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# Bio-rational control of red flour beetle *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) in stored wheat with Calneem® oil derived from neem seeds

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**Abstract** The red flour beetle, *Tribolium castaneum* (Herbst), is one of the most serious secondary pests that feeds on a wide range of durable stored products including cereals, cereal products and other high value produce such as cocoa beans and dried fruits. Toxicity and protectant potential of Calneem® oil derived from the seeds of the neem tree *Azadirachta indica* A. Juss. towards *T. castaneum* were evaluated in stored wheat in the laboratory using contact toxicity, grain treatment, persistency, progeny emergence and repellency assays. Calneem® contains about 0.3% azadirachtin as its major active ingredient. The Calneem® was applied at six dosages (0.1, 0.2, 0.5, 1.0, 2.0 and 3.0% v/v). The oil was emulsified with water using 0.07% soap. All dosages of Calneem® oil were toxic and highly repellent to *T. castaneum* with an overall repellency in the range of 52–88%. The highest dosage of 3.0% of Calneem® oil tested killed at least 90% of the beetles within 72 h on grain, and 88% mortality was obtained on filter paper. *T. castaneum* mortality was dose dependent. The development of eggs to adults on cracked wheat was significantly ($P < 0.05$) inhibited by Calneem® oil treatments. The effectiveness of Calneem® oil was significantly reduced by the length of storage after application. The results obtained suggest good potential for the practical use of Calneem® oil as grain protectant for stored product pest control. The use of plant materials such as neem oil may be a safe, cost-effective method of grain preservation against pest infestation amongst low-resource poor farmers who store small amounts of grains.

**Keywords** *Tribolium castaneum* · *Azadirachta indica* · Cereals · Repellency · Contact toxicity

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## Introduction

Insects are the major pests of cereal grains and grain products during storage (Bekele et al. 1995; Obeng-Ofori 2007). Damage caused by insects affects the quality, the quantity and the commercial value of the products. Many pests of stored products belong to the order Coleoptera and one of the most destructive secondary insect pests of durable stored products is the red flour beetle, *Tribolium castaneum* (Herbst). A commonly used method of controlling pests in stored products is the application of synthetic contact insecticides and fumigants (Chaube 2008). The indiscriminate use of broad-spectrum insecticides has created more problems than resolving them. The development of pesticide resistance by the pests, toxic residues in food and consequent health hazards, destruction of beneficial organisms, rapid resurgence of target pest populations and undesirable environmental pollution are critical problems that have arisen (Park et al. 2003). Further, the advent of maximum pesticides residue limits for food and horticultural products for

export have stimulated an intensive search for alternative measures such as the use of natural pesticides that are safer and environmentally acceptable (Schmutterer 1990; Isman 2006; Obeng-Ofori 2007). In addition, the synthetic pesticides are expensive to the users and may cause potential risk due to the lack of technical knowledge related to their safe use by resource-poor farmers in developing countries (Hodges and Carr 1999).

Botanical insecticides have long been touted as attractive alternatives to synthetic chemical insecticides for pest management, because botanicals are less harmful to the environment due to their biodegradation (Isman 2006). The body of scientific literature documenting bioactivity of plant derivatives to arthropod pests continues to expand, yet only a handful of botanicals are currently used in agriculture in the industrialized world, and there are few prospects for commercial development of new botanical products due to registration requirements. However, many small-scale farmers in Africa mix stored foodstuffs with different kinds of plant materials for protection against pest damage (Hassanali et al. 1990; Poswal and Akpa 1991; Talukder and Howse 1995; Obeng-Ofori 2007). The use of these traditional materials has recently stimulated research to establish the scientific basis for their continued use regarding their efficacy, active constituents and effective application technology (Weaver et al. 1991; Regnault-Roger et al. 1993; Schmidt and Streloke 1994; Bekele et al. 1995, 1996; Obeng-Ofori et al. 1997).

Neem products have been shown to be effective protectants of grains against infestation by grain weevils (Cobbina and Appiah-Kwarteng 1989), grain borers (Schmutterer 1985, 1990), cowpea beetles (Tanzubil 1987) and several species of storage moths (Schmutterer 1985, 1990). Native to India and Burma, the neem tree *Azadirachta indica* A. Juss. is a member of the mahogany family Meliaceae. It was introduced to Africa in the previous century and is now well established in at least 78 tropical and subtropical countries, including Ghana where it has become an important source of fuel, lumber and bioinsecticides. Calneem® oil is a new commercial product that was extracted from the seeds of the neem tree and is registered in Ghana for the control of field and storage pests (Obeng-Ofori 2007). It is an oil extract from pure neem seed kernels collected and crushed in Ghana. This study was carried out to assess the toxicity and protectant potential of Calneem® oil against the red flour beetle, *T. castaneum*, in stored wheat.

## Materials and methods

### Rearing of insects

Individuals of *T. castaneum* were cultured in a controlled environmental room at 25 ± 1°C and 65–70% RH under continued darkness. They were obtained from the laboratory stock culture at the Federal Research Centre for Cultivated Plants (Institute for Ecological Chemistry, Plant Analysis and Stored Product Protection, Berlin, Germany). Cracked wheat grain with a moisture content of 14.4% was used as food. One hundred *T. castaneum* adults of mixed sex were put in 500 ml glass jars containing 200 g of wheat feed. The jars were covered with nylon mesh held in place with rubber bands. After 1 week of oviposition period, the adults were removed and the progeny that emerged subsequently were used for the different assays. Insects were re-cultured after every 8 weeks. The ages of the insects were known to be 3–7 days.

### *Calneem® oil*

Calneem® oil is a biopesticide produced, registered and marketed in Ghana by AQUA AGRIC Community Projects (AACP). It is an in-house prepared, cold-pressed, double-filtered, pure and natural oil derived from high quality neem seeds. Calneem® oil contains about 0.3% azadirachtin (AZA) as its major active ingredient. According to Schmutterer (1985, 1990), neem products contain limonoids, including azadiractin, meliantriol, salamin, nimbin and nimbidin that act in different ways under different circumstances. Calneem® is a broad-spectrum insecticide, which is effective against several pests of vegetables, food crops, fruit and other tree crops, and durable stored products. Calneem® is marketed as 100% neem oil, and the recommended rate for application is 50 ml of neem oil + 10 ml of emulsifier in 15 l of water. The preparation of Calneem® dosages and its diluted AZA contents used in this investigation is shown in Table 1.

**Table 1** The Calneem® dosages and its diluted azadirachtin (AZA) contents used in the investigation

| | Amount per volume | | | | | |
|---|---|---|---|---|---|---|
| Calneem® dosages v/v (%) | 0.1 | 0.2 | 0.5 | 1.0 | 2.0 | 3.0 |
| Calneem® diluted azadirachtin (AZA) contents (%) | 0.03 | 0.06 | 0.015 | 0.3 | 0.6 | 0.9 |
| Calneem® oil content (ml) | 1.0 | 2.0 | 5.0 | 10.0 | 20.0 | 30.0 |
| Emulsifier (Soap) (ml) | 0.7 | 0.7 | 0.7 | 0.7 | 0.7 | 0.7 |
| Distilled water (ml) | 1,000 | 1,000 | 1,000 | 1,000 | 1,000 | 1,000 |

A preliminary experiment confirmed that the emulsifier (Palmolive Ultra soap, Henkel Deutschland) in water without the Calneem®oil had no effect on all stages of *T. castaneum.*

*Contact toxicity on filter paper*

Whatman® No. 1 filter paper (90 mm diameter) obtained from Schleicher & Schuell GmbH; Dassel, Germany was placed in a glass Petri dish (11.0 cm diameter). Six dosages of Calneem® oil (v/v), 0.1, 0.2, 0.5 1.0, 2.0 and 3.0%, respectively, were applied uniformly on the surface of the filter papers that were air dried for about 5 min prior to the introduction of 20 *T. castaneum* adults (3–7 days old of mixed sex) separately into each dish and were kept in the laboratory at 25 ± 1°C, 65–70% RH under an L16:D8 photo-regime. Actellic 50® (Syngenta Agro GmbH, Maintal, Germany) containing 500 g/l pirimiphos-methyl was selected as the reference insecticide because it is a commonly used synthetic insecticide registered and used in Ghana and some European countries against insects in stored products. A dose of 4 ppm pirimiphos-methyl was used. Water was used as a non-insecticide control. Each treatment was replicated five times. Insect mortality was recorded after 24, 48 and 72 h. Individuals were presumed dead if they remained immobile and did not respond to three probings with a blunt dissecting probe after a 5 min recovery period.

*Contact toxicity by topical application*

Tests for contact toxicity by topical treatment were carried out in the laboratory at 25 ± 1°C, 65–70% RH and under an L16:D8 photo-regime. Three to seven days old *T. castaneum* adults of mixed sex were first transferred into glass Petri dishes (11.0 cm diameter) lined with moist filter paper and chilled for 3 min to reduce their activity to enable topical treatment to be carried out. The immobilized insects were picked individually and 1 μm of each diluted Calneem® dosage was applied to the dorsal surface of the abdomen of each *T. castaneum* adult using a micro-pipette applicator. A total of 100 *T. castaneum* adult in five replicates of 20 insects each were treated with each Calneem® dosage. The same number of insects was treated with the reference product (pirimiphos-methyl) and water only to serve as the control. After treatment, the adults were transferred into glass Petri dishes (20 insects/Petri dish) containing 5% cracked wheat grains as food. The *T. castaneum* adults were examined daily for 3 days and those that did not move or respond to three probings with a blunt probe were considered dead. Insect mortality was recorded at 24, 48 and 72 h after treatment.

*Adult mortality in grain*

The effect of different dosages of Calneem®−treated wheat grains on adult mortality of *T. castaneum* was assessed in the laboratory. The six dosages (v/v) were 0.1, 0.2, 0.5, 1.0, 2.0 and 3.0%, equalling 0.006, 0.012, 0.030, 0.059, 0.118 and 0.175 ml of neem oil per 500 g wheat, respectively. In 1-l glass jars, 6 ml of the diluted Calneem® dosages (test solutions) were mixed with 500 g of whole wheat grains with 5% cracked wheat and stirred continuously on a mechanical roller for 5 min to ensure even spread of the material over the surface of the grains. In the reference insecticide treatment, 2.5 ml Actellic 50® was added per 500 g wheat. The samples were kept for about 5 min to allow the solvent to evaporate completely. The grains were then infested with 3- to 7-day-old *T. castaneum* adults of mixed sex (20 adults per jar) and each jar was covered with a perforated rubber lid. Each treatment was replicated five times. Mortality was recorded after 24, 48 and 72 h.

*Persistence of Calneem® oil in grain*

This experiment was designed to determine the duration of activity of Calneem® oil, which had been applied to wheat grain. To assess the persistence of the preparations, *T. castaneum* adults were exposed to treated grain, which had been stored for 1, 20, 30 and 60 days, as described above. Twenty adults were introduced into each treatment at the appropriate days and kept in the laboratory at 25 ± 1°C and 65–70% RH under an L16:D8 photo-regime. Each treatment was replicated five times. Mortality was recorded after 24 h of exposure.

*Toxicity of Calneem® oil to immature stages*

The toxicity of Calneem® oil to immature stages (eggs, larvae and pupae) of *T. castaneum* in wheat grains was evaluated. Batches of 100 g of whole wheat grain mixed with 5% cracked wheat were placed in 250 ml glass jars. These samples were infested with 50 unsexed *T. castaneum* adults (3- to 7-day-old) to allow egg laying to take place. The parental adults were removed after 7 days. One day after the removal of adults, five batches of the grain were treated with different dosages of Calneem® oil (0.5, 1.0, 2.0 and 3.0% v/v). Thereafter, these treatments were repeated at 1, 2 and 3 weeks after removal of adults to determine their effect on early instar larvae, late instar larvae and pupae, respectively. Adults subsequently emerging from the respective jars were counted for a period of 8 weeks following the removal of adults.

*Repellency test*

The area preference test described by McDonald et al. (1970) was used in evaluating the repellent action of Calneem® oil against *T. castaneum.* The test arena consisted of 9 cm Whatman's No 1 filter papers (surface area: 63.6 $cm^2$) cut in half. Different dosages of Calneem® oil, 1.57 µl/$cm^2$ from the respective dosages of 0.1–3.0% v/v, were applied to a half filter paper disc as uniformly as possible with a pipette. The other filter paper halves were treated with water to serve as control. Both halves were air dried for about 10 min after which the treated and untreated halves were re-made by attaching them together with sellotape. The joined filter papers were placed in (9 cm diameter) glass Petri dishes and 10 *T. castaneum* adults were released separately at the centre of each filter paper disc and covered. Each treatment was replicated ten times. All repellency assays were carried out in the laboratory at 25 ± 1°C and 65–70% RH under an L16:D8 photo-regime. The numbers of insects present on the control ($N_c$) and the number on the treated ($N_t$) area were recorded after 24 h of exposure. Percentage repellency (PR) values were computed as follows: $PR = [(N_c - N_t)/(N_c + N_t)] \times 100$.

*Statistical analysis of data*

Percentage mortality data of *T. castaneum* adults were not corrected for control mortality, because mortality in the control treatment was <2%. Percentage mortality data were transformed to arcsine $(chi)^{0.5}$ to stabilize heteroscedastic treatment variances. Data were subjected to two-way analysis of variance (ANOVA) using SigmaStat® 3.1 software. Treatments were considered significantly different at the alpha = 0.05 level. Means were separated using the Holm–Sidak test. Repellency data were subjected to one-way ANOVA and means separated using the Student Newman–Keuls test.

## Results

Contact toxicity on filter paper

Table 2 shows the percentage mortality of *T. castaneum* adults after exposure to increasing dosages of Calneem® oil on filter paper discs. Adult mortality was dosage dependent with the highest dosage of 3.0% causing 88% mortality of *T. castaneum* after 72 h, whilst pirimiphos-methyl (the reference insecticide) killed all the *T. castaneum* adults within 24 h and therefore it has been omitted in the table. All treatments with Calneem® oil differed significantly ($P < 0.05$) from the control after 48 and 72 h of exposure (Table 2). Significant differences were noted for all main effects and associated interactions (duration df = 2, $F = 602.2$; dosage df = 6, $F = 87.2$; duration × dosage df = 12, $F = 20.7$; in all cases $P < 0.001$).

**Table 2** Contact toxicity of Calneem® oil (0.1–3.0% v/v) on filter paper to *T. castaneum* at a temperature of 25°C and 65–70% relative humidity, data are means ± SE of five replicates of 20 insects each

| Dosage % (v/v) | Exposure period (h) % mortality (mean ± SE) | | |
|---|---|---|---|
| | 24 | 48 | 72 |
| Control | 0.0 ± 0.0aA | 0.0 ± 0.0aA | 0.0 ± 0.0aA |
| 0.1 | 1.0 ± 1.0aA | 15.0 ± 2.7bB | 31.0 ± 1.9bC |
| 0.2 | 3.0 ± 1.2aA | 16.0 ± 2.9bB | 43.0 ± 1.2bcC |
| 0.5 | 1.0 ± 1.0aA | 24.0 ± 2.4bcB | 54.0 ± 1.9cdC |
| 1.0 | 1.0 ± 1.0aA | 31.0 ± 1.0cB | 72.0 ± 3.0deC |
| 2.0 | 2.0 ± 1.2aA | 37.0 ± 4.4cdB | 83.0 ± 3.7eC |
| 3.0 | 1.0 ± 1.0aA | 51.0 ± 1.0 dB | 88.0 ± 6.2eC |

* Means in the same column followed by the same lowercase letter do not differ significantly; means in the same rows followed by the same uppercase letter do not differ significantly at $P < 0.05$ (Holm–Sidak test)

Contact toxicity by topical application

Contact toxicity of different dosages of Calneem® oil applied topically to *T. castaneum* depending on exposure time is shown in Table 3. Mortality of *T. castaneum* was significantly affected by duration of exposure and dosages of Calneem® oil applied topically to the dorsal abdomen. Significant differences were noted for all main effects and associated interactions (duration df = 2, $F = 50.6$; dosage

**Table 3** Contact toxicity of different dosages of Calneem® oil applied topically to the dorsal surface of *T. castaneum* using a micro-pipette applicator at a temperature of 25°C and 65–70% relative humidity, data are means ± SE of five replicates of 20 insects each

| Dosage %(v/v) | Exposure period (h) % mortality (mean ± SE) | | |
|---|---|---|---|
| | 24 | 48 | 72 |
| Control | 0.0 ± 0.0aA | 0.0 ± 0.0aA | 0.0 ± 0.0aA |
| 0.1 | 12.0 ± 1.2bA | 16.0 ± 1.0bB | 17.0 ± 1.2bB |
| 0.2 | 26.0 ± 1.9cA | 30.0 ± 1.6cB | 32.0 ± 1.2cB |
| 0.5 | 30.0 ± 1.6dA | 38.0 ± 1.2 dB | 39.0 ± 1.9 dB |
| 1.0 | 36.0 ± 1.0eA | 40.0 ± 1.6dA | 40.0 ± 0.0dA |
| 2.0 | 39.0 ± 1.9efA | 46.0 ± 1.0eB | 50.0 ± 1.6eB |
| 3.0 | 44.0 ± 1.0fA | 54.0 ± 1.9fB | 59.0 ± 1.9fC |

* Means in the same column followed by the same lowercase letter do not differ significantly, means in the same rows followed by the same uppercase letter do not differ significantly at $P < 0.05$ (Holm–Sidak test)

df = 6, $F = 1346.4$; duration × dosage df = 12, $F = 2.4$; in case of duration and dosage $P < 0.001$, and interaction of duration × dosage $P < 0.009$). Toxicity of Calneem® oil increased significantly with increasing dosage. Calneem® oil applied at the highest dosage of 3.0% induced 59% mortality within 72 h of application. Pirimiphos-methyl used as the reference insecticide killed all the *T. castaneum* adults within 24 h of exposure.

### Adult mortality in grain

Mortality of *T. castaneum* adults was affected by duration of exposure, dosage and their interactions (duration df = 2, $F = 302.6$; dosage df = 6, $F = 77.2$; duration × dosage df = 12, $F = 8.8$; in all cases $P < 0.001$). After 48 h, all treatments caused significantly higher mortality than the untreated control (Table 4). For all dosages tested except for 0.1%, mortality increased with increasing exposure time. A mortality of 60% or more was only achieved at 0.5% or more and 72 h (Table 4). The reference insecticide killed all the *T. castaneum* adults after the 24 h exposure period.

### Persistence of Calneem® oil in grain

Duration of storage (1, 20, 30 and 60 days), dosage, and their interactions had significant effects on mortality of *T. castaneum* adults in grain (duration df = 3, $F = 771.4$; dosage df = 6, $F = 484.7$; duration × dosage df = 18, $F = 29.6$; in all cases $P < 0.001$). After 20 days, mortality of the adults ranged from 40 to 64% in grains treated with Calneem® oil at the dosages of 0.5–3.0% (Table 5). Except for the highest dosage tested, mortality decreased after 20 days compared to 1 day after treatment. The effectiveness of Calneem® oil was significantly reduced after 30 days for all dosages tested (Table 5). After 60 days, the highest dosage of 3.0% achieved only 20% mortality of *T. castaneum*. For grains tested after 60 days of storage following application, mortality decreased to less than 20% even at the highest dosage of 3.0% (Table 5).

**Table 4** Toxicity of different dosages of Calneem® oil in wheat grains to *T. castaneum* adults at a temperature of 25°C and 65–70% relative humidity, data are means ± SE of five replicates of 20 insects each

| Dosage % (v/v) | Exposure period (h) % mortality (mean ± SE) | | |
|---|---|---|---|
| | 24 | 48 | 72 |
| Control | 0.0 ± 0.0aA | 0.0 ± 0.0aA | 0.0 ± 0.0aA |
| 0.1 | 3.0 ± 1.2abA | 7.0 ± 2.6bA | 28.0 ± 1.2bB |
| 0.2 | 3.0 ± 2.0abA | 9.0 ± 1.0bcB | 43.0 ± 1.2bcC |
| 0.5 | 4.0 ± 1.0bcA | 11.0 ± 1.0bcB | 59.0 ± 1.9cdC |
| 1.0 | 5.0 ± 2.2bcA | 17.0 ± 2.6cdB | 65.0 ± 1.6cdeC |
| 2.0 | 7.0 ± 3.0bcA | 25.0 ± 1.6deB | 79.0 ± 1.9deC |
| 3.0 | 10.0 ± 1.6cA | 39.0 ± 2.9eB | 90.0 ± 0.0efC |

* Means in the same column followed by the same lowercase letter do not differ significantly, means in the same rows followed by the same uppercase letter do not differ significantly at $P < 0.05$ (Holm–Sidak test)

### Effect of Calneem® oil on eggs and immature stages of *T. castaneum*

The treatment of wheat with Calneem® oil significantly reduced the number of *T. castaneum* adults emerging when eggs stage, late stage larvae or pupae stage was exposed (Table 6). Comparing the efficiency against eggs stage and late stage larvae, an equal control effect of Calneem® oil was observed (Table 7). In the case of pupae stage, only dosages of 2.0 and 3.0% affected adult emergence (Table 6). Mortality of late larvae stage and pupae stage was significantly lower at 0.5% (v/v) neem oil compared to all other treatments. The level of inhibition was dose dependent with the highest dosage of 3.0% inducing the highest suppression of progeny development by 64% in the case of beetles exposed from the egg stage onwards (Table 7). Calneem® significantly increased the mortality of *T. castaneum* in wheat. Significant differences were noted for all main effects and associated interactions (developmental stage df = 3, $F = 23.0$, $P = 0.115$; dosage of neem df = 3, $F = 44.1$, $P < 0.001$) except for developmental stage × dosage (df = 9, $F = 1.2$, $P = 0.595$).

### Repellency

Repellency due to Calneem® was shown for *T. castaneum* at all dosages tested with overall repellency in the range of 52–88% (Table 8). Repellency was dose dependent (one-way ANOVA, $P < 0.001$, df = 6, $F = 22.1$). The highest dosages of 2.0 and 3.0% v/v repelled significantly more compared to 0.1 and 0.2%. There was significant difference amongst the treatments (Student Newman–Keuls test, $P < 0.05$) (Table 8).

## Discussion

In this study, the toxicity and protection potential of Calneem® oil to *T. castaneum* have been demonstrated in stored wheat in the laboratory using contact toxicity, grain treatment, persistency, progeny emergence and repellency bioassays. These results confirm the findings of several workers who had demonstrated the toxic and highly repellent action of different neem preparations against a

**Table 5** Mortality of adult *Tribolium castaneum* in wheat treated with different dosages of Calneem® oil applied and stored for 1, 20, 30 and 60 days prior to exposure, a temperature of 25°C and 65–70% relative humidity, data are means ± SE of five replicates of 20 insects each

| Dosage % (v/v) | Exposure period (days) % mortality (mean ± SE) | | | |
|---|---|---|---|---|
| | 1 | 20 | 30 | 60 |
| Control | 0.0 ± 0.0aA | 0.0 ± 0.0aA | 0.0 ± 0.0aA | 0.0 ± 0.0aA |
| 0.1 | 45.0 ± 1.6bA | 25.0 ± 1.6bB | 5.0 ± 1.6bC | 0.0 ± 0.0aC |
| 0.2 | 45.0 ± 1.6bA | 30.0 ± 1.6bB | 10.0 ± 1.6cC | 5.0 ± 0.0bD |
| 0.5 | 50.0 ± 1.5bcA | 40.0 ± 1.6cB | 10.0 ± 1.6cC | 5.0 ± 1.6bD |
| 1.0 | 60.0 ± 1.6cdA | 40.0 ± 1.6cB | 15.0 ± 1.6dC | 10.0 ± 0.0cD |
| 2.0 | 65.0 ± 1.6dA | 50.0 ± 1.6 dB | 15.0 ± 0.0dC | 15.0 ± 1.6dD |
| 3.0 | 70.0 ± 1.6dA | 64.0 ± 2.9eA | 20.0 ± 1.6 dB | 20.0 ± 0.0dB |

* Means in the same column followed by the same lowercase letter do not differ significantly, means in the same rows followed by the same uppercase letter do not differ significantly at $P < 0.05$ (Holm–Sidak test)

**Table 6** Mean number of *T. castaneum* adults that emerged from wheat treated with various dosages of Calneem® oil at different times after oviposition period, temperature 25°C and 65–70% relative humidity, data are means ± SE of five replicates of 20 insects each

| Dosage % (v/v) | Number of progeny emergence (mean ± S.E.) | | | |
|---|---|---|---|---|
| | Eggs | Early larvae | Late larvae | Pupae |
| Control | 244.0 ± 28.8aA | 269.4 ± 11.8aA | 227.6 ± 11.9aA | 140.0 ± 12.2aB |
| 0.5 | 138.8 ± 21.8bA | 151.8 ± 17.7bA | 152.8 ± 9.4bA | 106.0 ± 14.6abA |
| 1.0 | 139.8 ± 8.2bAB | 148.2 ± 13.0bA | 149.4 ± 11.2bA | 88.4 ± 12.3abB |
| 2.0 | 120.8 ± 28.2bAB | 142.8 ± 8.7bA | 123.2 ± 15.6bA | 68.2 ± 6.5bcB |
| 3.0 | 86.8 ± 13.8bA | 106.2 ± 5.7bA | 112.4 ± 3.4bA | 62.2 ± 7.5bcA |

* Means in the same column followed by the same lowercase letter do not differ significantly, means in the same rows followed by the same uppercase letter do not differ significantly at $P < 0.05$ (Holm–Sidak test)

**Table 7** Mean corrected % morality of *T. castaneum* progeny emerged from wheat treated with various dosages of Calneem® oil at different times after oviposition period, temperature 25°C and 65–70% relative humidity, data are means ± SE of five replicates of 20 insects each

| Dosage % (v/v) | % mortality (mean ± SE) | | | |
|---|---|---|---|---|
| | Eggs | Early larvae | Late larvae | Pupae |
| 0.5 | 43.11 ± 9.0aA | 43.43 ± 6.7aA | 32.86 ± 4.1aAB | 24.89 ± 10.4aB |
| 1.0 | 42.70 ± 3.4aA | 44.99 ± 4.8aA | 34.36 ± 4.9aA | 36.86 ± 8.8abA |
| 2.0 | 50.49 ± 11.6aA | 46.99 ± 3.2aA | 45.87 ± 6.9aA | 51.29 ± 4.6bA |
| 3.0 | 64.43 ± 5.7aA | 60.58 ± 2.1aA | 50.62 ± 1.5aA | 55.57 ± 5.4bA |

* Means in the same column followed by the same lowercase letter do not differ significantly, means in the same rows followed by the same uppercase letter do not differ significantly at $P < 0.05$ (Holm–Sidak test)

wide range of storage pests (Jilani and Malik 1973; Jilani et al. 1988; Schmutterer 1990; Isman 2006; Obeng-Ofori 2007). Our experiments show that Calneem® oil is amongst the preparations that could be expected to reduce infestation by *T. castaneum*. Perhaps the only study showing direct effects of neem oil on larvae and pupae of *T. castaneum* 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 neem oil at rates of 100, 500 or 1,000 ppm of the oil. They also reported failure of the larvae to pupate, delayed development and abnormal pupae and adults. In our study, dosages of Calneem® oil of 0.5, 1.0, 2.0 and 3.0% v/v, equalling 5,000, 10,000, 20,000 and 30,000 ppm showed a significant dose-dependent effect. We continued with higher dosages than those of Jilani et al. (1988) and observed a significant reduction in the number of progeny emergence of *T. castaneum* when eggs, early larvae or late larvae were exposed

**Table 8** Mean % repellency (PR) of different dosages of Calneem® oil against *T. castaneum* adults in a choice arena after 24 h of exposure at 25°C and 65–70% relative humidity, data are means ± SE of 10 replicates of 10 insects each

| Dosages %(v/v) | % Repellency (PR) (mean ± SE) after 24 h |
|---|---|
| Control | 0.0 ± 0.0a |
| 0.1 | 52.0 ± 2.0b |
| 0.2 | 59.0 ± 9.0b |
| 0.5 | 68.0 ± 7.3bc |
| 1.0 | 74.0 ± 8.1bc |
| 2.0 | 86.0 ± 2.4c |
| 3.0 | 88.0 ± 4.9c |

* Means in the same column followed by the same letter do not differ significantly at $P < 0.05$ (Student Newman–Keuls test)

(Table 6). The dosages between 20,000 and 30,000 ppm affected adult emergence in the case of pupae as a parameter measured. The highest tested dosage of 30,000 ppm induced the greater portion of suppression of progeny development by 64.4% (Table 7). The effect of these higher dosages was not studied previously. However, this dose-dependent effect might not be due to the AZA-content. Xie et al. (1995) studied 98% AZA and three neem extracts (48, 23, and 7% AZA) and found no increase in mortality of *T. castaneum* with increasing AZA content. Other active ingredients of neem oil (Schmutterer 1990) and the physical effect of the oil may be responsible for the dose-dependent effect found here and in other studies applying neem oil.

Lale and Mustapha (2000) reported significant reduction in egg laying and adult emergence in *Callosobruchus maculatus* treated with neem seed oil at different rates (25, 50, 75 and 100 mg/5 g seed). They found that treatment of seeds with neem oil at the rates of 50 mg/5 g and 75 or 100 mg/5 g reduced seed damage from over 25% in controls to less than 10% and less than 5%, respectively. In our study, Calneem® dosages applied at much lower dosages of 0.5–3.0% v/v (0.3, 0.6, 1.18, and 1.75 mg/5 g of wheat grain) reduced progeny production in *T. castaneum* by 43.1, 42.7, 50.5 and 64.4%, respectively, in all the treatments compared to the control. The level of reduction in adult emergence was dose dependent (Table 7); however, the impact of this progeny reduction on product loss remains to be studied.

Dunkel et al. (1990) observed more than 70% mortality for adult *Sitophilus oryzae* exposed for 2 weeks to wheat treated with the neem-based insecticide Margosan-O at a concentration of 0.2%, 6 ppm AZA. We conducted a test similar to Dunkel et al. (1990), with neem oil diluted to an AZA content of 0.03, 0.06, 0.015, 0.3, 0.6 and 0.9% and obtained 43% mortality with *T. castaneum* within 72 h with the AZA content of 0.06 representing 0.2% of the formulated Calneem® dosage (Table 5). This confirms the major influence of AZA in the effectiveness. Further differences between the studies are insect strains, insect ages (0–3 days vs. 3–7 days), test conditions (25°C and 65–70% RH versus 30°C and 65% RH) and a AZA-based insecticide (Margosan-O) versus pure neem oil. Another example for an AZA-based insecticide that is effective at lower dosages in grain compared to neem oil is the study of El-Lakwah and El-Kashlam (1999). They tested the effect of NeemAzal-W, a powder containing 10% AZA, on mortality and progeny reduction of *S. oryzae*, *Rhyzopertha dominica*, *C. maculatus* and *T. castaneum* adults and reported maximum mortality values of 100% for all the test species at 1,000 ppm (1 g/kg). Maximum progeny reduction values ranged between 94.6 and 100%.

Xie et al. (1995) showed that azadirachtin and neem concentrates repelled three insects in stored products, *Cryptolestes ferrugineus*, *S. oryzae* and *T. castaneum*, using a product which contained 98% AZA and the three neem extracts. In a choice test, filter paper strips treated with $\geq$200 μg/cm$^2$ of neem oil repelled *T. castaneum* adults and in a food preference chamber fewer adults settled in grains treated with >100 ppm of neem oil (Jilani et al. 1988). Although, the AZA content was very low in our study, it was found to cause overall repellency ranging from 52 to 88% for *T. castaneum* adults compared to the control.

Due to the fact that pupae do not feed, the obtained mortality could not be explained by any of the known modes of action of neem: antifeedant effects and growth regulator effects. Antifeedant effects on insects in stored products have been extensively investigated (Saxena et al. 1988). Treating stored grain can disrupt insect feeding by making the treated materials unattractive or unpalatable and, as a consequence, insect growth, survival and reproduction are adversely affected (Norris 1986; Saxena 1987). Neem products have been reported to cause growth inhibition, malformation and mortality, especially when applied to the larval stages, as well as repellency. The effects of insect growth regulators (IGR) include slow growth, delayed moulting, moult abnormalities, inability to complete moulting, insects remaining as "over-aged" larvae for a greatly extended period of time and, finally, mortality. Generally, IGR effects are more consistent between species than antifeedancy effects (Nicol and Schmutterer 1991; Mordue and Blackwell 1993; Mordue 1998). Azadirachtin, the major component in neem, inhibits the release of prothoracicotropic hormones and allatotropins, thereby affecting metamorphosis in insects (Banken and Stark 1997). In our studies, the growth regulator effect was demonstrated by low mortality of late larvae and pupae exposed to 0.5% Calneem® compared to

egg and early larvae. In Ghana, Tanzubil (1987) demonstrated that cowpea treated with neem oil remained undamaged by *C. maculatus* over a 12-week storage period with 2 ml/kg and for a 16-week storage period with 5 ml/kg, whilst untreated cowpea had 90% grain damage. In our study we used much lower dosages of Calneem® oil (0.012, 0.024, 0.060, 0.120, 0.236 ml and 0.35 ml/kg) in wheat grain and observed persistence for only a 4-week storage period with *T. castaneum*. If higher doses are used, it could have better persistence to *T. castaneum*, too.

We suggest that Calneem® oil may have good potential for the practical use as grain protectant for stored product pest control. However, a single application would not be persistent enough for long-term protection of grain during storage. The second application needs to be done after 30 days after the first treatment. In warehouse trials, wheat grain treated with neem oil at a proportion of 8 ml to 1 kg grain, prior to storing for 8 months in gunny bags, had 50–70% less infestation by *S. oryzae*, *R. dominica*, *T. castaneum* and *Cryptolestes* spp. (Ketkar 1976). In our study, the maximum dosage of neem oil tested was 2% of the dosage applied by Ketkar (1976), so the concentrations tested here need to be verified under field conditions. The use of plant materials may also be a safe, cost-effective and environmentally friendly method of grain preservation against pest infestation amongst low-resource poor farmers who store small amounts of grains in Ghana. However, unlike synthetic insecticides (for example, pirimiphos-methyl and deltametrin) these alternatives often do not provide effective or rapid suppression of pest populations and may not be effective against all species of pests.

Nonetheless, a good level of control of *T. castaneum* was achieved with the pure neem seed oil extract. The results from the current study revealed that for practical application of Calneem® on stored grains, according to label data of Calneem®, for example, 1 ton of grains require 350 ml of Calneem® oil and 105 l of water. Applying this to grain with a moisture content of even 11% will lead to moulding of the grain because of the quantity of water used. The development of a formulation suitable for application in stored product protection using less water is therefore required. Further areas requiring attention are possible changes in the quality of grains treated with the Calneem® oil and the exact application procedure for improving insecticidal potency and stability, and for reducing cost.

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