and 0.01588mW/larva with a standard deviation of 0.00036 for a 10 hour period, respectively. Metabolic heat production varied from a minimum of 0.00917mW/larva to a maximum of 0.01465mW/larva (Figure 4.37).
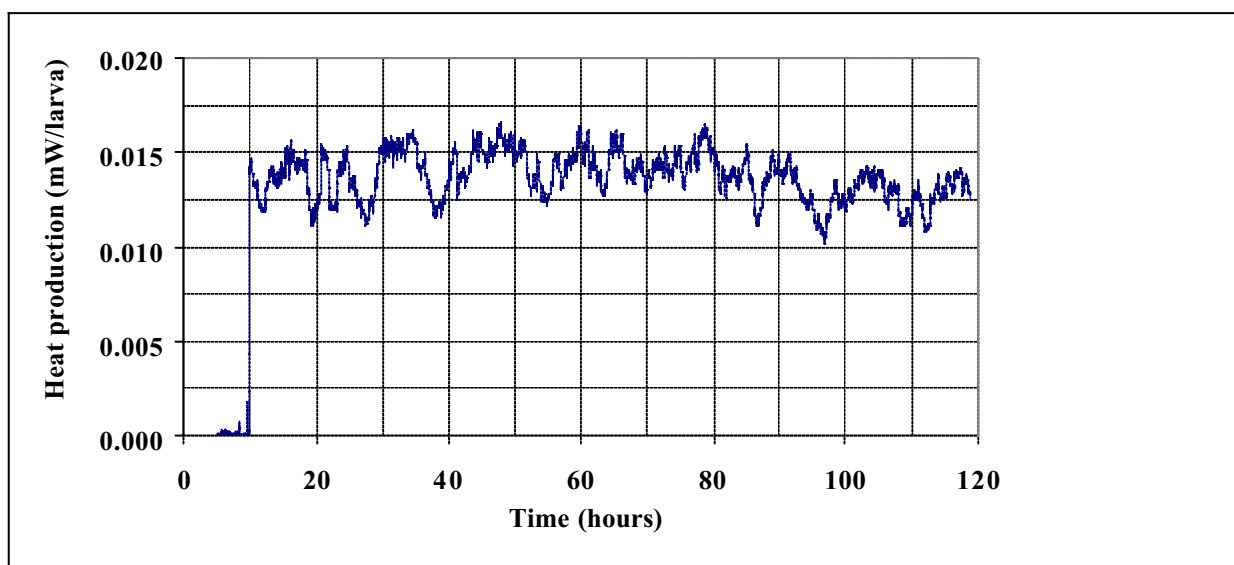
Figure 4.34: Metabolic heat production of larvae kept on non-treated maize flour in a micro-calorimeter at 303°K
Heat production (H) = 0.01429 - 1.037 x 10^{-5}t, t = time, Adj. r^2 = 0.07112
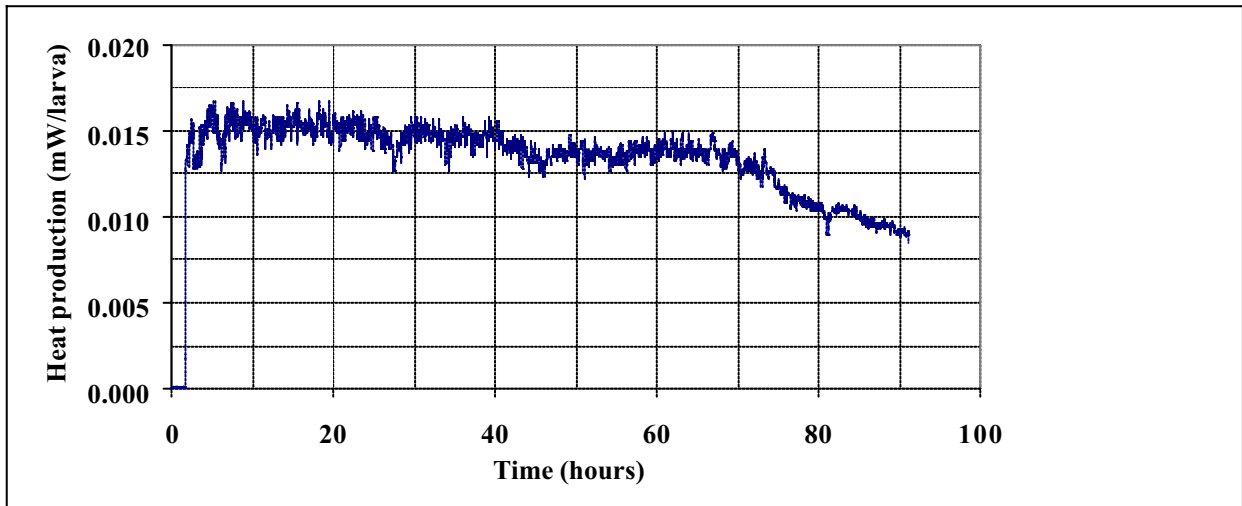
Figure 4.35: **Metabolic heat production of larvae kept on maize flour treated with neem oil in a micro-calorimeter at 303°K**

Heat production (H) = 0.01617 - 5.393 x 10^{-5}t, t = time, Adj. r^2 = 0.69166
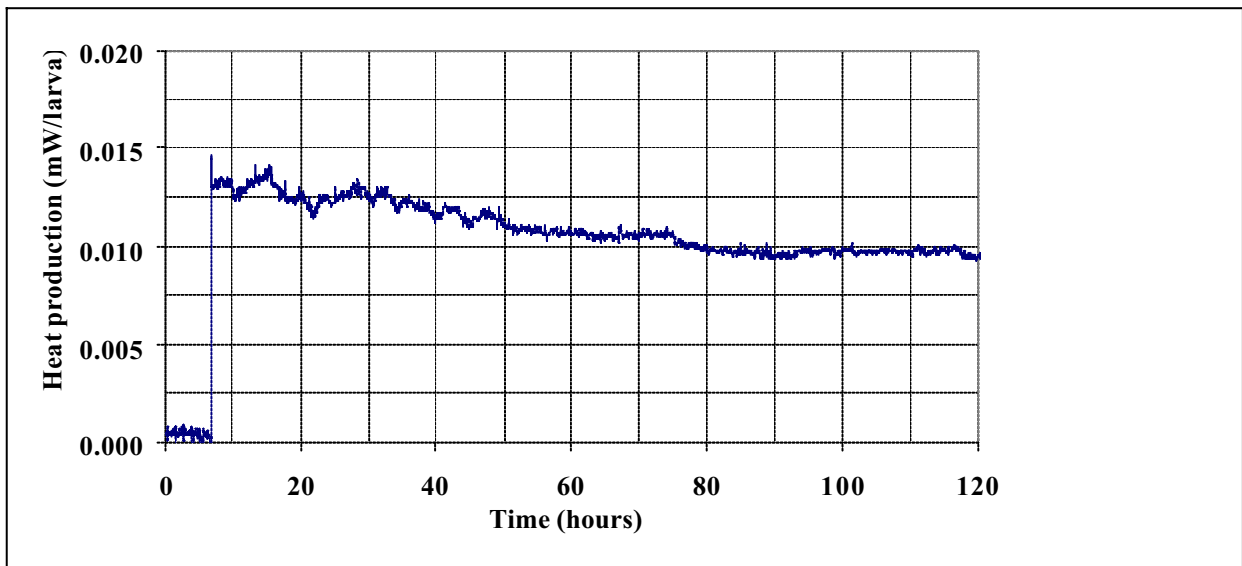
Figure 4.36: **Metabolic heat production of larva kept without flour in a micro-calorimeter at 303°K**

Heat production (H) = 0.01292 - 3.4669 x 10^{-5}t, t = time, Adj. r^2 = 0.90425
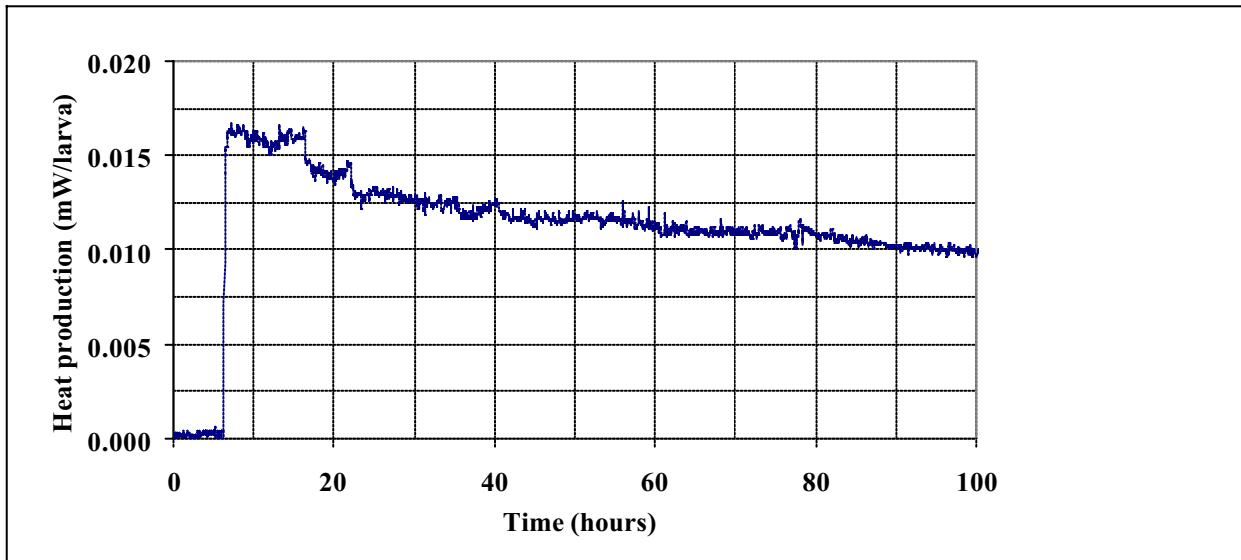
Figure 4.37: **Metabolic heat production of larva kept on treated filter paper without flour in a micro-calorimeter at 303°K**
Heat production (H) = 0.014650 - 5.7651 x 10⁻⁵t, t = time, Adj. r^2 = 0.90425

Generally, the trend displayed by all the three treatments without non-treated flour was similar and different from the trend displayed by the treatments with non-treated flour; similar coefficient of variation, gradient and coefficient of correlation, all of which were higher than in the non-treated flour samples (Table 4.13). Metabolic heat production (Joules/mg/h) was determined by integrating the area under the curve and multiplying its value by 3.6 to convert it from mWh to Joules and finally dividing the values in Joules by the time and the body weight of the larvae.

$$\text{Metabolic heat production (H)} = \frac{\text{area under curve x 3.6}}{\text{time x larval body weight}}$$

Table 4.13: **Mean metabolic heat production, coefficients of variation, and gradients for the fitted linear relationships between metabolic heat production of *P. truncatus* larvae on various treatments and time in a micro-calorimeter at 303°K**

	Mean heat production (mW/larva)	Mean heat production (J/mg/h)	CV(%)		Gradient
			Whole period	10 hours	
Non-treated flour	0.01373	0.024	8.91	7.80	-1.0371 x10⁻⁵
Treated flour	0.01354	0.019	13.54	7.47	-5.8934 x10⁻⁵
No flour	0.01067	0.017	12.82	2.60	-3.4669 x10⁻⁵
Treated filter paper	0.01191	0.018	14.61	2.26	-5.7651x10⁻⁵

5   Discussion

5.1   Effect of neem products on mortality and reproduction of *P. truncatus*

Effect of neem seed cake powder

Previous studies have reported highly varying effects of neem products on insects in general and on *Prostephanus truncatus* as discussed below, presumably due to lack of uniformity in the experimental materials used. In this study, efforts were made to determine the azadirachtin content in the products for ease of comparison between products and also with previous results. The ineffectiveness of neem seed cake powder in the control of *P. truncatus* may have been due to its inability to properly coat the maize grains, which are relatively smooth. Up to 5% w/w content of neem seed cake powder was required to cause 12.7% mortality, which although statistically significant, still remains an unacceptable level of control in farm stores. Similar low activity by the powders on *P. truncatus* and *Sitophilus zeamais* was reported by Niber (1994) and Cobbinah and Appiah-Kwarteng (1989), the latter attributing the poor performance to the settling of the powder particles at the bottom of the treated product, a phenomenon that was also observed in this study.

On the contrary, neem powders have given much better results in the control of many other pests of maize and other products, such as *Rhyzopertha dominica, Sitophilus oryzae, S. zeamais, Trogoderma granarium, Cryptolestes ferrugineus* (Jotwani and Sirca, 1967; Pereira and Wohlgemuth, 1982; Sharma, 1995, 1999) and *Callosobruchus spp.* (Ahmed and Ahamad, 1992; Prakash *et al*., 1993; Singh and Pandey, 1995; Chiranjeevi and Sudhakar, 1996). Against these pests, the powders exhibited toxicity to adult insects and reduced productivity. According to Pereira and Wohlgemuth (1982), 4% dosage level of neem seed cake powder caused mortality of more than 50% in *R. dominica*, *C. ferrugineus* and *S. oryzae*. *P. truncatus* hence seemed to have higher tolerance to neem powders than the other pests, most probably because it lays its eggs and the larval stages develop, inside the grains. This may generally reduce the contact with the control materials and limit effect. Niber *et al.* (1992) also reported higher tolerance against crude ethanolic extracts of neem by *P. truncatus* than by *Acanthoscelides obtectus* and *S. oryzae*. For effective utilization of neem powders in the control of *P. truncatus*, a formulation that will improve its attachment to the grain is

required. This view was also supported by the work of Niber (1994) in which slurries of neem products were reported to provide better protection than powders against *P. truncatus* and S. *oryzae*.

Effect of neem oil

In this study, neem oil caused high mortality of *P. truncatus* at high dosages, up to 100% at the 20ml/kg dosage level in seven days. The increase in mortality with increasing exposure time was similar to that reported by Dey and Sarup (1993) for various oils on *S. zeamais* where the highest increase occurred between the first and second day irrespective of the oil used. Neem oil at lower rates significantly reduced maize grain weight loss even at dosages that caused insignificant mortality, implying that the product either inhibited feeding or caused insect inactivity (Schmutterer and Wilps, 1995) or both.

The observed effects of neem oil on mortality of *P. truncatus* in this study differ from previous reports in which the effects were found to be non-profound or only slight in some experiments and much more significant in others. Maredia *et al*. (1992) reported a non-profound effect of neem oil at a dosage of 10ml/kg on *P. truncatus* up to seven days after treatment while Niber (1995) reported a reduction in *P. truncatus* population increase of about 29% within three months on maize using neem oil at a dosage of 20ml/kg. It may thus be concluded that neem oil is ineffective in the control of *P. truncatus* adults at low dosages (<10ml/kg). In this study, 20ml/kg dosage level of neem oil resulted in 100% adult mortality and nil insect population increase compared to only 29% reduction in population obtained by Niber (1995). Such a large difference may have resulted from a number of factors, including the azadirachtin and other antifeedant components' content, the variety of maize used, the difference in the susceptibility of the strains used and even the time between application of the oil and introduction of the insects. Otherwise, it is difficult to explain such a low effect of the oil at a dosage level of 20ml/kg since at such a dosage a significant physical effect due to the oil is expected even if no bioactivity is exhibited.

In their study with *S. oryzae* on maize, Dey and Sarup (1993) showed that all the oils tested (mustard, soybean, coconut, neem, groundnut, cotton, sesame and castor) resulted in more than 20% decrease in population and more than 26% decrease in grain damage compared to

the untreated controls, at a maximum dosage of 3.3ml/kg. Shaaya *et al.* (1997) also reported that 10g/kg of cotton and soybean oil gave full protection of maize against *S. zeamais* for a period of 4-5 months. Sharma (1999) reported that neem oils at a dosage of 1% by weight were toxic to *S. oryzae*, *R. dominica*, *S. cerealella*, *T. granarium* and *T. castaneum* on maize grains. Tembo and Murfitt (1995) reported similar mortality of *S. granarius* due to exposure to oils of groundnut, rapeseed and sunflower and concluded that the mode of action for all the oils was similar.
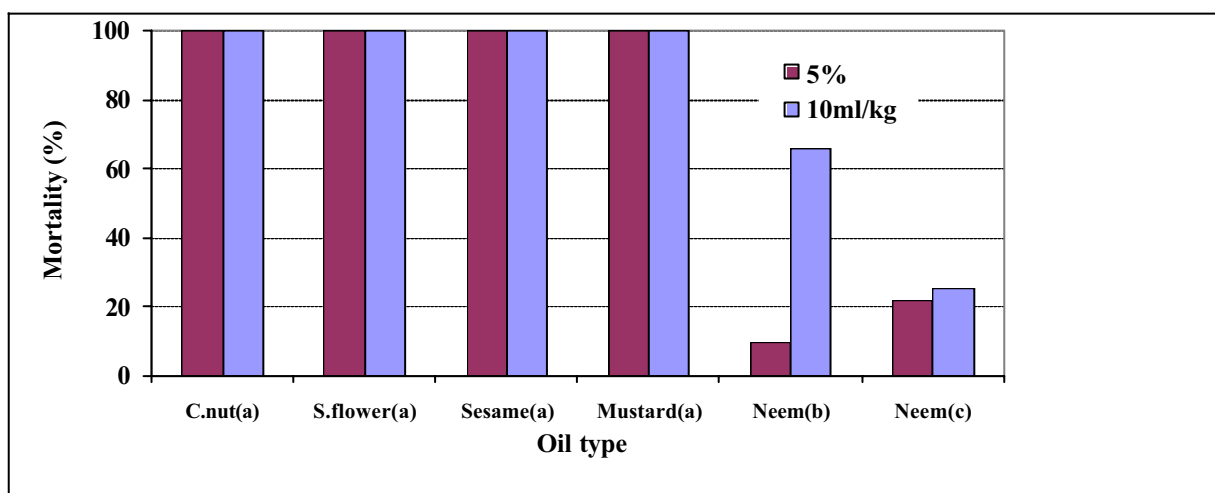
Figure 5.1: Effect of various plant oils on mortality of *P. truncatus* in various studies
        a-Obeng-Ofori and Reichmuth (1999)(27°C, 65-70%r.h.)
        b-This study (30°C, 65-70%r.h.)
        c-Maredia *et al*. (1992) (25°C, 65-75%r.h.)

Niber (1995) concluded that the effect of neem oil at 1.5% dosage, which was not significantly different from that of 2% in the same study, was chemical rather than physical. Cobbinah and Appiah-Kwarteng (1989) concluded that neem oil contained some insecticidal properties not found in ordinary edible oils. From this study, the two arguments remain valid, but in addition, it is important to note that the toxic effect of neem oil declined very significantly with time, reaching a minimum in just about two weeks. Considering this fact, it becomes more likely that mortality of the adult insects was due to physical rather than chemical effects, such that after the amount of oil on the surface of the grains declined, the toxic effect also fell accordingly regardless of the fact that the oil actually did not evaporate from the grains. This view is supported by work reported by Don-Pedro (1989) using vegetable oils against *S. zeamais* on wheat. In his study, grains treated with groundnut and traditional coconut oil resulted in mortality of up to 30% at a dosage of 17.5ml/kg, but the

toxic effect was not appreciable after 14 days. Just like in this study, he reported that dead adults were heavily coated with oil. Ivbijaro *et al*. (1984) also reported that very high mortality of *S. oryzae* occurred within 24 hours due to exposure to vegetable oils. Helwitt (1975) showed that refined mineral oil could enter the tracheae of *Sitophilus spp*. and cause anoxia. It is highly likely that neem oil caused a similar effect on *P. truncatus.*

In the control of insect population increase, there is agreement between the results of this study and those of many others already reported, that oils cause a significant reduction of the progeny of various insects. This could be as a result of the oils affecting oviposition by the adult insects, exerting ovicidal effects, or affecting hatching or larval and pupal development. Most of the studies in this area have dealt with bruchids, especially *Callosobruchus spp*. on legume grains, with only few reports on cereals and particularly maize. In their review on the effect of non-volatile oils on storage pests, Boeke *et al.* (2001) cited 343 references out of which less than ten did not involve *Callosobruchus spp.* This may suggest that oils are a lot more successful in the control of these species than any other group of species of storage pests.

Perhaps the only study showing direct effects of neem oil on larvae and pupae on a cereal is the one by Jilani *et al.* (1988), who reported a reduction in the number of larvae, pupae and adults of *T. castaneum* in rice treated with oils of turmeric, sweet flag and neem as well as Margosan-O (commercial neem oil). They also reported failure of the larvae to pupate, delayed development and abnormal pupae and adults. Most of the other reports are either on non-cereal products or on general effects on insect population. Schoonhoven (1978) reported a reduction in oviposition, egg hatching and number of adult progeny of *Zabrotes subfasciatus* on beans by 1-5ml/kg of cottonseed and palm oils. Qi and Burkholder (1981) demonstrated a reduction in progeny of *S. granarius* by 10ml/kg of various vegetable oils. Pandey *et al.* (1981) showed that oils of sal (*Shorea robusta*), cotton seed and rice bran at the rate of 0.3-0.5% by weight gave full protection of green gram against *C. maculatus*. Khaire *et al.* (1992) reported complete prevention of adult emergence of *C. chinensis* on pigeon peas by 0.5% neem oil and 0.75% karaj oil. Pereira (1983) reported that only neem oil out of the six plant oils investigated significantly reduced oviposition while all the other oils exhibited significant ovicidal activity in *C. maculatus* on cowpea.

Cobbinah and Appiah-Kwarteng (1989) suggested that the oils were more successful against bruchids, which lay their eggs outside the grains than against insects such as *S. zeamais*, which insert their eggs inside the grains. This would imply that the eggs have to come into contact with the oil in order to be affected. This seems also to be the view of Boeke *et al.* (2001) who suggested that the film of oil prevents attachment of eggs to the seed coat and plugs the respiratory system of the eggs and adults. Hence, the effect of neem oil on pests that lay eggs outside the grains has been attributed to causing insecure attachment of the eggs (Don-Pedro, 1989: Boeke *et al.,* 2001), clogging the egg openings (Credland, 1992; Murdock *et al.*, 1997), causing death of the developing embryo through asphyxiation (Lale and Abdulrahman, 1999) as well as toxicity to eggs and young larvae (Boeke *et al.*, 2001). For insects that lay eggs inside the grains like *S. zeamais* and *P. truncatus*, however, the mode of action may not be explained by these examples.

In this study, larvae of *P. truncatus* died inside the grains at an early stage, most likely during the first instar stage. It is important also to note that the death of these larvae occurred even at relatively low dosages of 2.5ml/kg and on grains that had been kept prior to exposure of the larvae until there was no more oil on the surface. The conclusion that can be drawn from this phenomenon is that the oil penetrated into the grains and caused the death of the larvae. Don-Pedro (1989) suggested that this penetration occurred even through the waxy plugs that are used by the females to seal the egg holes and exerted some lethal action on the eggs. This is a possible explanation assuming a similar function between the waxy plugs of *S. zeamais* and frass of *P. truncatus* although it may not apply in the case of this study because of three reasons. Firstly, the effect was observed at very low dosages that may not exert any physical action. Secondly, hatching of the eggs actually occurred only for the death to follow at larval stage. Thirdly, there was great similarity between larvae in oil-treated flour and starved larvae in the treated flour experiment.

According to Cobbinah and Appiah-Kwarteng (1989), neem oil seemed to contain insecticidal properties, which accounted for much higher effectiveness against *S. zeamais* than one would expect from treatment with ordinary edible oils. Prakash *et al.* (1993) also reported that neem powder was most effective in reducing insect populations of *S. oryzae* among the twenty plant products tested. This may just explain the effect of neem oil in this study. Neem oil seemed to contain properties that interfered with the development of *P. truncatus* larvae after hatching.

The fact that these properties exerted toxic effects on larvae cannot be ruled out, but lack of any abnormal symptoms makes it highly unlikely. Furthermore, there were no any partial effects such as incomplete development as was reported by Jilani *et al.* (1988) and as has been reported in many other insects that have shown susceptibility to toxic effects of neem (Steets, 1975; Schmutterer and Rembold, 1995; Ladd *et al.*, 1984; Kaethner, 1992). All beetles that initially survived pupated and emerged into normal adults. The similarity between treated and starved larvae points to the possibility of starvation. It is hereby proposed that the mortality of larvae of *P. truncatus* in treated grains is caused by antifeedant properties in the oil and the larvae die due to starvation after total rejection of all nutrition containing a minimum amount of the antifeedant factors. These antifeedant factors may just be the insecticidal properties referred to by Cobbinah and Appiah-Kwarteng (1989).

Effect of NeemAzal® PC KG 01

There are no reports on the effect of NeemAzal® PC KG 01 and NeemAzal® PC 05 on storage pests, although some reports on the effects of other types of NeemAzal® do exist (El-Lakwah *et al.*, 1994; Mansour, 1997; Wudtke, 1997; Hawala *et al.*, 1998; El-Lakwah and El-Kashlan, 1999; Shemais 2000; Aviles Pacheco *et al.*, 2000). In this study, treatment of maize grains with NeemAzal® PC KG 01 resulted in higher mortality of *P. truncatus* adults up to six months. The product controlled the pest by direct toxicity, causing high mortality at dosages as low as 1.5g/kg. NeemAzal® PC 05 was much less effective than NeemAzal® PC KG 01. This is likely to have been due to the formulation difference between the two products. The particle size of NeemAzal® PC KG 01 was much smaller than that of NeemAzal® PC 05 and the former tended to adhere better onto the grains. It therefore seems that NeemAzal® PC 05 suffered from shortfalls similar to those of neem seed cake powder. NeemAzal® PC KG 01 contained 1% azadirachtin while NeemAzal® PC 05 contained 5%. El-Lakwah and El-Kashlan (1999) tested the effect of NeemAzal-W, a powder containing 10% azadirachtin, on mortality and progeny reduction of *S. oryzae*, *R. dominica*, *C. maculatus*, and *T. castaneum* adults and reported maximum mortality values of 100% for all the test species at 1000ppm (1g/kg). Maximum progeny reduction values ranged between 94.6 and 100%. In this study, maximum mortality values were 9% and 98% for NeemAzal® PC 05 and NeemAzal® PC KG 01, respectively. The two NeemAzal® products in this study were produced by incorporation of azadirachtin into silica gel. The composition of NeemAzal®-W was not given. Comparison

of the NeemAzal® products in this study with blank silica gel powders showed a non-significant difference, suggesting that the lethal effect could largely be as a result of the silica gel rather than the presence of azadirachtin. The effects of such gels on various storage pests without azadirachtin have often been reported, starting from early in the 20th century (Zacher and Kunike, 1931) up to current years (Prasantha, 2003). The results of this study show that NeemAzal® PC KG 01 did not affect egg laying at sub-lethal dosages, and that treating of the grains after oviposition allowed significant numbers of adults to emerge and did not affect the larvae inside the grains. This indicates that the insects could avoid the toxic effects of the control product as long as they were not in direct contact with it. The beetles were only affected after they emerged and came into direct contact with NeemAzal® PC KG 01. The results from this study can also be compared with those described by Shemais (2000) in which the mortality of *T. granarium* adults treated with a NeemAzal® powder (composition not given) and an inert dust "Kabeljous" ranged between 93% and 97%. The effect of the NeemAzal® powder was reported to be similar to that of the inert dust Kabeljous. It can therefore be concluded that the lethal effect of NeemAzal® depends mostly on the formulation of the product. Other studies utilised other NeemAzal® formulations such as liquids (El-Lakwah *et al*., 1994; Mansour, 1997; Wudtke, 1997; Hawala *et al*., 1998; Aviles Pacheco *et al*., 2000) and their results can therefore not be easily compared with the results from this study.

5.2   Effect of neem oil on *Teretrius nigrescens*

This study was unique since no such study had previously been reported and was intended to investigate the effect of neem oil on the ability of *T. nigrescens* to control *P. truncatus*. This would give an indication as to whether *T. nigrescens* would safely survive and effectively predate in an environment where neem oil were being used for the control of *P. truncatus*. The results showed that *T. nigrescens* adults were slightly but significantly less susceptible to neem oil than *P. truncatus* adults. The real reason for this difference is not known but it could be due to the visibly different body surfaces between the two insects. *P. truncatus* has a rough rugged body surface while *T. nigrescens* is extremely smooth. It is possible that more oil could be held on the rough body of *P. truncatus* and hence easily block the trachea as suggested by Helwitt (1975) than on that of *T. nigrescens*. This is also supported by the fact that the difference between the two decreased as the dosage of the oil increased. At high

dosages such as 20ml/kg, there is sufficient oil to adversely affect both insects equally. It is also possible that the size, number or surface area of the spiracles is different leading to different effects.

T. nigrescens managed to significantly control the population of *P. truncatus* in both the presence and absence of neem oil. The total number of *P. truncatus* resulting from *T. nigrescens* predation in the absence of neem oil was 29 from the initial number of 50 after eight weeks compared to 471 in the untreated control and compared favourably with those reported by Rees (1985, 1990). The difference in the number of *P. truncatus*, weight loss of the infested maize grains and number of damaged grains between the presence and absence of *T. nigrescens* was generally not significant apart from at the 2.5ml/kg dosage level. This lack of significance may be interpreted as being unnecessary to use both neem oil and *T. nigrescens* simultaneously. However, it is important to note that *T. nigrescens* is released into the surroundings where it is intended to control *P. truncatus* both in the surroundings as well as inside the stores just in case *P. truncatus* finds its way there. So, it is more likely that one may supplement the effect of *T. nigrescens* with the effect of the oil, but not vice-versa. It should also be noted that generally, the oil caused a higher degree of control on its own than *T. nigrescens*. Under such circumstances, the lack of significance becomes important since in a situation where a farmer does not expect satisfactory results from the effect of *T. nigrescens*, he may supplement its effect with that of neem oil with the confidence that he will not adversely affect the predator.

The effect of neem oil on the larvae of the two insect species was significantly different. The initial impression given by the results with *T. nigrescens* may lead to the conclusion that neem oil affects the development of predator larvae just as it affects prey larvae. However, when additional prey larvae were supplied to the predator as additional nutrition, predator populations increased normally. This may hence imply that in treated samples, the population of *T. nigrescens* was limited by the absence, or shortage, of *P. truncatus* larvae. This conforms to the work of Detmers (1993) who reported that *T. nigrescens* could not lay eggs in the absence of, or in the presence of very few, *P. truncatus* larvae. Provision of additional larvae to *T. nigrescens* confirmed that their larvae are not adversely affected by neem oil at the tested dosages. Previous reports showed that *T. nigrescens* is susceptible to the insecticides used to control *P. truncatus*, both pirimiphos-methyl and permethrin (Golob

et al., 1990). This may limit the application of *T. nigrescens* in areas that utilise these chemicals on a large scale. Neem oil could hence be considered for use where *T. nigrescens* needs to get, or is, established. The difference in the susceptibility of the two larvae may lie in the fact that their sources of nutrition are different. As already discussed, the results from this study suggest that *P. truncatus* larvae are disrupted from feeding by the antifeedant properties of neem oil and hence starve to death. This may explain the ability of the predator to survive despite its feeding on the affected larvae, which did not incorporate significant amounts of neem oil. These results also confirm that there is no contact toxicity of neem oil on the larvae under these conditions.

The effects of various neem products on various beneficial and non-target species have already been reported in several reports, although mostly not involving pests of stored products. Among Coleoptera, *Coccinella septempuctata*, an important predator of aphids has been reported not to be adversely affected by NSCP and neem oil (Kaethner, 1991). Spraying of the sorghum aphid, *Melanaphis sacchari* (Srivastava and Parmar, 1985) as well as the green peach aphid, *Myzus persicae* (Eisenlohr *et al*., 1992) did not affect the coccinellids and syrphids. Among egg-parasitoids, *Telenomus remus*, a parasitoid of *Spodoptera litura,* was also not affected by neem products (Fernandez *et al*., 1992). However, negative effects on parasitization were observed when *Trichogramma pretiosum* was treated with NSCP in the laboratory, but not in the field. Mansour *et al*. (1997) showed that Neemguard, a commercial neem product, was highly toxic to *Tetranychus cinnabarinus*, a phytophagous mite, but not harmful to two of its predators. The effect of neem products on beneficial and non-target species therefore seems to be largely by coincidence or natural adaptation.

5.3   Persistence of the effects of neem products

Effect of neem oil

The persistence of the effects of neem products varied with the product and the type of effect. In neem oil for example, the lethal effect on adults declined very fast while the effects on egg production, weight loss of treated maize grains and progeny reduction of *P. truncatus* persisted over a period of more than six months. These results have some similarities with others that have already been reported. The lethal effect of groundnut and coconut oils on

S. zeamais adults was significantly high when the insects were exposed to freshly treated seeds but it disappeared when seeds were kept for 14 days (Don-Pedro, 1989). Similarly, Qi and Burkholder (1981) reported that significant mortality occurred only when adults of *S. granarius* were exposed to grains freshly treated with a high rate (>10ml/kg) of groundnut oil. They also noted that reduction in insect population was the same even after 60 days. Dey and Sarup (1993) also reported that the highest mortality due to all oils tested occurred in the first day. Obeng-Ofori (1995) reported that plant oils (cotton seed, soybean, corn, groundnut and palm) caused 100% mortality of *Cryptolestes pusillus* and *R. dominica* within 24 hours. Mansour (1997) showed that NeemAzal-S oil gave 100% mortality of *C. chinensis* on mung bean (*Vigna radiata*) up to three months, but protected the beans for up to one year. These results lead to the conclusion that the effects of the oils on adult pests are mainly physical and when the amount of the oil on the surface of the grains decreases, the lethal effects decline as well. These reports and the results of this study show that mortality of adults of various pest species due to treatment with various oils declines until it is negligible after a given period. This study shows also that the rate of loss of this effect is variety dependent, especially for maize. Figure 5.2 shows comparisons between effects of various oils and dosages on various insect pests obtained from different studies.
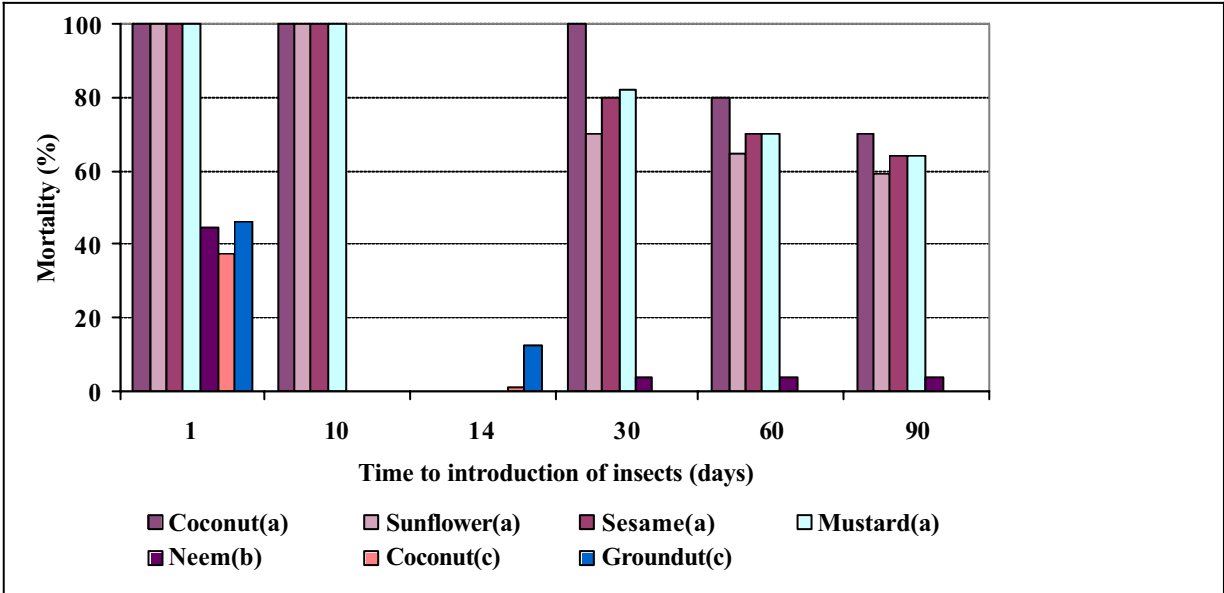
Figure 5.2: Effect of various oils (10ml/kg) on mortality of *P. truncatus* and *S. zeamais* introduced at various durations after treatment of grains:
      a-Obeng-Ofori and Reichmuth (1999) (*P. truncatus*)
      b-This study (*P. truncatus*.)
      c-Don-Pedro (1989) (*S. zeamais*)

In the case of other effects such as weight loss, progeny reduction and egg production, results from this study show that they persist much longer than toxic effects. This suggests that the cause of such effects that persist for long is different from the physical cause of mortality. Since it has already been suggested that these effects are due to antifeedant properties contained in neem oil, it is logical to believe that these properties persist for a long period. Azadirachtin may just be one of these factors since it has been demonstrated to have antifeedant properties (Butterworth and Morgan, 1968). Studies on the persistence of azadirachtin have largely been performed in field crops where its residual effect tends to decline very rapidly, mostly in less than seven days (Kumaran and Ramesh, 1999; Markandeya *et al.*, 2001; Blumel and Hausdorf, 2002; Karmarkar *et al.*, 2002). Even the bitterness associated with neem oil has also been reported to disappear within this time (Jalaluddin, 1999). This is possibly due to translocation and active transport of chemical compounds that occur within the plants especially in the presence of water and instability under UV light. The situation is however different in stored products. The effects of neem products, including azadirachtin have been reported to last for much longer periods. For example, biological activity of azadirachtin-enriched neem kernel extracts against *R. dominica* in stored wheat, determined as inhibition of F_1 progeny production, was reported to persist for 48 weeks (Rahim, 1998), in spite of the fact that the product did not cause any mortality even on freshly treated products. Sharma (1999) observed that neem oil at 2% by weight on maize grains effectively prevented the emergence of F_1 of all the pests investigated for nine months. Jilani and Saxena (1990) also singled out, from all the products investigated, neem oil and Margosan-O, a neem-based insecticide, for what they referred to as greater persistence against *R. dominica* and recommended that the two products deserved further evaluation. This persistence is likely to be the one responsible for the progeny reduction for up to one year as reported by Mansour (1997). It is therefore hereby suggested that the chemical constituents of neem oil are responsible for the antifeedant properties of neem oil and that these properties are responsible for the persistent effect on *P. truncatus*.

Effect of NeemAzal[®] PC KG 01

The results of this study show that the effects of NeemAzal[®] on *P. truncatus* as indicated by all the parameters recorded were not affected for the whole period of six months. However, unlike in the case of neem oil, it retained its lethal effect on the adults for the whole period.

The effects on progeny and grain weight loss were wholly related to its effect on adults. There is no evidence that the presence of azadirachtin played an important role in the effects of NeemAzal® and hence the persistence has to be explained differently. As previously suggested, silica gel may have played the leading role and hence the persistence may also be related to silica gel. This is likely considering that dusts are generally very persistent (Korunic *et al*., 1996). Stathers *et al.* (2002) have also reported that two diatomaceous earths, Protect-It and Dryacide gave good protection to threshed maize, sorghum and cowpea in Zimbabwe against insect attack for eight months. It can hence be concluded that the persistence of NeemAzal® against *P. truncatus* was largely because of the presence of silica gel.

5.4 Repellent effect of neem oil

Having clarified the difference between volatile and contact repellent effects (chapter 2), the experiments on the repellent effect of neem oil were designed to distinguish between the two. The repellent effects of neem products have often been reported for various pests of both harvested stored products and crops in the field without any such distinction. Jilani *et al.* (1988) and Jilani and Saxena (1990) reported repellence of oils of turmeric, neem and sweet flag against *T. castaneum* and *R. dominica* and noted that the effect of neem oil persisted longer than the others. They suggested that this could be caused by the high molecular weight of azadirachtin. Pandey *et al*. (1986) showed that petroleum ether extracts of neem leaves and twigs were highly repellent to *C. chinensis*. Khatre *et al.* (1993) reported a significant repellent action against egg laying in *C. maculatus*. Ignatowicz and Wesoloska (1996) demonstrated the repellent effect of powdered neem kernels against *C. chinensis*, *S. oryzae* and *S. granarium*. Xie *et al.* (1995) showed that azadirachtin and neem concentrates repelled three stored-product insects, *C. ferrugineus*, *S. oryzae* and *T. castaneum*.

In most of these studies, there was no distinction between olfactory and gustatory effects. Generally, olfactory repellent effects are common in products containing volatile components such as plant essential oils in which the effects of such volatile products have widely been described (Norris, 1990; Ojimelukwe and Adler, 1999; Andronikashvilli and Reichmuth, 2002). Since azadirachtin has been reported to be non-volatile (Blaney and Simmonds, 1995), it would sound logical to discard the olfactory effect and attribute all the repellence on contact effect. Unfortunately, the effect of volatiles is not out-rightly conclusive since there are a lot

more components involved than just azadirachtin. In their report, Xie *et al.* (1995) suggested that the repellent effect, although clearly chemo-sensory, could be either olfactory or gustatory. An analysis of the volatiles of neem oil and neem seed showed that they contained very many organosulphur constituents, the most prominent being di-n-propyl disulphide (76%). It has been suggested that these organosulphur compounds may be responsible for repellent properties of neem (Balandrin *et al.*, 1988; Saxena, 1995). In addition, reports exist of adverse effects of neem volatiles on insects. Ban *et al.* (2000) reported that despite the lack of effect on oviposition and survival of immatures of *C. chinensis*, the volatiles of neem oil caused significant repellence. Reddy and Singh (1998) reported that neem volatiles caused mortality to larvae and adults of *C. maculatus*. The volatiles also showed various effects on *Schistocerca gregaria* (Nicol, 1994). Saxena and Khan (1986) reported the odour of neem oil disrupting normal feeding behaviour of a green leafhopper, *Nephotettix virescens*. It was hence necessary to separate the two effects. From the results of this study, no volatile repellent effect on both *P. truncatus* and *T. nigrescens* by neem oil was observed. Careful observation of data from the volatile repellence experiments (section 4.4) showed that both insects were not repelled by neem oil but were attracted by treated and/or non-treated maize. For the case of *P. truncatus*, the oil seemed to interfere with its ability to respond to maize grain odours. There was a masking effect whereby the pest failed to get attracted to maize despite it having got attracted in the absence of the oil. These results are in accordance with work by Keyserlingk (1982) on bark beetles, *Scolytus scolytus*, where it was reported that neem oil seemed only to be a "disturber" and not a "repellent". This disturbance is likely to be the masking effect of neem oil. It is therefore hereby concluded that neem oil does not cause volatile repellent effect against both *P. truncatus* and *T. nigrescens*.

Other non-volatile oils have also been reported to exhibit repellent action against pests of stored products. Obeng-Ofori (1995) reported repellent action of plant oils against *C. pusillus* and *R. dominica*. Qi and Burkholder (1981) showed repellent effect of vegetable oils (cotton seed, soybean, maize and peanut) against *S. granarius*. Pandey *et al.* (1976) demonstrated the repellent effect of neem oil against *C. maculatus*. Under contact repellence, an insect is expected to come into contact with the repellent agent before it is repelled. The results from this study show clearly that neem oil exhibited contact repellent properties against *P. truncatus*. The design of the contact repellence experiments was intended to determine whether the insects preferred to move away from the repellent agent or not. The utilisation of

a food preference chamber enabled the insect to select between treated and non-treated grains. There is also the possibility that the insects moved to non-treated grains to get acceptable food. Dethier *et al.* (1960) defined an insect repellent as a chemical substance that causes an insect to make oriented movements away from the source. An insect repellent can therefore also act as a feeding deterrent (Warthen and Morgan, 1990). Using alternate grain-filled and empty chambers, it was possible to demonstrate that *P. truncatus* actually moved away from treated product after coming into contact with it. Whether the repellent effect was due to azadirachtin, other constituents or simply the oil could not be distinguished. Since all these components have been shown to repel insects, it can only be concluded that they may have acted either separately or in combination.

5.5   Antifeedant effect of neem oil

From the results of this study, neem oil exhibited antifeedant properties against both larval and adult *P. truncatus*. For adults, there was reduced feeding with increasing dosage of neem oil until total rejection at about 40ml/kg on maize grains. At this dosage, the insects opted to starve to death rather than feed on treated grains. These antifeedant properties fit the description for feeding deterrents given by Munakata (1977), "a chemical that inhibits feeding but does not kill the insect directly", and by Dethier (1960), "a chemical that inhibits feeding or oviposition when present in a place where insects would, in its absence, feed or oviposit. The insect remains near the treated plant and dies from starvation". In this study, only 16.4% of the adults survived up to 28 days at a dosage of 40ml/kg compared to 95.6% in the untreated control. All the same, the 0% weight loss implies that they did not attempt to feed on the grains and it is hence logical to argue that they would finally die leading to 0% survival. It is important to note that the feeding deterrence exhibited by neem oil on the grains was much higher than on maize flour. At the same rate of 40ml/kg, survival on flour was 66%. The possible explanation for the difference may lie in the distribution of the antifeedant factors. It is highly likely that the antifeedant factors on the flour were uniformly distributed in the product making the content in any portion of the product uniform while on maize grains, more antifeedant factors remained on the surface making the content on the surface higher than in maize flour. As a result, the maximum acceptable amount was attained on the grains and not on the flour in spite of the fact that the same dosage per unit weight of grains and flour was applied.

104

Based on previous studies, much of the antifeedancy can be attributed to azadirachtin since it has been reported to be the most important antifeedant compound in neem products (Butterworth and Morgan, 1968; Rembold, 1995; Warthen, 1989). Azadirachtin has been demonstrated to stimulate the deterrent neurones and to inhibit the phagostimulatory neurones in the chemo-receptor cells of various insects (Haskell and Schoonhoven, 1969; Schoonhoven, 1982; Simmonds and Blaney, 1985; Blaney and Simmonds, 1990). The 100% unpalatability response elicited by azadirachtin against *L. migratoria* was demonstrated by Blaney (1981) and helped to explain the different responses that may be exhibited between different insects. *Schistocerca gregaria* exhibited a labelled line response in which the level of unpalatability did not change with azadirachtin level, while *L. migratoria* exhibited an across-fibre response in which both phagostimulant and deterrent inputs played a part.

These observations may be used to explain the response of *P. truncatus* to neem oil. The insect seemed to exhibit the across-fibre-kind of response whereby the antifeedant effect of azadirachtin in neem oil increased with increasing amounts of azadirachtin. There was, however, a maximum content beyond which the insects could just not feed. This maximum lied between 30 and 40ml/kg (Figure 4.29) on whole grains for adults but could not be attained on flour. In larvae, the younger they were the lower was the maximum acceptable level of azadirachtin at which they stopped feeding. This may explain why the first instar larvae could not survive on treated flour, the second instar larvae only survived on treatments up to 5ml/kg and the third instar larvae were not significantly affected. There may be a distinct dose-bodyweight relationship that could be more clearly demonstrated in further experiments. In the experiments with larvae, there seemed to be a significant similarity between the treatment-affected insects and the starved ones, supporting the view that the insects actually starved after rejecting treated flour. Again, although the toxic effect or any other effect of neem oil or azadirachtin cannot be conclusively ruled out, similarity between treated and starved larvae suggests antifeedancy.

The micro calorimetric observations of insect activity also support this view. Generally, starving insects reduce their energy expenditure by, among other effects employed, reducing total metabolism (Ziegler, 1985). This is finally reflected in the reduction of metabolic heat production. In adult *Manduca sexta*, respiration decreased by 50% in three days while in

larvae it decreased by 70-75% in 20 hours (Siegert and Ziegler, 1982) due to starvation. The results from this study showed that the rate of reduction in metabolic heat production was similar for larvae kept on treated flour and those starved, but significantly different from those kept on non-treated flour. This implies that the larvae on treated flour reduced their metabolism in a similar manner as starved larvae. The pattern was also the same for starved larvae exposed to neem oil. Metabolic heat production fell by about 50% in five days for the non-feeding insects while it showed constant fluctuations for the feeding ones. *P. truncatus* larvae therefore seem to starve in the presence of neem oil.

5.6   Seed-oil properties

The results of this study show that neem oil behaves differently on different varieties of maize. It came out clearly that the evaporation of the oil from the grain is negligible. This can be expected considering that neem oil is generally non-volatile (Kumar and Parmar, 1997). The volatiles responsible for the highly unpleasant odour account for a negligible part of the oil. Similar results were obtained by Don-Pedro (1996) who reported negligible evaporation of vegetable oils. This leads to the conclusion that the oil is actually absorbed into the grains. The results of this study show that the rate of absorption differs significantly with variety. The real cause of this difference is not known but is likely to be related to the differences in the structure of the seed coat and other properties of the grain such as hardness and oil content of the aleuron layer. A study on varietal differences with respect to their holding of vegetable oils was also carried out by Dey and Sarup (1993). They reported significant interactions between variety and dosage and variety and type of oil; Pusa Arun variety was better protected by mustard and neem oil than VL-42 and AEB composite. Such differences in absorption of the oil may have been responsible for the differences reported between different studies under similar conditions. For example, whereas Obeng-Ofori (1995) and Obeng-Ofori and Reichmuth (1999) reported 100% mortality of *R. dominica, P. truncatus* and *S. zeamais,* using 10ml/kg, Dey and Sarup (1993) reported a maximum mortality of 32% using 3.3ml/kg on *S. oryzae.* Maredia *et al.* (1992) reported a non-profound effect on *P. truncatus* using 10ml/kg while Niber (1995) reported less than 50% mortality using 20ml/kg against *P. truncatus*, and in this study, 100% mortality was obtained with 20ml/kg against *P. truncatus.* It is hereby suggested that for future studies using neem oil on maize, the varietal effects need to be taken into consideration for better comparison purposes.

5.7   General discussions

In this study, no insect growth regulation effect due to all the neem products used against *P. truncatus* was observed. In the whole list of various pests affected by neem products (Chapter 2), very few studies reported effects due to growth and metamorphosis on stored products except on *Callosobruchus* spp. Although growth and metamorphosis effects are very widely reported among Lepidoptera and generally among pests of crops in the field, they are very rare among stored products. Even the studies that reported some growth and metamorphosis effects among stored product pests mostly utilised artificial diets such as flour and flour discs (Mukherjee *et al.*, 1993; Singh *et al.*, 1996; Xie *et al.*, 1996). It could hence be argued that neem products and azadirachtin will most likely not cause growth and metamorphosis defects against pests of stored products under natural storage conditions. The exact reason for this is not known, but the difference in the form of food consumed is of immediate interest. Reports on interspecific differences in susceptibility to neem and azadirachtin are not common in spite of the large number of studies conducted. The most likely reason for this difficulty to compare results is based on the use of non-standardized products in these studies. Isman (1995) reviewed interspecific differences among Lepidoptera and noted non-conclusive differences with respect to different bioassays, both physiological and behavioural. No concrete causes of variability are given.

Most of the stored product pests listed (Chapter 2) belong to the order Coleoptera and one is tempted to argue that Coleopteran pests are less susceptible to neem products than Lepidoptera and Homoptera (Mordue and Blackwell, 1993). However, in a review of effects of neem products on Coleoptera, Schmutterer (1995) showed that many beetles on field crops are excellent objects in bioassays for studies on metamorphosis, fecundity and activity. The difference in the activity of neem and azadirachtin between the two groups of pests may be related to the nature of their respective food diets. It is highly likely that the field pests consume more azadirachtin than storage pests due to the uniform distribution of the ingredient in the real consumed plant parts. In most stored products, there is minimal penetration of the active ingredients into the food material hence their uptake is limited. This may explain the few cases of IGR effects observed in stored-product pests when artificial diets were used since the mixing ensured higher amounts of azadirachtin or other antifeedant factors in the food that was actually consumed by the insects. In the review by Mordue and Blackwell

(1993), the antifeedant range of selected species of Coleoptera was given as ranging from 100 to 600ppm while that of Lepidoptera ranged from 1 to 50ppm. In the same review, the range of IGR was given as 1-4µg/g body weight for all species listed although Coleoptera were not included. It was also concluded that interspecific ranges are much more similar for IGR effects than for antifeedancy. A summary of these values is given in Table 5.1.

Table 5.1: **Antifeedant and insect growth regulation effect of azadirachtin on Lepidoptera and Coleoptera**

Insect group	Antifeedancy		Insect growth regulation	
	Azadirachtin content (ppm)	Antifeedant effect (%)	Azadirachtin content (µg/g body weight)	Moult Inhibition and mortality (%)
Lepidoptera	<1-50	37-100	1-4	5-100
Coleoptera	100-600	0-100	-	-

Adapted from Mordue and Blackwell (1993)

With these figures in mind, several points can be noted. Firstly, antifeedancy requirement can be easily attained by the use of neem oil. Secondly, when adequately mixed with flour, the azadirachtin content per given amount of flour falls far below azadirachtin content per given amount of oil. Thirdly, the amount of azadirachtin consumed from the treated product is very low, 6µg/g of food consumed. The actual food consumption by *P. truncatus* was 142mg/insect per lifetime (Pradzynska, 1993). In this study, the azadirachtin content of neem oil was about 300ppm (0.3g/kg) (Table 3.1). This just falls within the range for antifeedancy. This calculated as amount of azadirachtin in completely mixed flour yields a content of only 6ppm (0.06g/kg), and may explain why the percentage of surviving adults was higher on flour than on grains. It also shows that the youngest stages of *P. truncatus* larvae are sensitive to <0.06g/kg, but sensitivity declines with growth and is least at the adult stage. It can hence be concluded that the antifeedant components of neem oil tend to accumulate on the surface of the grains as the oil is absorbed, and that the little antifeedant factors that manage to penetrate into the grain are sufficient to cause antifeedancy among the first instar larvae.

This study has demonstrated the effectiveness of neem oil on larvae of *P. truncatus*, a fact which hitherto, has received little attention and which has a significant bearing on the whole process of management of *P. truncatus*. It is important to note that in most cases, pest

population increase during storage is normally responsible for the largest proportion of grain damage by *P. truncatus*. Attack by this pest may occur as early as when maize still has moisture content of 40-50% (Giles and Leone, 1975). Borgemeister *et al*. (1998) reported that leaving maize in the field for extended periods after physiological maturity resulted in severe grain losses after eight months of storage, implying that most of the losses, even as late as eight months after harvest, could still be attributed to the initial infestation in the field. The Ministry of Agriculture's extension services in Kenya recommend to farmers to harvest their maize as early as possible so that they commence the storage with as little infestation as possible to avoid rapid multiplication of pests. With strict observation of proper pre-storage practices, minimal adult infestation can be expected and hence more emphasis will be put on minimizing insect population increase. The most limiting factor in the use of neem oil has been the bitter test it exerts on food. As long as there is a real positive effect of neem oil, it is highly likely that alternatives would be developed to avoid this shortcoming. For example, the reduction of the amount utilized to only 2.5ml/kg could significantly reduce the bitter test. Dunkel *et al*. (1990) performed sensory tests with a trained panel of consumers on palatability of Margosan-O (neem kernel extract)-treated beans and observed that although preference for the neem-treated beans was 30%, acceptability was 70%. This serves to illustrate the importance of neem under no-alternative situation in which most of Kenyan rural farmers find themselves. In an integrated pest management situation, neem oil could play a very significant role in the control of *P. truncatus* and possibly many other pests.

6   Conclusions and Recommendations

The results from this study demonstrate that neem seed cake and neem leaf in powder state are not effective in controlling *P. truncatus* most likely because of poor attachment onto the grains. The effect of NeemAzal® PC 05 was also poor due to similar reasons. For better control of *P. truncatus* using these products, a formulation that will improve their attachment onto the grains needs to be developed. NeemAzal® PC KG 01was effective in controlling *P. truncatus* since it resulted in high mortality of adults, suppressed insect population increase and persisted for more than six months. Its effectiveness at very low dosage levels compared to other studies demonstrates its potential of being developed into an effective insecticide. Its effect however, was not related to the amount of azadirachtin.

Neem oil caused high mortality of *P. truncatus* adults at high dosage levels, and the mortality declined rapidly with time. It can therefore be concluded that the effect of neem oil on *P. truncatus* adults was mainly physical. At dosages above 40ml/kg, neem oil inhibited the insects from feeding on treated maize grains leading to their starvation to death. On larvae, neem oil suppressed insect development by exerting antifeedant properties, which persisted for more than six months. The antifeedant effect of neem oil on *P. truncatus* was therefore age dependent, the younger insect, were more affected than the older ones. First instar larvae were not able to develop on grains treated with more than 2.5ml/kg. The persistence of neem oil on the surface of maize grains was related to maize varietal properties and varied significantly between maize varieties. It can be concluded that maize variety is an important factor that must be taken into account when oils are used in the control of storage pests. Volatiles of neem oil did not have any effect on the development and survival of *P. truncatus*. From the results of this study, it can be concluded that the effect of neem products on *P. truncatus* depends largely on the formulation of the product and is not directly related to the content of azadirachtin in the product.

The results of this study also demonstrate that the predatory ability of *T. nigrescens* is not affected by the treatment of maize grains with neem oil at dosages that do not cause significant mortality of *P. truncatus*. In the presence of abundant food supply for *T. nigrescens*, its reproduction is not affected as well. The supplementary effect of the two treatments was generally not profound.

The volatiles of neem oil did not exhibit any repellent effect on both *P. truncatus* and *T. nigrescens*. *P. truncatus* was repelled by neem oil only when it came into direct contact with neem oil-treated grains. The oil however suppressed the attraction of *P. truncatus* by volatiles of maize grains and by volatiles from males infesting non-treated grains. *P. truncatus* preferred staying outside maize grains to inside neem oil-treated grains. It can hence be argued that only contact repellence is exhibited by neem oil against *P. truncatus*.

From the results of this study it can be argued that although neem oil does not cause significant mortality of adult *P. truncatus* at low dosages, it can still play an important role in integrated pest management of *P. truncatus* without causing undesirable effects on its predator, *T. nigrescens*. Neem oil can inhibit insect population increase and feeding and repel *P. truncatus*. The adverse effects of bitterness and smell could be minimized by using lower dosages on the grains. The antifeedant and repellent effect of neem oil demonstrated in this study could be utilized directly by farmers. Farmers to whom palatibility is not a serious concern could apply the oil directly onto grains that are not infested with *P. truncatus* adults and control the development of new insect populations. Where palatability is a concern, some other indirect methods of application could be considered such as treatment of storage bags and baskets. The effectiveness of NeemAzal® in this study and of diatomaceous earth in other studies should encourage local researchers to consider utilising locally available diatomaceous earth deposits for the control of *P. truncatus*. Certainly, field trials need to be performed to investigate the effectiveness of these methodologies in addition to determining how best to combine the desired qualities of neem oil with other pest management practices for improved control of stored-product pests.

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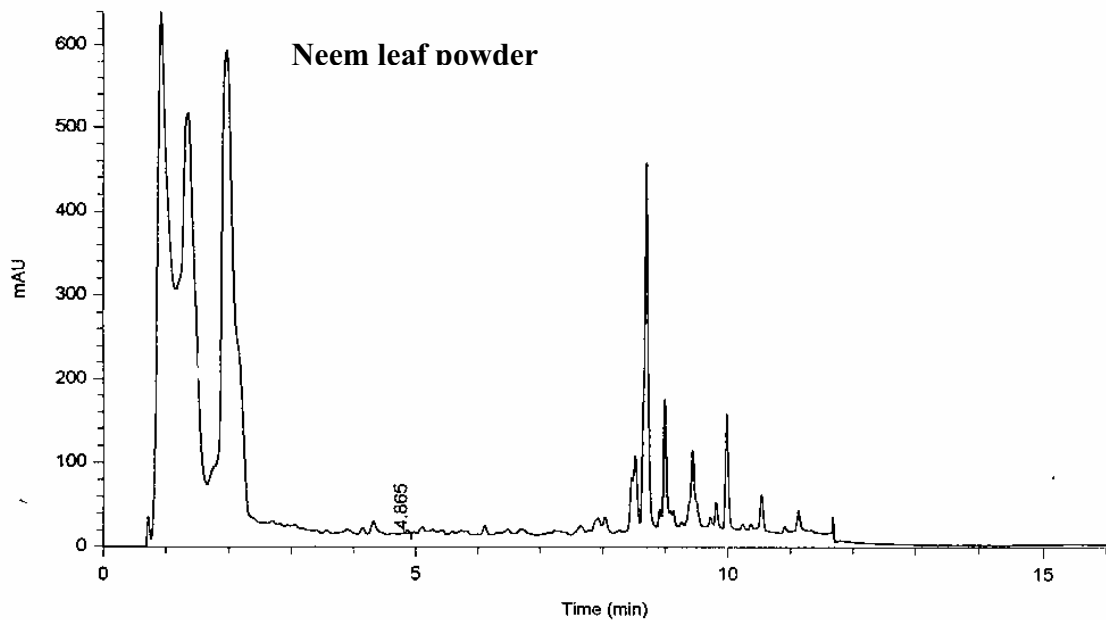
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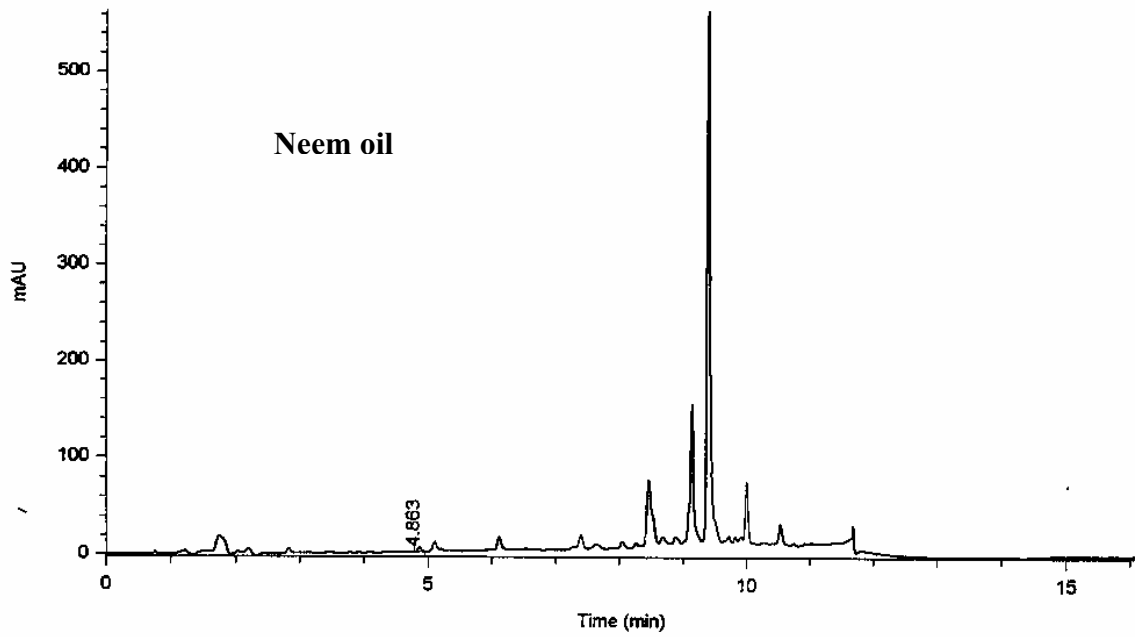
(i) Sample mass spectrometer diagram for azadirachtin content in neem oil
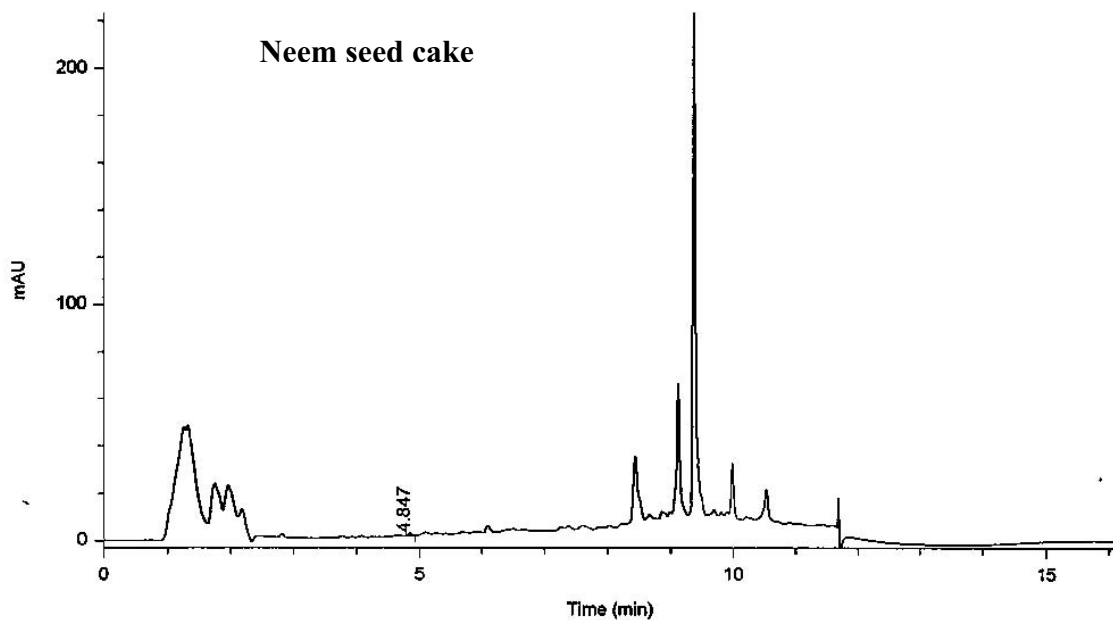
(ii) Sample mass spectrometer diagram for azadirachtin content in neem seed cake powder

Neem leaf powder

(iii)   Sample mass spectrometer diagram for azadirachtin content in neem leaf powder