

# Reproduction Inhibition Effects of Neem Products on the Larger Grain Borer (*Prostephanus truncatus*) (HORN) in Stored Maize Grains

Ogemah, V.<sup>1\*</sup>, Reichmuth, C.<sup>2</sup>, Buttner, C.<sup>2</sup>, Ayiecho, P.O.<sup>3</sup> and Keya, N.C.O.<sup>1</sup>

<sup>1</sup>Masinde Muliro University of Science and Technology, Department of Sugar Technology, P.O. Box 190-50100 Kakamega, Kenya

<sup>2</sup>Humboldt University of Berlin

<sup>3</sup>Maseno University

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## Abstract

The larger grain borer (LGB), *Prostephanus truncatus* (HORN), has become one of the most important insect pest of stored maize in tropical Africa where it was introduced in the early 1980s. It has the major effect of seriously damaging dry stored maize grains and so increasing post-harvest losses and contributing towards food shortage in this region. Losses of up to 85% by weight and 100% by food value have been reported. A study was conducted to investigate the effects of two neem products, neem oil and NeemAzal® powder on egg laying, hatching, larval development and adult emergence of the pest on shelled maize. Treatment of the grains with sub-lethal dosages resulted in 76.3% and 0.6% reduction in egg laying by neem oil and NeemAzal®, respectively. No adults emerged from samples treated with 0.3 and 0.6% w/w NeemAzal® and ca 2% v/w of neem oil. Generally, neem oil reduced the progeny population more than the adult population, the contrast of which was true for NeemAzal®. When the grains were treated after oviposition, no progeny emerged in neem oil samples while 4.4 and 20 larvae, 3.4 and 27.8 live adults and 17.6 and 1.4 dead adults were observed in NeemAzal® and control samples, respectively. The grain weight loss recorded was significantly lower for neem oil than for NeemAzal®. The two products have different effects on LGB, neem oil being effective in controlling early stages while NeemAzal® is more effective in controlling the adults. It can therefore be concluded that the effect of the neem products used in this study on LGB depended more on the formulation than on the content or dosage of azadirachtin used. The results of this study will help refocus research on the control of LGB towards design of appropriate formulations of neem products, which will make them useful in the control of the management of LGB by small scale, resource poor farmers in Africa.

**Key Words:** neem, *Azadirachta indica*, larger grain borer, *Prostephanus truncatus*, insect population, Azadirachtin

## Introduction

The larger grain borer, *Prostephanus truncatus* HORN, is a serious pest of stored maize and cassava with increasing importance in tropical Africa since it was introduced to this region from its natural habitat of central America in the early 1980s. Its control under small-scale production is limited because most such farmers cannot afford effective insecticides, and there is limited access to such chemicals in rural areas. There is also the possibility of pesticide poisoning due to inappropriate use by such farmers. Synthetic insecticides also have the general disadvantages of high possibility for insect resistance development and environmental hazards. These limitations have increased the need for alternative methods of control, especially under small-scale production. Such methods include biological control and the use of plant products. Neem tree, *Azadirachta indica*, is one of the plants that have been found to exhibit a wide range of effects on various insects, including repellence, feeding deterrence, mortality and various effects on reproduction. In addition, neem products have also been reported to affect development hence limiting the overall increase in insect population (Kraiss and Cullen, 2008; McKenzie *et al.* 2010).

Neem tree products and azadirachtin in particular, which is the most bioactive substance in the tree, have shown several adverse effects on ovarian development, fecundity, and fertility of various insects (Isman, 2008;2006; Kausik *et al.*, 2002; Subapriya and Nagini, 2005). Azadirachtin has been shown to inhibit oogenesis and ovarian ecdysteroid synthesis in *Locusta migratoria* (Rembold and Sieber, 1981). Reduced fecundity was demonstrated in *Plutella xylostella* (Charleston *et al.*, 2006), *Oncopeltus fasciatus* (Dorn, *et al.* 1987) *Cordyceps capitata* and *Liriomyza trifolii* (Parkman and Pienkowski, 1990) among many other insects. Antifeedant activity was observed in *Spodoptera littoralis*, *Spodoptera frugiperda*, and *Helicoverpa armigera* (Simmonds *et al.*, 2004) Toxicity has also been reported on *Tribolium castaneum* (Adarkwah *et al.*,2010)among many other effects. Neem products have been reported to cause growth inhibition, malformation and mortality especially when applied to the larval stages of many insects. Typical insect growth regulation (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. This study was intended to investigate the effects of neem seed oil from trees in Kenya and NeemAzal<sup>®</sup>, manufactured in Germany by

incorporation of azadirachtin into silica gel, on egg laying, larval development and adult emergence of *P. truncatus*.

## Materials and Methods

### Neem Products

The products used in this study were neem oil and NeemAzal<sup>®</sup> PC KG 01. Neem oil was obtained from the International Centre for Insect Physiology and Ecology (ICIPE), Nairobi, Kenya. It was prepared by pressing neem seed kernels of the Kenyan neem tree, collected from the Coastal and North Eastern regions 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. NeemAzal<sup>®</sup> was provided by Triflio GmbH, Lahnau, Germany. The amount of azadirachtin in the oil was determined at the same company by High Performance Liquid Chromatography (HPLC) to be 0.29mg/gram. NeemAzal<sup>®</sup> contained 0.1% azadirachtin A according to the specification of the manufacturer.

### Insects

*P. truncatus* 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 gauze (0.5mm) to prevent the insects from chewing through them.

### Determination of Effect of Neem Products on Oviposition

The experiment was set up in the laboratory in a 2x3 factorial design with five replicates. 50 unsexed adults of ages between one and seven days were added into maize samples in 250 ml glass jars with perforated metal lids containing sub-lethal dosages of 0.25, 0.5 and 0.75 v/w neem oil and 0.01, 0.02 and 0.03 w/w NeemAzal<sup>®</sup> and kept at 30°C and 70 %r.h. for seven days. The sub-lethal dosages were determined by fitting prediction curves for previously determined motility values using Table Curve 2D version 5.01 software. 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 0.2 mm sieve to hold the eggs. 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:

$$\% \text{ egg reduction} = \left[ 1 - \left( \frac{TLE}{TEE} \right) \times 100 \right]$$

where TLE = number of eggs laid in the treated sample  
 TEE = expected number of eggs in the treated sample  
 = CLE  $\left( \frac{TS}{CS} \right)$

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

Percentage population reduction was determined by fitting curves using Table Curve 5.01 software.

### 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. The experiment was set up in a completely randomised design with three treatments of five replicates each. Fifty *P. truncatus* were kept on 100-g 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 250 ml jars with perforated metal lids and treated with the highest dosages of neem oil (2% v/w) and NeemAzal<sup>®</sup> (0.6% w/w). 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.

Data obtained were subject to analysis of variance using SPSS analysis tool and means separated mostly by modified LSD (Bonferroni test). For two-sample tests with normal distribution data, student's t-test was performed. Data were presented in form of bar and line graphs with standard error bars.

## Results

### Oviposition

The effect of neem products on oviposition of *P. truncatus* is given in Fig. 1 and 2. Although the products affected the total number of eggs laid, the eggs were 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:  $p > 0.05$ ). The mean number of eggs laid in samples treated with neem seed oil and NeemAzal<sup>®</sup> was 25.6 and 111, respectively, compared to 116.4 in the control. In terms of percentage reduction in egg production, neem seed oil effected the greatest reduction of 76.3% while NeemAzal<sup>®</sup> resulted in a non-significant reduction of only 0.6%.

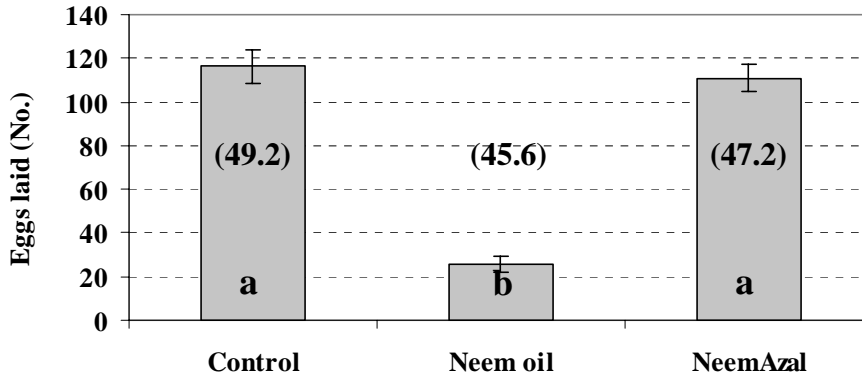


Figure 1: Egg laying by *P. truncatus* adults on maize treated with NeemAzal<sup>®</sup>, n=5, bars denoted by the same letter are not significantly different (t-test, p > 0.05)

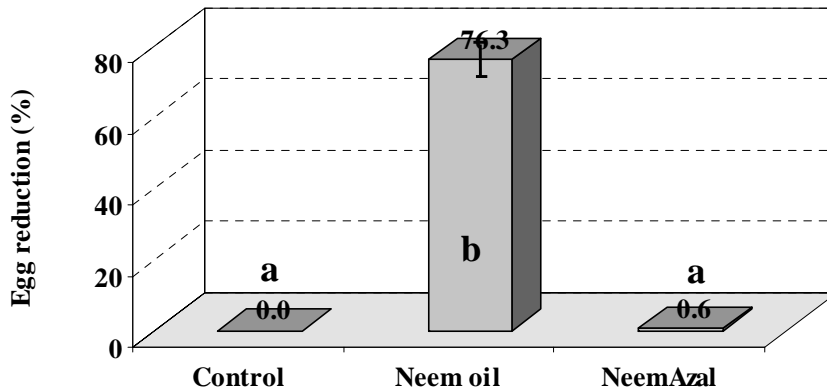


Figure 2: Reduction of egg laying by *P. truncatus* on maize treated with NeemAzal<sup>®</sup> n=5, bars denoted by the same letter are not significantly different (t-test, p > 0.05)

**Insect Population Increase**

Effect of pre-oviposition treatment: the total number of *P. truncatus* adults obtained from samples treated with different dosages of neem seed oil and NeemAzal<sup>®</sup> are given in Fig. 3. ANOVA was performed using transformed values ( $y = \sqrt{x}$ ) since the data obtained originally did not conform to normal distribution. All the treatments at all the dosage levels resulted in significantly ( $P < 0.01$ ) less adults than the control Treatment with NeemAzal<sup>®</sup> at 0.3% and 0.6% w/w and neem oil at 2%v/w dosage levels resulted in nil insects. The samples treated with 0.5% v/w dosage of neem seed oil resulted in significantly more progeny than those of 1%. Sieving of the frass at the end of the experiment revealed many larvae in the neem seed

oil treated samples which, because of their body size, seemed to have died in their first few days after hatching. They never showed any abnormality in shape or characteristics and were uniform in body size and shape. The surviving adults also did not show any degree of morphological malformation.

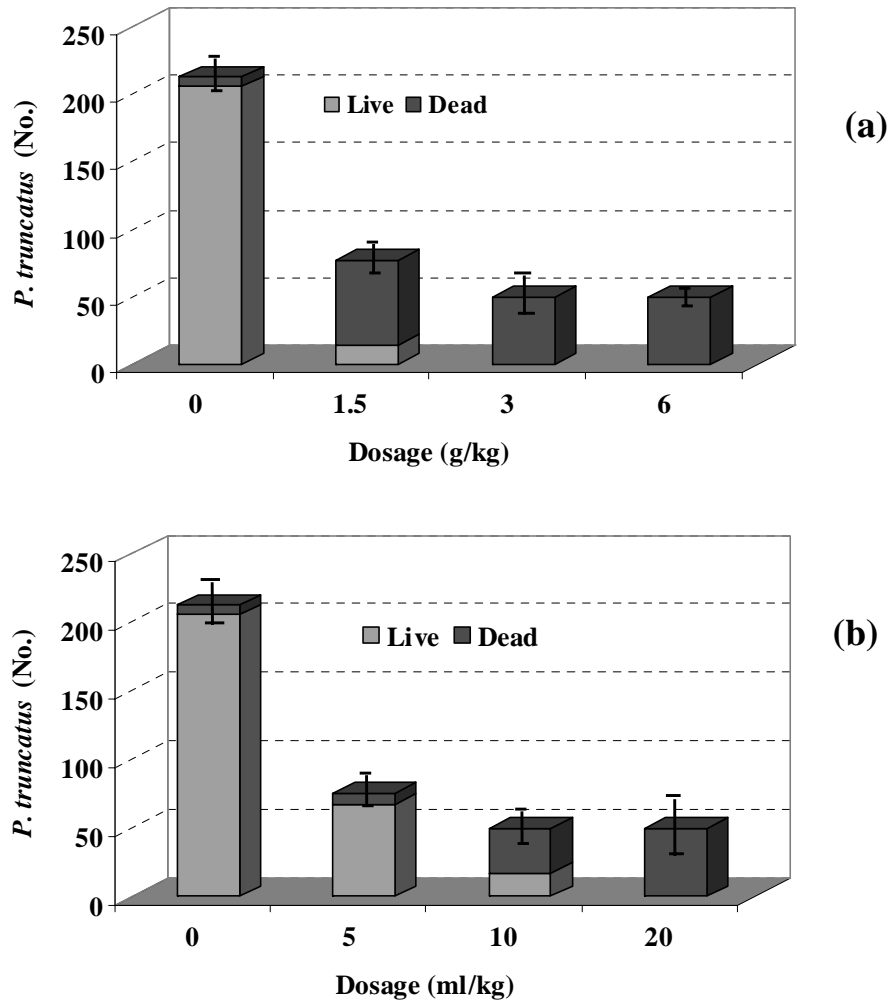


Figure 3: Effect of (a) NeemAzal® and (b) neem oil on the population increase of 50 *P. truncatus*, n=5

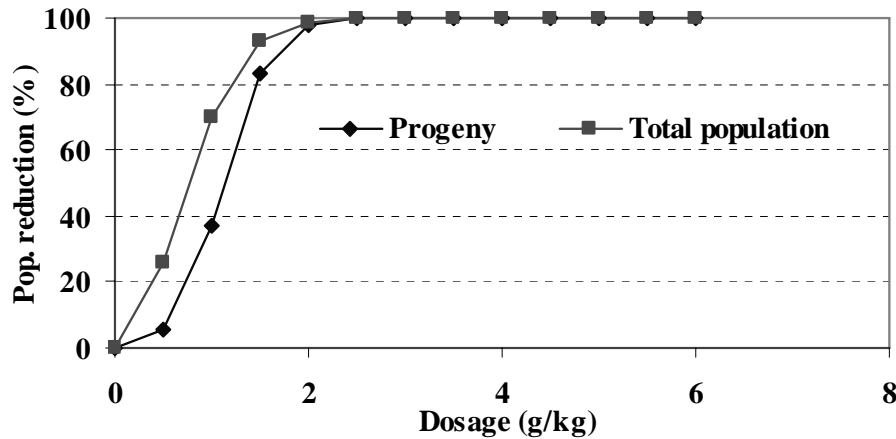


Figure 4: Insect population reduction by NeemAzal® n=5, Adjusted  $r^2=0.99$

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 (Fig. 4 and 5. For NeemAzal®, total population was reduced more than the progeny at lower treatment dosages while the reverse was true for neem seed oil.

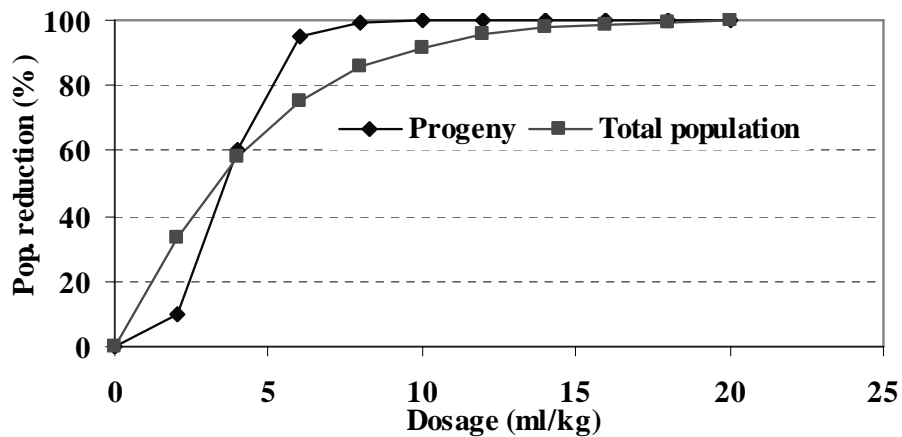


Figure 5: Insect population reduction by neem seed oil. n=5, Adjusted  $r^2=0.99$

Effect of post-oviposition treatment: the population of *P. truncatus* was significantly ( $P<0.01$ ) affected when grains containing eggs were treated with neem products. In the neem seed oil treated grains, no progeny was observed while the number of progeny observed in the NeemAzal® treated

samples was significantly ( $P < 0.01$ ) lower than that in the control. Adults formed the largest percentage of the insects observed in both the NeemAzal<sup>®</sup>-treated and control samples, but the NeemAzal<sup>®</sup> samples contained more dead insects than living ones (Fig. 6). Sieving of the neem seed oil treated samples revealed tiny dead larvae similar to those observed in the pre-oviposition treatment samples.

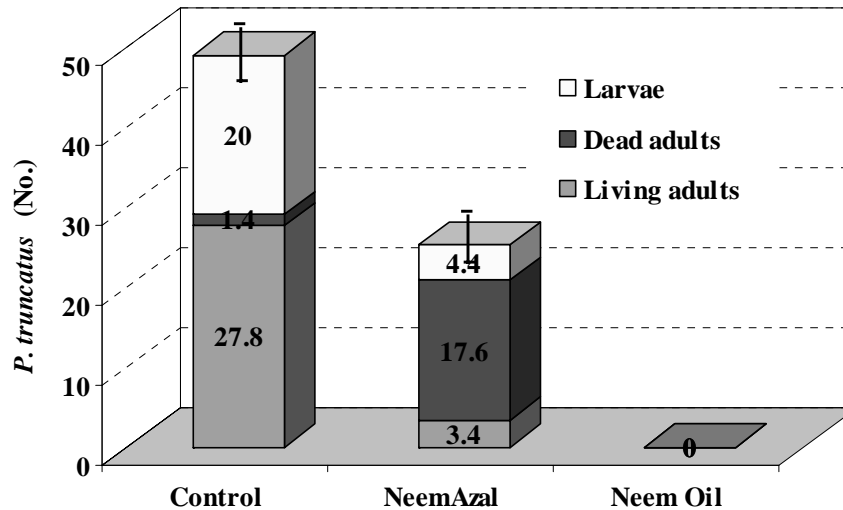


Figure 6: Number of *P. truncatus* obtained from samples treated with 0.6% w/w NeemAzal<sup>®</sup> and 2% v/w neem oil after egg laying by *P. truncatus*, n=5

## Discussion

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.

Perhaps the only study showing direct effects of neem seed 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), and 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 1-5 ml/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. Maculates*. 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.

In this study, larvae of *P. truncatus* died inside the grains during the first instar stage. It is important also to note that larvae mortality occurred even at relatively low dosages of 0.25% v/w 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 larvae mortality. 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 of *Sitophilus zeamais*. 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 two 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.

There are no reports on the effect of NeemAzal<sup>®</sup> on *P. truncatus*, although some reports on the effects of other types of NeemAzal<sup>®</sup> do exist (Karnavar, 1987; El-Lakwah *et al.*, 1994; El-Lakwah and El-Kashlan, 1999; Mansour, 1997; Wudtke, 1997; Halawa, *et al.* 1997; Shemais, 2000). In this study, treatment of maize grains with NeemAzal<sup>®</sup> 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 1.5 g/kg. 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 (0.1% w/w). Maximum progeny reduction values ranged between 94.6 and 100%. In this study, maximum mortality values were 98% for NeemAzal<sup>®</sup>. The NeemAzal<sup>®</sup> product in this study was produced by incorporation of azadirachtin into silica gel. The composition of NeemAzal<sup>®</sup>-W was not given. Comparison of the

NeemAzal<sup>®</sup> products in this study with blank silica gel powders showed a non-significant difference, suggesting that the toxicity 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 20<sup>th</sup> century up to current years [Zacher and Kunike, 1931; Prasantha, 2003]. The results of this study show that NeemAzal<sup>®</sup> 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<sup>®</sup>. 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<sup>®</sup> powder (composition not given) and an inert dust “Kabeljous” ranged between 93% and 97%. The effect of the NeemAzal<sup>®</sup> powder was reported to be similar to that of the inert dust Kabeljous. It can therefore be concluded that the lethal effect of NeemAzal<sup>®</sup> depends mostly on the formulation of the product. Other studies utilised other NeemAzal<sup>®</sup> formulations such as liquids and their results can therefore not be easily compared with the results from this study (Karnavar, 1987; El-Lakwah *et al.*, 1994; Mansour, 1997; Wudke, 1997; Halawa, *et al.*, 1998).

## Conclusions and Recommendations

Based on the results of this study it can be concluded Neem oil and NeemAzal<sup>®</sup> can significantly control population increase of *P. truncatus* at low dosages depending on the stage of the target insect. Neem oil affects both oviposition and development while NeemAzal<sup>®</sup> causes mortality of adults. As a result of these effects, neem oil affected the progeny more than the adults and vice versa for NeemAzal<sup>®</sup>. It is therefore recommended that in the use of neem products, the formulation of the product should be considered in relation to the intended use of the product.

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