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EFFICACY OF NEEM OIL FOR CONTROL OF STORED COWPEA SEED BEETLE

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ABSTRACT

Cowpea beetle, *Callosobruchus maculatus*, is a major pest of stored cowpea. Farmers use mostly synthetic insecticides to control the pest in store, although biopesticides such as neem oil have been proven to be a sustainable alternative. The objective of this study was to determine the effective and feasible rates to enhance cost-effectiveness of neem oil in the management of *C. maculatus* in stored cowpea grains. A laboratory experiment was conducted, in which sterilised healthy cowpea grains (50 g each) were treated with neem oil at 0, 0.5, 0.75, 1 ml and 0.05 g ACTELLIC Gold Dust® (16 g kg⁻¹ Pirimiphos methyl + 3.6 g kg⁻¹ Thiamethoxam). The experiment was laid out in a completely randomised design, with four replications. Results revealed that adult female *C. maculatus* laid eggs on all the treated cowpea grains, except those treated with 1.0 ml of neem oil. However, no adult emergence and damaged grains were recorded from grains treated with 0.50, 0.75 and 1.0 ml of neem oil. This study has shown that cowpea grains treated with 0.50, 0.75 and 1.0 ml of neem oil outperformed those treated with insecticide actellic dust. Among the rates of neem oil assessed, there was no significant difference between their effect on the management of *C. maculatus*. Thus, 0.50 ml of neem oil per 50 g of cowpea grains is the most cost-effective rate for the management of *C. maculatus* in stored cowpea grains.

Key Words: *Callosobruchus maculatus*, cowpea, neem oil

RÉSUMÉ

Le coléoptère du niébé, *Callosobruchus maculatus*, est un ravageur majeur du niébé stocké. Les agriculteurs utilisent principalement des insecticides synthétiques pour contrôler l'infestation du niébé par *C. maculatus*, bien que les biopesticides tels que l'huile de neem s'est avérée être une alternative durable. L'objectif de cette étude était de déterminer la taux efficaces et réalisables pour améliorer la rentabilité de l'huile de neem dans la gestion de *C. maculatus* dans les grains de niébé stockés. Une expérience en laboratoire a été menée dans laquelle du niébé sain et stérilisé les grains (50 g chacun) ont été traités avec de l'huile de neem à raison de 0, 0,5, 0,75, 1 ml et 0,05 g d'ACTELLIC Gold Dust® (16 g kg⁻¹ Pirimiphos méthyle + 3,6 g kg⁻¹ Thiaméthoxame). L'expérience s'est déroulée de

manière complètement conception randomisée, avec quatre répétitions. Les résultats ont révélé que la femelle adulte *C. maculatus* a pondé sur tous les grains de niébé traités, à l'exception des grains de niébé traités avec 1,0 ml d'huile de neem ; cependant, aucun adulte la levée et les grains endommagés ont été enregistrés à partir de grains de niébé traités avec 0,50, 0,75 et 1,0 ml l'huile de neem. Par conséquent, cette étude a montré que les grains de niébé traités avec 0,50, 0,75 et 1,0 ml de neem le pétrole a surpassé celui traité avec de la poussière insecticide actélique. Parmi les taux d'huile de neem évalués, il n'y avait pas de différence significative entre leur effet sur la gestion de *C. maculatus*. Ainsi, 0,50 ml d'huile de neem pour 50 g de grains de niébé est le seuil et donc le taux le plus rentable pour le gestion de *C. maculatus* dans les grains de niébé stockés.

Mots Clés : *Callosobruchus maculatus*, niébé, huile de neem

INTRODUCTION

Cowpea (*Vigna unguiculata* (L.) Walp) is a major staple crop in Africa and Asia; providing critical nutritional components including 23.4% protein, 1.8% fat, and 60.3% carbohydrate content (Gupta and Gopalakrishna, 2010; Adeyemi *et al.*, 2012). Unfortunately, enormous quantities of cowpea grain (> 50%) are lost to the cowpea beetle (*Callosobruchus maculatus*), accounting for untold economic and nutritional losses (Duan *et al.*, 2014; Gad, 2019).

The most destructive stage in the life cycle of *C. maculatus* is the larval stage (Srivastava and Subramanian, 2016), at which cowpea grain is destroyed through beetle feeding activities (Hamzei *et al.*, 2023). When the larvae develop into the adult inside the cowpea grains, the former chew their way-out, leaving exit holes on the grain surface (Devi and Devi, 2014); this reduces the quality and quantity of the grain.

In order to curb these grain losses during storage, substantial quantities of synthetic insecticides have been used by actors in the cowpea value chain, despite their being costly and risky to environmental safety (Ngegba *et al.*, 2022). Besides, over-reliance on these synthetic pesticides have resulted in insect pests developing resistance to the commonly used pesticides and rates (Nikolaou *et al.*, 2021). This has prompted the need for safe and sustainable alternative management methods (Fening *et al.*, 2013).

Biopesticides provide good alternatives to their synthetic counterparts, because they are ecologically friendly (Damalas and Koutroubas, 2020), leave no residues in stored grains, and are readily available to farmers (Adarkwa *et al.*, 2017). Extracts from cashew, neem, orange, moringa, lemongrass and candlewood, among others, have been reported to be effective in the management of *C. maculatus*. However, extracts from neem are frequently applied due to their efficacy and ready availability.

Neem (*Azadirachta indica*) is a tree in the mahogany family, Meliaceae, which mostly grows in the tropical and semi-tropical regions (Ojebode *et al.*, 2016). Extracts of neem; aqueous, powder and oil possess insecticidal properties that are effective against *C. maculatus* (Ekoja *et al.*, 2020). Among these extracts, neem oil is reported to be the most potent (Waghmare *et al.*, 2007). The objective of this study was to assess the efficacy of neem oil on the control of *C. maculatus* and the quality of stored cowpea grains.

MATERIALS AND METHODS

The study was carried out in the Entomology laboratory of the Department of Crop Science, University of Ghana under ambient conditions: temperature 25 - 32 °C, a relative humidity of 65-70%, and a photoperiod of 12-hour light: 12-hour darkness. The study was carried out over a month period (4th July to 5th August 2022).

The stock of adult *C. maculatus* (500) was obtained from the Entomology laboratory and used in raising new progenies of the same cohort. Adult unsexed *C. maculatus* (200) were introduced on 300 g of sterilised untreated cowpea grains, in two different 2 litre sterilised Kilner jars. Each Kilner jar was covered with a muslin cloth and held tight with a rubber band to prevent adult beetles from escaping (Fig. 1). The culture was left to stand on the shelf in the laboratory for 7 days.

The adult beetles were sieved out at 7 days after their introduction to the cowpea grains in the jar. The time frame of 7 days was to allow the adult beetles to lay enough eggs. The culture was then kept back on the shelf in the laboratory for adult emergence. After 21 days, newly emerged adult beetles were used to set up the main experiments.

A cold-pressed neem oil, containing 1% azadirachtin, was obtained from Green-Gro Limited, Accra, Ghana; and applied at three

different rates, namely 0.50, 0.75 and 1.00 ml to 50 g of maize grains. These rates were adopted and modified from the study conducted by Ojebode *et al.* (2016). ACTELLIC Gold Dust® (16 g kg⁻¹ Pirimiphos methyl + 3.6 g kg⁻¹ Thiamethoxam) at 0.05 g was also included as a positive control; while no treatment added acted as a negative control.

Untreated cowpea grains, sterilised with a hot air oven, weighing 50 g, were treated with the different rates of neem oil, using a pipette. The grains were mixed thoroughly to ensure even coverage with the treatments. Likewise, the grains treated with actellic dust were admixed to ensure an even coverage of the treatment. The treated grains were kept in sterilised 0.3 L glass jars (11.00 x 11.51 x 12.29 cm), and left to stand for 1 hour to dry, to prevent the wet seeds from soaking adult beetles when introduced.

After treating the grains, 2-day-old, unsexed adult *C. maculatus* (20 in number)



Figure 1. Culture of *Callosobruchus maculatus* on cowpea grains.

were introduced into each experimental unit. The experimental units were covered with muslin cloth and held tight by a rubber band. Each treatment was replicated 4 times in a Completely Randomised Design (CRD). The setups were kept on benches in the entomology laboratory for 30 days under the conditions stated above.

The data collected included adult mortality, oviposition of adults, adult emergence and grain damage. Adult mortality was assessed every 24 hours after setting up the experiment, for 7 days, by sieving out the adult beetles and counting dead ones among the 20 unsexed adults initially introduced. The dead insects were identified when they did not respond to probing by a camel hairbrush, on their abdomen (Gariba *et al.*, 2021). Surviving adults were reintroduced into their respective experimental units. Adult mortality was monitored and assessed till the treated cowpea grains induced mortality on the introduced adult beetles.

Percentage mortality was corrected using Abbott’s formula (Abbott, 1925) when control mortality was greater than 5% and less than 20%. Percentage mortality was calculated using the formula adopted by Gever and Echezona (2023):

$$\text{Mortality (\%)} = \frac{\text{Number of dead adults}}{\text{Total number of adults}} \times 100$$

..... Equation 1

Oviposition was assessed by determining the number of grains with eggs laid on them by *C. maculatus* adult females. This was done after assessing adult mortality, because it is reported that adult females begin to lay eggs from 2 days up to 7 days after their introduction to stored grains (Deshwal *et al.*, 2020). This time frame was to allow the adult beetles to lay enough eggs. The number of grains with eggs was assessed by randomly selecting 20 grains and counting the grains with eggs, with the aid of a hand lens. The grains were kept back in their respective experimental

units on the shelf in the laboratory, for adult emergence.

After 23 days, the F1 progeny began emerging and newly emerged adult beetles were sieved out and counted every 24 hours, until there was no further emergence. The newly emerged adults were removed from the experimental units to prevent double counting.

Damage to the cowpea grains was estimated using the exit holes produced by the newly emerged adult beetles (F1 progeny), as an indicator of damage (Oluwafemi, 2012). Damage of the grains was estimated using the method adopted by Gever and Echezona (2023). Maize grains (20) were selected at random, and grains with exit holes were sorted and counted. Percentage grain damage was obtained by adopting the formula used by Kemabonta *et al.* (2010):

$$\text{Damage (\%)} = \frac{\text{Number of grains with exit holes}}{\text{Number of grains sampled}} \times 100$$

..... Equation 2

All data collected were transformed prior subjecting them to the analysis of variance (ANOVA) test. Data on percentage adult mortality and percentage grain damage were arsine and square root transformed, respectively; while data on oviposition and adult emergence were transformed using the log transformation. R version 4.3.3 was used to perform the ANOVA test, using the agricolae package. Tukey’s Honestly Significant Difference (HSD) at a 5% significance level was used for means separation.

RESULTS

Cumulative mortality. Neem oil application had a highly significant (P < 0.0000) effect on mortality of adult *C. maculatus* (Table 1). Also, the days following the introduction of adult *C. maculatus* to the treated cowpea grains had a highly significant (P < 0.0000) effect on the cumulative mortality of the adult beetle.

TABLE 1. Effect of the treatment on the percentage cumulative mortality of adult *C. maculatus*

Treatments	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
0.50 ml neem oil	68.00fg	76.81ef	76.81de	92.50bcd	96.25abc	96.25abc	96.25abc
0.75 ml neem oil	88.00cd	95.66abc	98.46a	100.00a	100.00a	100.00a	100.00a
1.0 ml neem oil	96.00abc	98.55a	98.55a	98.75a	100.00a	100.00a	100.00a
0.05 g actellic dust	77.33ef	94.20bcd	94.20ab	98.75a	100.00a	100.00a	100.00a
Control	6.25l	13.75kl	13.75jk	28.75a	40.00hi	47.50h	55.00gh

Values with the same alphabet in the columns are not significantly different from each other at a 5% significance level

Furthermore, the adult *C. maculatus* mortality varied greatly ($P < 0.0000$) among the treated cowpea grains at different days following their introduction to the treated grains.

There were no significant differences ($P > 0.05$) between cowpea grains treated with neem oil at 0.75 and 1.0 ml on the 1st day of assessing cowpea beetle mortality, but were significantly ($P < 0.05$) different from cowpea grains treated with 0.5 ml neem oil, 0.05 g actellic dust, and the control. On the 2nd day, cowpea grains treated with 0.75 and 1.0 ml neem oil, 0.05 g actellic dust did not differ significantly ($P > 0.05$) from each other, except between 0.50 ml neem oil and control, which was significant ($P < 0.05$). Moreover, cowpea grains treated with 0.50 ml neem oil did not vary significantly from control. On the 3rd day, there was no significant variation ($P > 0.05$) among the mortality values of adult *C. maculatus* recorded on cowpea grains treated with 0.75 and 1.0 ml neem oil, 0.05 g actellic dust, but differed significantly ($P < 0.01$) from cowpea treated with 0.50 ml neem oil and control. On the 4th day, there was no significant variation ($P > 0.05$) among the cumulative mortality of cowpea grains treated with 0.75 and 1.0 ml neem oil, 0.05 g actellic dust and control; but differed significantly ($P < 0.01$) from cowpea grains treated with 0.50 ml neem oil. On the 5 - 7th days after initiation of the study, there were no significant differences in the mortality of adult *C. maculatus* among cowpea grains treated with the various rates

of neem oil, and 0.05 g actellic dust but differed significantly ($P < 0.05$) from the control.

Eggs laid by adult beetles. The treatments had a significant effect on the number of grains with eggs laid by *C. maculatus*. The mean number of cowpea grains with eggs of *C. maculatus* varied significantly ($F_{(4,15)} = 129.00$, $P = 0.0000$) among the treatments. Following the multiple mean comparison test, no significant difference was found in the mean number of grains with adult *C. maculatus* eggs among cowpea grains treated with 0.50 ml neem oil and 0.05 g actellic dust, respectively. However, the mean number of grains with adult *C. maculatus* eggs recorded on cowpea grains treated with 0.50 ml neem oil and actellic dust, respectively differed significantly ($P < 0.05$) from the mean number of grains with adult *C. maculatus* eggs recorded on cowpea grains treated with 0.75- and 1.0-ml neem oil, as well as control, respectively. The mean number of grains with adult *C. maculatus* eggs recorded in cowpea grains treated with 0.75 ml neem oil was significantly ($P < 0.05$) different from all the other treatments. Furthermore, the mean number of grains with adult *C. maculatus* eggs recorded in cowpea grains treated with 1.0 ml neem oil was significantly ($P < 0.05$) different from all the other treatments. Likewise, the mean number of grains with adult *C. maculatus* eggs recorded in control was significantly ($P < 0.05$) different from all the other treatments. The

highest oviposition occurred on control, followed by neem oil at 0.50 ml and actellic dust at 0.05 g, neem oil at 0.75 ml, and the least being neem oil at 1.0 ml (Fig. 2).

Adult emergence. Neem oil treatments had a highly significant effect ($P < 0.0000$) on the total number of emerged adults. The number of F_1 adults from the grains treated with 0.50, 0.75 and 1.0 ml of neem oil, did not differ significantly ($P > 0.05$) from each other; but was significantly different ($P < 0.05$) from the overall mean number of adults that emerged from cowpea grains, treated with 0.05 g actellic dust and control. The highest number of adults emerged from the control, followed by application of 0.05 g actellic dust, and the least was application of neem oil at 0.50 - 1.0 ml (Fig. 3).

Cowpea grains damaged. The treatments had a highly significant effect ($P < 0.0000$) on the percentage of cowpeas damaged in store. Damage of cowpea grains treated with 0.50,

0.75, and 1.0 ml neem oils did not differ significantly ($P > 0.05$) from each other; but was significantly different ($P < 0.05$) from the damage recorded in the 0.05 g actellic dust and control, respectively. The highest grain damage occurred on control, followed by 0.05 g actellic dust, with the grain treated with neem oil 0.50-1.0 ml recording (Fig. 4).

DISCUSSION

Adult mortality. The considerable effectiveness of neem oil and actellic dust on mortality of adult *C. maculatus* found on cowpea grains in store (Table 1), could be attributed to their insecticidal properties reported earlier (Rumbos *et al.*, 2013; Sakka and Athanassiou, 2021; Gever and Echezona, 2023). Neem oil is reportedly made up of many active ingredients; along with azadirachtin, which is toxic to many insects and induces mortality (Ekeh *et al.*, 2013; Sokame *et al.*, 2015).

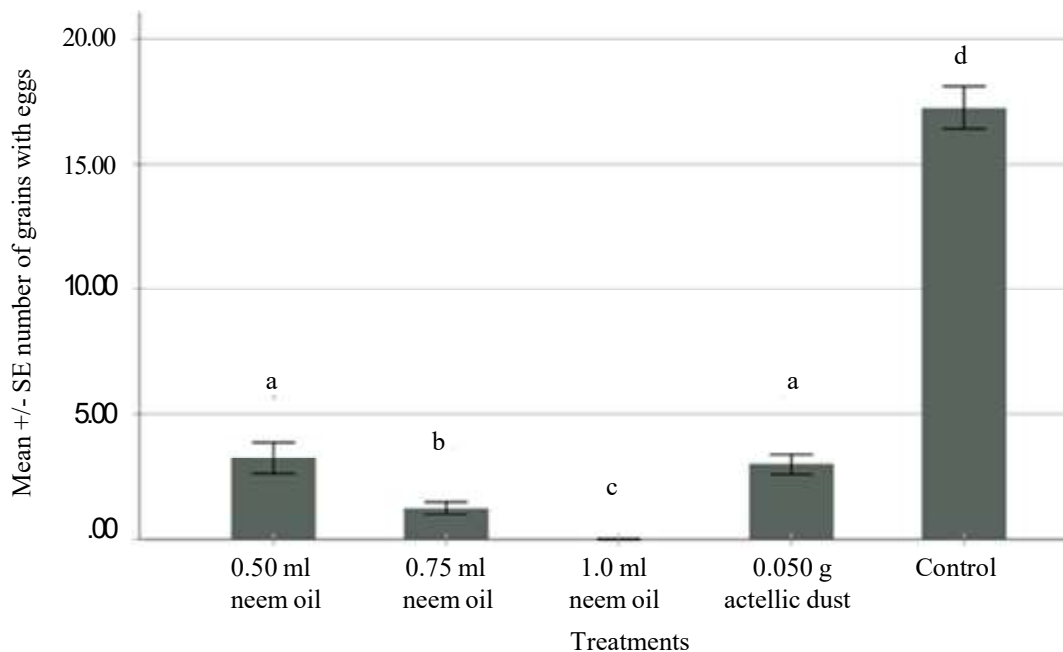


Figure 2. Effect of neem oil application on the number of grains with *Callosobruchus maculatus* eggs.

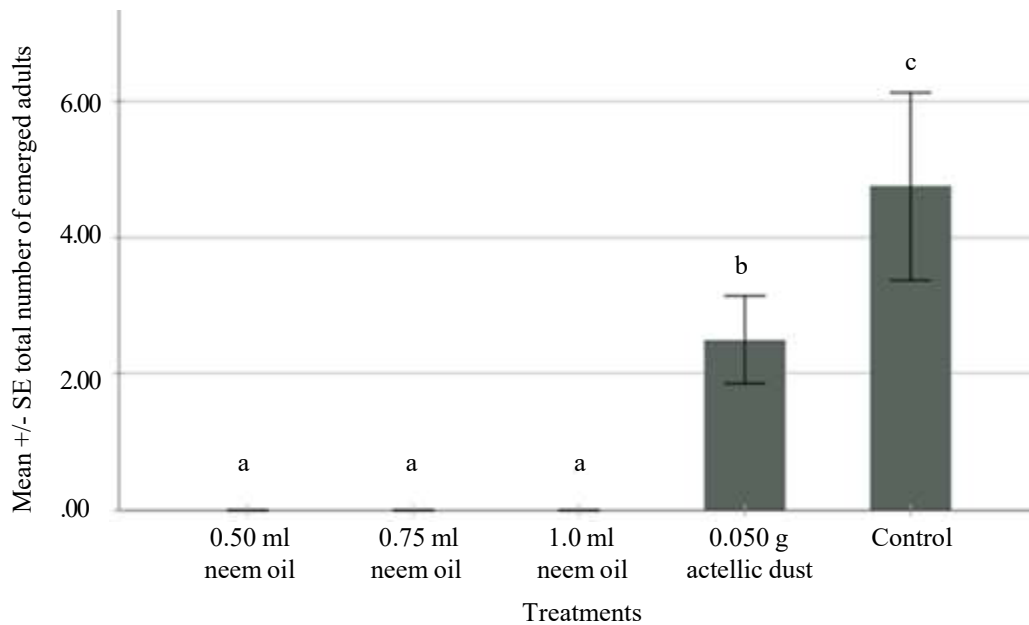


Figure 3. Effect of neem oil application on the total number of *Callosobruchus maculatus* F₁ adults feeding on cowpea grains in store.

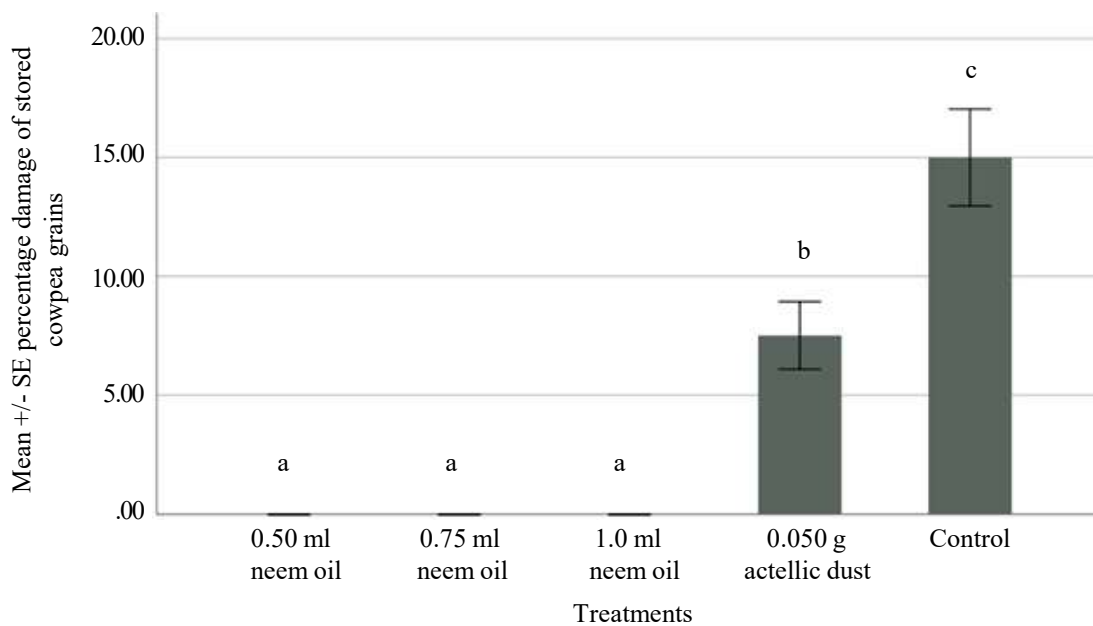


Figure 4. Effect of neem oil application on damage of cowpea grains in store, by *Callosobruchus maculatus*.

Likewise, actellic dust consists of pirimiphos-methyl; which is toxic and inhibits acetylcholinesterase in insects, causing mortality and thiamethoxam which interferes with the nicotinic acetylcholine receptor and therefore have specific activity against the insect nervous system (Pope, 1999; Cabello *et al.*, 2001). Significant differences were found in the mortality patterns of adult *C. maculatus* over time, from day 1 to day 7; following their introduction to the treated cowpea grains. These findings corroborate with those of several other studies (Boeke *et al.*, 2004; Mbaiguinam *et al.*, 2006; Ojebode *et al.*, 2016), in which was reported that neem oil applied on cowpea grains decimated virtually all the adult *C. maculatus*. Therefore, neem oil applied to cowpea grains in store at the rate of 0.50 ml per 50 g of cowpea appears to be the threshold for controlling adult *C. maculatus* in store, at the conditions (temperature, relative humidity, etc.) prevalent in the laboratory. It is imperative that a similar study is done in the field to ascertain the effectiveness of the materials under real farm conditions, before compelling recommendations are advanced to end users.

Oviposition of adults. The significant effectiveness of neem oil on the number of cowpea grains with eggs of adult *C. maculatus* (Fig. 2), is suggestive that the oviposition stage could be a strategic point at which to control *C. maculatus* using neem oil. The mean number of cowpea grains with eggs of adult *C. maculatus* was zero in the cowpea grains treated with 1.0 ml neem oil; which differed significantly from all the other treatments. This suggests that cowpea grains treated with 1.0 ml neem oil outperformed all the other treatments. These results connote that 1.0 ml neem oil applied to 50 g of dry cowpea grains effectively prevent oviposition of *C. maculatus* adult females on cowpea grains in storage facilities.

These findings conform to those of Boeke *et al.* (2004) and Ekoja *et al.* (2020), who

reported that cowpea grains treated with neem oil effectively reduced oviposition by *C. maculatus*. Nevertheless, our findings contradict reports made by Ivbijaro (1990). Ivbijaro (1990) reported that there were no significant differences between oviposition of 200 g of cowpea grains treated with neem oil at 2 and 3 mg kg⁻¹ rate of application. The variations in the findings of this study and previous reports could be attributed to the differences in the neem concentrations used in both studies. Further investigations may be necessary to confirm the cause-effect relationship in this particular case.

Adult emergence. The total absence of emergence of adult *C. maculatus* in all the neem oil treated cowpea grains in store, could be attributed to the insecticidal properties of Azadirachtin present in neem oil (Brahmachari, 2004; Campos *et al.*, 2016; Gever and Echezona. 2023). Azadirachtin in neem oil is the most active triterpenoid, which modifies growth by inhibiting the release of prothoracic hormones (ecdysone) (Schmutter, 1990; Khattak and Rashid, 2006). Similarly, azadirachtin in neem oil aids in the manufacture of ecdysteroids, which hinders insect growth and development (moulting and metamorphosis) (Sarwar, 2020); thereby resulting in no emerged adults. Therefore, the adult emergence results in the present study could be presumed as confirmatory test for neem oil as an efficacious organic pesticide for controlling *C. maculatus*. This could be adopted to contribute to resolution of the risks caused by the prevalence use of synthetic pesticides, which are increasingly blamed for many livelihood disorders reported globally (Kim *et al.*, 2017).

Adult damage. The lack of differences in damage in cowpea grains treated with different concentrations of neem oil, suggests that concentrations of neem oil used were generally effective in reducing damages caused by *C. maculatus*. It can be presumed that it induced

antifeedancy by activating deterrent cells in chemoreceptors (Koul, 2008; Bezzar-Bendjazia *et al.*, 2017), thereby resulting in no damaged cowpea grain.

Neem oil causes anomalies in eggs, larvae and adults (Mitcheli *et al.*, 2004); which reduces the emergence of new progenies, eventually affecting the damage caused by *C. maculatus* on stored grains. Indeed, the mean quantity of cowpeas damaged by *C. maculatus* was significantly less in the cowpea grains treated with different concentrations of neem oil than in cowpea grains treated with actellic dust and control, respectively. This is in line with earlier studies, where it was reported that the application of neem oil on cowpea grains significantly reduced the number of grains damaged by *C. maculatus*; compared to untreated cowpea grains (Ekoja *et al.*, 2020; Gever and Echezona, 2023).

This finding suggests that the different concentrations of neem oil, namely 0.50, 0.75 and 1.0 ml used in this study outperformed the actellic dust rate and the control in effectively controlling the damages caused by *C. maculatus* on cowpea grains. The actellic dust-treated grains suffered some damage probably due to reduced efficacy and possibly the development of insecticide resistance by *C. maculatus* to actellic (Pirimiphos methyl), although this was not verified in the present study. It has, however, been established in an earlier study (Odeyemi *et al.* (2006). Moreover, a study conducted by Zongo *et al.*, (2021) proved that resistance was detected in several strains of *C. maculatus* in Burkina Faso.

Our results suggest that any of the three concentrations of the neem oils could be applied to effectively control the damages caused by *C. maculatus* on cowpea grains in storage facilities. However, the level of infestation could be taken into consideration to select the most cost-effective rate of application, ranging between 0.50-1.00 ml for 50 g of dry cowpea grains.

CONCLUSION

This study has demonstrated that neem oil is a very efficacious organo-pesticide applicable to control *C. maculatus* under storage conditions. Neem oil is effective in intervening in virtually all stages in the life cycle of the beetle (oviposition and adult emergence) The threshold concentration for effecting the control at the various points in the life cycle of the beetle is 0.50 ml neem oil treated on 50 g of cowpea grains. However, further studies are necessary to ascertain the most cost-effective concentration for achieving convincing results under actual field conditions.

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