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Evaluation of the Potency of *Azadirachta indica* (Neem) Leaf and Fruit Powders as Natural Alternatives to Synthetic Insecticides in Controlling *Callosobruchus maculatus* (Fabricius)

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Original Research Article

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Abstract

Background: The cowpea weevil (*Callosobruchus maculatus*) poses a serious threat as a significant post-harvest pest of stored cowpeas, causing quantitative and qualitative losses in sub-Saharan African countries. Control of these insects has relied on synthetic insecticides, which are costly, harmful to human health, and unsustainable due to the development of insecticide resistance. While there is increasing attention on using plant-based alternatives, few empirical studies have investigated the efficacy of *Azadirachta indica* (neem) fruit and leaf powder against this pest especially in the Upper East Region of Ghana.
Aim: This study aimed to evaluate the insecticidal efficacy of neem fruit and leaf powder against *C. maculatus*.
Methodology: Cowpea seed infested with adult weevils was treated with neem fruit and leaf powder (15 g, 25 g and 45 g each). Mortality rate and seed damage were determined at intervals of seven days for four weeks in a storage experiment.
Results: The findings showed that both neem leaf and fruit powder caused significant ($p < 0.05$) increase in the mortality of cowpea weevils and significantly ($p < 0.05$) reduced the number of perforated seeds in the treated samples. Both the 25 g and 45 g treatments showed greater effectiveness than 15g. The 45 g neem leaf powder had an average mortality of 8.0 ± 2.8, while the fruit powder attained a mean mortality of 14.50 ± 2.70.
Conclusion: While a high concentration produced better results, there was no significant difference ($p > 0.05$) between the effectiveness of 25g and 45g. This shows that the optimum dosage required for effective control of cowpea weevils is 25g.

Keywords: Callosobruchus maculatus (Cowpea weevil); Azadirachta indica (Neem); cowpea storage; biopesticides; post-harvest losses.

1. Introduction

Vigna unguiculata (Cowpea) is widely grown in tropical climates and especially prominent in the savanna areas of West Africa because it is an important source of food and cash crops (Amadi *et al*., 2018). The crop is valuable because it supplies an excellent source of plant protein to humans and animals without much antinutritional properties (Jehajo *et al*., 2022; Nana *et al*., 2014).

However, the productivity and preservation of cowpea seeds are hampered by insect pests resulting in great losses through reduction of seed weight, quality, and market value (Goudoungou *et al*., 2022). Pest management strategies that can be used include physical, biological, mechanical, chemical, host plant resistance, and botanical extract applications.

Callosobruchus maculatus (Cowpea Weevil) is the most destructive storage pest for cowpea, which may lead to complete loss of grain after only six months of unprotected storage (Nboyine *et al*., 2024; Bawa *et al*., 2025). The major damage consists of direct consumption of the seeds resulting in substantial weight reduction, reduced germination rate, poor nutritional value, and contamination of the grain with insect excrements and debris (Nboyine *et al*., 2024; Ekeh et al., 2013; Hakeem et al., 2017; Tlale et al., 2025).

Although synthetic pesticides have been widely used for *Callosobruchus maculatus* (cowpea weevil) control in storage, overreliance on them has several drawbacks such as increased pest resistance, adverse impacts on consumer health, pollution, and toxicity to non-target organisms (Adarkwah *et al*., 2010). In addition, synthetic pesticides are expensive, technically challenging, and difficult to access for the majority of smallholder farmers.

These challenges underscore the need for safer, affordable, and environmentally sustainable alternatives. Botanical pesticides have long been used by farmers around the world to control stored product pests before the use of synthetic insecticides (Ahmad *et al*., 2015). Plant-based pesticides are increasingly favored because they

are relatively less toxic, degradable, affordable, and accessible (Nkechi *et al*., 2013). There has been renewed global interest in the exploration of plant-based derivatives to manage insect pests.
Azadirachta indica (neem), have shown considerable promise in pest control (Adu-Acheampong *et al*. 2024; Abiola & Boluwatife, 2025). Although various neem-based products, such as leaves, seeds, and oils, have been studied in other parts of the country (Adu-Acheampong *et al*. 2024), there is limited information on the use of neem fruit and leaf powder, especially in the Upper East Region of Ghana.

Therefore, this study sought to address this research gap by evaluating the efficacy of neem fruit and leaf powder against *C. maculatus* on stored cowpea in Navrongo.

2. Materials and Methods

2.1 Study Site

The study was conducted at the University of Technology and Applied Sciences, Navrongo, in the Kassena-Nankana Municipal of the Upper East Region, Ghana (Fig. 1).

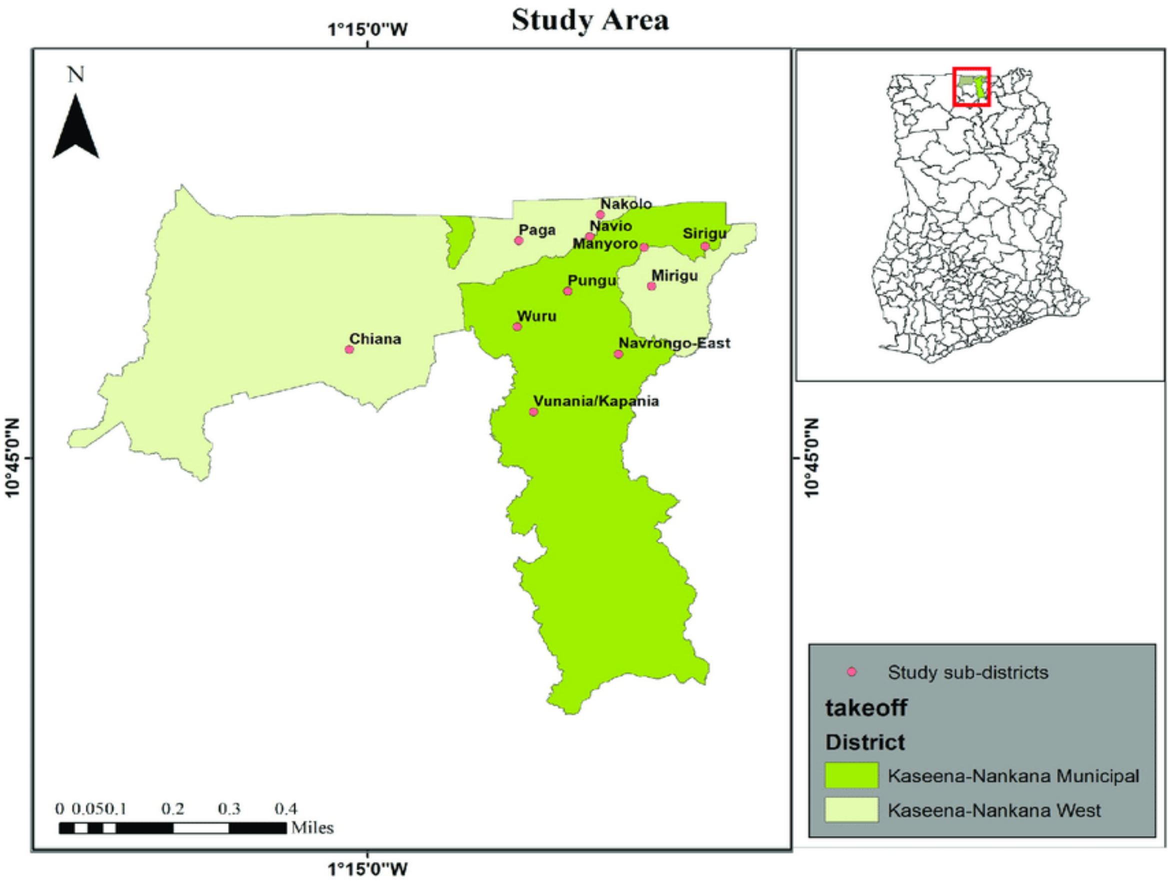

Fig. 1. Map of Kassena - Nankana Municipality

2.2 Sample Collection and Preparation

2.2.1 Cowpea Collection

Healthy non-infested cowpea (*Vigna unguiculata)* was obtained from the Navrongo local market and was transported to the Entomology laboratory of the Department of Applied Biology, University of Technology and Applied Sciences. It was thoroughly hand-picked to remove foreign materials and damaged seeds. The healthy seeds were sun-dried for 6 days and stirred several times a day to ensure proper drying and reduce microbial load. The seeds were then transferred to an airtight plastic container and kept at room temperature for further use.

2.2.2 Laboratory Rearing of *Callosobruchus maculatus*

Initial populations of *C. maculatus* were obtained from naturally infested Black-eye cowpea seeds purchased from the Navrongo local market. This stock was transported to the Entomology Laboratory at the University of Technology and Applied Sciences, where it was maintained as a starter culture. This setup allowed for the emergence of a new generation of adults. To ensure a homogenous cohort for the bioassays, newly emerged adults from this laboratory-reared population were selected for the experiment.

2.2.3 Plant Samples Collection and Preparation

Fruits and leaves of *A. indica* were collected from the Navrongo Senior High School campus, after the plant was identified and authenticated by a taxonomist. The plant materials were shade-dried for 14 days and subsequently pulverized using a mortar and pestle. The resulting material was sieved through a 1.50 mm mesh to obtain a uniform particle size. The processed powder from the fruits and leaves was stored in airtight containers at ambient temperature under dark conditions until further use.

2.3 Treatment of Cowpea with Plant Samples

Uninfected healthy cowpea seeds (n= 200 seeds) were transferred to three sets of dried and sterile plastic containers. Each set of seeds was treated with neem fruit powder of varying masses (15, 25 and 45 g), respectively, and gently rocked to ensure that the seeds were thoroughly coated with the powder, followed by the introduction of adult weevils (n=15) to each set. Each container was then sealed with a muslin cloth and tied with a rubber band to allow proper ventilation and prevent the insects from escaping. The procedure was repeated for the neem leaf powder. A control experiment consisted of the same number of cowpea seeds and adult weevils but without plant samples. Each experiment had three replications.

Mortality data were recorded based on the number of dead weevils as those that failed to show signs of motion upon stimulation of either their legs or antennae. Data were taken from both treated and control samples. Testing was done in daytime when the weevils were actively moving due to high temperatures and humidity. Data gathering was done after every seven days starting from the day of treatment until the fourth week after treatment. Furthermore, seed damage was measured based on the number of seeds showing holes. Only those holes that could be easily distinguished from the natural characteristics of the seed and germination pores were considered damage to the seeds. Seeds without any observable holes were considered non-damaged. During data gathering, dead weevils and damaged seeds were removed from the test samples to avoid double-counting.

2.4 Statistical Analysis

The data were analyzed using SPSS (V:22.0). The data were expressed in mean ± SD and were analyzed using ANOVA, while the treatment means were compared using LSD at 5% probability level.

3. Results

3.1 Effect of Neem Samples on Mortality (%) of Cowpea Weevil

The effects of neem fruit powder and neem leaf powder on cowpea weevil mortality at different doses (15 g, 25 g, 45 g) and exposure times (7, 14, 21, and 28 days) are presented in Table 1.

For neem fruit powder, on day 7 after the treatment, mortality was still insignificant in the control (0.00 ± 0.00), and mortality in the treatments was higher but not consistent. Mortality in the treatments of 25 g and 45 g were significantly higher ($p < 0.05$) than the control, and mortality in the treatment of 15 g was somewhat between the control and the higher dosages.

After 14 days of treatment, the mortality levels of all the neem fruit treatments were significantly higher than the control, which shows progression of toxicity over time. While mortality in the treatments was relatively higher, there was no statistical difference among them, which suggests a possible limit in efficacy past 15 g at this point. On the 21st and 28th days, mortality continued to increase in all the neem fruit treatments but remained significantly higher than in the control. While the maximum mortality levels were registered in 25 g and 45 g, there was no statistical difference between them, implying that increasing the dose past 25 g had no corresponding mortality levels.

Table 1. Effect of Neem samples on Mortality (%) of Cowpea Weevil

Sample	Dose (g)	Days of Treatment (Mean ± Standard Deviation)			
		7	14	21	28
Neem fruit powder	Control	0.00±0.00^{a}	1.00±0.00^{a}	0.50±0.00^{a}	1.00±0.00^{a}
	15	3.25±2.99^{ab}	8.00±2.99^{ab}	5.50 ± 2.98^{ab}	10.33±2.98^{ab}
	25	6.00±3.27^{b}	10.25±3.26^{b}	10.00±3.26^{b}	13.15±3.27^{b}
	45	8.25±2.71^{b}	13.00±2.70^{b}	13.66±2.71^{b}	14.50±2.70^{b}
Neem Leaf Powder	Control	0.00±0.00^{a}	1.00±0.00^{a}	0.50±0.00^{a}	1.00±0.00^{a}
	15	3.35±2.99^{ab}	4.00±1.80^{b}	7.50±5.30^{b}	8.50±5.30^{b}
	25	5.00±3.27^{b}	5.50±0.70^{b}	8.00±3.26^{b}	8.01±1.90^{b}
	45	5.80±0.70^{b}	7.75±2.10^{b}	8.50±1.60^{b}	8.0±2.80^{b}

For neem leaf powder, mortality increased with both increasing dosage and duration of exposure. At 7 days after treatment, all neem leaf-treated groups recorded significantly higher mortality compared to the control. The highest initial mortality was observed at 45 g (8.25 ± 2.71), followed by 25 g (6.00 ± 3.27), while the lowest dose (15 g) recorded 3.25 ± 2.99. In contrast, no mortality was recorded in the control at this stage (0.00 ± 0.00). Statistical analysis indicates that the treated groups (particularly 25 g and 45 g) differed significantly from the control. By day 14, mortality increased markedly across all treatments. The 45 g dosage recorded the highest mortality (13.00 ± 2.70), followed by 25 g (10.25 ± 3.26) and 15 g (8.00 ± 2.99). The control remained significantly lower (1.00 ± 0.00). This trend of increasing mortality with dosage and time continued through days 21 and 28.

At 21 days, the 45 g and 25 g neem leaf treatments maintained high mortality levels (13.66 ± 2.71 and 10.00 ± 3.26, respectively), while the 15 g treatment showed a slight decline (5.50 ± 2.98), suggesting possible variability or reduced residual efficacy at the lowest dose. The control remained negligible (0.50 ± 0.00). By 28 days, maximum mortality was observed, particularly at 45 g (14.50 ± 2.70), followed by 25 g (13.15 ± 3.27) and 15 g (10.33 ± 2.98). The control still showed minimal mortality (1.00 ± 0.00). Statistical analysis indicates that the 25 g and 45 g treatments consistently belonged to the same significance group (b), showing no significant difference between them, but both were significantly higher than the control (a).

Overall, the results indicated that neem fruit powder had more bioactivity on cowpea weevil compared to neem leaf powder.

3.2 Effect of Neem Samples on Cowpea Seed Damage by Cowpea Weevil

The effect of different treatment concentrations of neem fruit and leaf powder on seed damage by adult *C. maculatus* is presented in Table 2. The treatments showed a lower mean number of damaged seeds as compared to the controls.

Table 2. Effect of neem samples on cowpea seed damage by cowpea weevil

Sample	Dose (g)	Days of Treatment (Means ± S.D)			
		7	14	21	28
Neem Fruit Powder	Control	5.45±7.89^{a}	12.00±7.89^{a}	21.44±7.90^{a}	35.24±7.89^{a}
	15	4.54±5.17^{ab}	4.50±5.16^{ab}	5.90±5.17^{ab}	5.00±5.17^{ab}
	25	3.22±1.71^{b}	3.05±1.70^{b}	3.05±1.71^{b}	2.25±1.71^{b}
	45	2.25±0.96^{b}	2.25±0.96^{b}	2.10±0.95^{b}	2.06±0.96^{b}
N ee m Le	Control	5.45±7.89^{a}	12.0±7.89^{a}	21.0±7.90^{a}	35.24±7.89^{b}

15	12.50±1.76^{b}	14.0±4.51^{b}	16.5±1.76^{b}	18.5±1.76^{b}
25	10.00±2.08^{b}	10.0±3.33^{b}	10.0±2.08^{b}	11.0±2.08^{b}
45	8.5±3.02^{b}	9.00±5.00^{b}	9.5±3.02^{b}	10.5±3.02^{b}

The result of the neem fruit treatments indicated that 25g and 45g had the lowest number of damaged seeds compared to 15g. There was no significant difference in the number of damaged seeds between 25g and 45g after a storage duration of 28 days.

For the neem leaf powder, the control had the least seed damage at day 7 after treatment (6.45 ± 3.89), but all the treated seeds already had significantly higher seed damage protection compared to the control. Amongst the treatments, the treatment that recorded the least seed damage was the 45g treatment, followed by the 25g and 15g treatments. There were no significant differences among the treatments ($p > 0.05$). On days 21 and 28, the differences became increasingly evident. The control had an increasing amount of seed damage, with values of 21.0±7.90 and 35.24±7.89, respectively. On the other hand, the treatments had only marginal increases in their seed damage values. The 25g and 45g treatments had the least seed damage values, which were significantly similar, whereas the 15g treatment had the highest damage rates despite still being effective.

4. Discussion

The current study examined the effectiveness of fruit and leaf powders of the neem (*A. indica*) tree against the Cowpea weevil (*C. maculatus*) that causes post-harvest loss to cowpeas. It was established that neem fruit and leaf powders were effective in suppressing the number of weevils and protecting cowpea seeds from damage, but their effectiveness depended on the applied doses and period of exposure.

It was observed that neem fruit powder was more effective compared to the neem leaf powder in killing *C. maculatus*. These results agree with findings from current literature review. According to Adu-Acheampong *et al*. (2024) study about the effect of botanical extracts against the *C. maculatus* in stored cowpea, neem had different formulations that varied greatly in effectiveness.

Moreover, Perzada *et al*. (2025) observed that botanical powders of *Azadirachta indica* achieved 100% mortality of the target pest (*C. maculatus*) in a laboratory setting within seven days. It was also noted that samples treated with botanical powder suffered less loss of weight compared to the control samples. Despite the high effectiveness of neem formulations observed in these studies, the current findings reveal less efficiency. In particular, the maximum mortality rate recorded in the current experiment was 14.50 (at 28 days post-treatment) due to fruit powder. Differences in formulation (powder vs. extract), application rates, and experimental settings could have resulted in such a variation.

Dosage dependency was evident among both fruit and leaf powders because higher application rates (25g and 45g) were associated with higher percentages of weevil mortality as compared to 15g dose. Nevertheless, there were no statistically significant differences between 25g and 45g powders over most time points. It implies the plateau in the effectivity of the tested powders after a certain point.

Accordingly, there is evidence from the literature concerning this phenomenon. According to Akbar *et al*. (2024) comprehensive study involving effectiveness of five different plant extracts in controlling *C. maculatus*, the most effective formulation belonged to *A. indica*. The effectiveness of *A. indica* was defined as the lowest infestation (16.65%) and host seed weight loss (7.85%). It was also stated that repellency increased with dose and time (100.00% repellency was attained with 3% formulation after 48 hours). Critically, the authors reported that toxicity analysis indicated that efficacy plateaus beyond certain concentrations, supporting the present study's observation of diminishing returns at higher application rates (Akbar *et al*., 2024).

Temporal analysis of the weevils' mortality rate showed continuous progression of mortalities from day 7 to day 28 in all the neem-exposed groups, implying prolonged efficacy of the product. This result is in agreement with Adu-Acheampong *et al*. (2024), who observed that neem preparations remained active for a period of up to 6 weeks post-application with persistent inhibition of the weevil resurgence (Adu-Acheampong *et al*., 2024).

Another study investigated the use of neem and moringa seed oils as plant preservatives for cowpea. The researchers found that, depending on the variety, cowpea treated with these natural compounds retained efficacy against *C. maculatus* even during 360 days of storage (Ilesanmi *et al*., 2017).

Further, some studies have tested neem extracts along with other botanical extracts. For example, according to a study done in 2025, the oils extracted from balanite, black seed, and neem proved very effective in deterring oviposition by *C. maculatus*, and the oil from neem was just as effective as synthetic insecticides (Ubani & Muhammad, 2025). The bioactive components in these oils included tetradecane, oleic acid, and octadecanoic acid, which have insecticidal and repellent properties, and were identified through GC-MS (Ubani & Muhammad, 2025).

In another study, neem extracts, neemsol, and cypermethrin were compared for their effectiveness in controlling pests in cowpeas. The study revealed that neem-based treatments exhibited efficacy comparable to cypermethrin (synthetic insecticide) in pest management (Abiola & Boluwatife, 2025). More importantly, the cowpea plants treated with neem extracts showed maximum seed weight, weighing 79.72 grams, while those treated with cypermethrin weighed 74.03 grams (Abiola & Boluwatife, 2025).

The lack of statistical significance when comparing treatments of 25 g and 45 g implies that a smaller dose (of 25 g per experimental unit) might be enough for efficient seed protection. Economically speaking, the finding is quite valuable for impoverished farmers. Adu-Acheampong *et al*. (2024) advised using biopesticides as economically feasible and eco-friendly storage means for cowpea, especially those grown in high-production areas. Based on the effectiveness of the treatment lasting 28 days with no increase in damage after the fourteenth day, it can be said that a single use of neem powder can protect the seeds for a month.

Some inconsistencies exist between the current results and literature in recent times that need clarification. First, the mortality rates associated with neem leaf powder in this research seem to be relatively low in comparison with other studies. According to Perzada *et al*. (2025), a 100% mortality rate for *C. maculatus* was observed after one week using *A. indica* as an active substance. This inconsistency probably lies in the difference in formulations and other variables in the experiments, including the drying process. According to Adu-Acheampong *et al*. (2024), the amount of azadirachtin may vary depending on the way plant material is dried.

There are several factors responsible for the insecticidal properties of neem against *C. maculatus*, which have been further understood through recent research findings. Azadirachtin works as an ecdysone agonist, hindering molting and metamorphosis processes, along with serving as an antifeedant and oviposition inhibitor. A current research study conducted by Abkar *et al*. in 2024 proved that *A. indica* was highly toxic (LC_{50} = 1.09%, LC_{90} = 68.52%) when compared to other plant extracts after 96 hours of treatment duration, where repellent activity increased as per concentration and duration (Akbar *et al*., 2024).

There are several limitations of the current experiment which must be taken into account. For instance, recent findings showed that nanoformulation of neem products could increase their effectiveness as compared to powdered products (Ekwueme *et al*., 2025; Izuagie *et al*., 2025). Therefore, the application of nanoparticles, nanoemulsions, and neem-based bio-nanocomposites in storage pest management should be studied. Moreover, the use of neem together with other plant materials such as neem with moringa oil (Ilesanmi *et al*., 2017) and silver nanoparticles (Ekwueme *et al*., 2025) could be considered in further research.

5. Conclusion

In the current study, it is evident that neem fruit and leaves' powders are capable of controlling cowpea weevils and protecting cowpea seeds from any damages caused by the cowpea weevils. It was found that neem fruits' powders are more effective than leaves in terms of their insecticide properties. No statistical differences were observed between the two dosages (25 g and 45 g) used, which means that the lesser dosage might be more suitable in practice. In accordance with recent research, neem products have proved to be efficient and sustainable substitutes for chemical pesticides, with nanoformulation technology being introduced to further increase efficiency.

We recommend that the use of neem fruit powder should be encouraged as a cowpea protectant. It is recommended to use 25g of neem fruit powder as the dosage. Further studies need to be carried out to investigate the efficiency of the neem fruit powder over a period of time in the field conditions.

Disclaimer (Artificial Intelligence)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

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Competing Interests

Authors have declared that they have no known competing financial interests OR non-financial interests OR personal relationships that could have appeared to influence the work reported in this paper.

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