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Research Article

Exploring the Repellency Activity and the Effectiveness of Lower Concentrations of Certain Botanical Insecticides for the Control of *Callosobruchus maculatus* Fab

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ABSTRACT

The adoption of plant-derived insecticides will advance if they deliver optimal activity at minimal concentrations. The aim of this study was to assess the repellency and insecticidal efficacy of lower concentrations of azadirachtin, myristicin, and α-humulene based insecticides against *Callosobruchus maculatus* (Bean beetle). Insect mortality and repellency tests were determined using standard procedures. Notably, optimal repellency was observed at the 6th hour of insect exposure to 100 µg/mL of each botanical insecticides. Furthermore, complete insect mortality (100.00 ± 0.00%) was achieved at the 96th hour following exposure to significantly low concentrations: 0.0015 µg/mL (azadirachtin), 0.0061 µg/mL (myristicin), and 0.0122 µg/mL (α-humulene). LC_{50} values determined for the insecticides were 0.1 µg/mL for azadirachtin, 2.8 µg/mL for α-humulene, and 4.4 µg/mL for myristicin. These findings demonstrate that even at low concentrations, botanical insecticides can effectively manage *C. maculatus*, albeit over a prolonged exposure period, with azadirachtin emerging as the most potent among the tested compounds.

Keywords: *Azadirachtin, Myristicin, α-Humulene, Repellency, Insecticide*

INTRODUCTION

Callosobruchus maculatus is the most prominent insect pest of cowpea (*Vigna unguiculata*) in the tropics, causing significant postharvest losses (Togola *et al.*, 2020). This pest poses a serious threat not only to food and nutrition security, but also to the livelihoods of millions of people in West and Central Africa who depend on cowpea cultivation (Bolarinwa, 2022). Although, synthetic insecticides have been widely used and shown to be effective in controlling insect infestation in stored seeds (Leivas *et al.*, 2020), their continued use is increasingly discouraged due to issues related to residual toxicity and associated health and environmental risks (Joko *et al.*, 2020; Muhammed *et al.*, 2022). Therefore, there is a need for a substitute to these conventional chemicals with less or no detrimental effects.

Botanical insecticides have gained attention as safer alternatives due to their biodegradability, broad-spectrum insecticidal property, and lower toxicity to non-target organisms (Srinivasan *et al*., 2021). However, the assumption that plant-derived insecticides are inherently safe is misleading. Toxicity is often dose-dependent, and excessive concentrations, even of natural substances, can be harmful. Therefore, determining and applying the minimum effective concentrations while avoiding direct contact with the treated seeds is essential for safe and sustainable pest control.

Azadirachtin, a prominent compound found in the leaves, flowers, and seeds of *Azadirachta indica* (neem), is a complex tetranortriterpenoid limonoid with notable agro-medicinal properties (Chatterjee *et al*., 2023; Acharya *et al*., 2017). Another plant-based compound, myristicin (naturally occurring alkenylbenzene), is commonly found in nutmeg and mace (Ramirez-Alarcon *et al*., 2023), while α-humulene (also known as caryophyllene) is a biologically active monocyclic sesquiterpene extracted from shampoo ginger (*Zingiber zerumbet*) (de Lacerda Leite *et al*., 2021). These compounds have demonstrated sustained insecticidal activity against *C. maculatus* at concentrations of 0.0061 μg/mL (azadirachtin), 0.0122 μg/mL (myristicin), and 0.0244 μg/mL (α-humulene) (Ogbonnaya *et al*., 2024). Continued research into their minimum effective concentrations and safe application techniques remains crucial in advancing food safety and securing crop protection for the growing human global population.

MATERIALS AND METHODS

Plant-based active ingredients

Alpha-humulene (PHL83351-100MG); purity 96.0%, MW: 204.3511, Myristicin (09237-10MG-132 F); purity>97% MW: 192.21 and Azadirachitin A7430-5MG; purity>95% MW: 720.71 were obtained from Sigma Aldrich, USA.

Insect

Callosobruchus maculatus infested cowpea grains were obtained from the Storage Entomology Laboratory, Department of Crop Protection Faculty of Agriculture, Ahmadu Bello University Zaria Kaduna State.

Culturing of insect

Fifty (50) pairs (male and female) of parent stock of *C. maculatus* were introduced into a Kilner Jar containing 500 g of cowpea grains. The jar was covered with muslin cloth for seven (7) days at 27.5±2°C and relative humidity of 49%. Emergence of the First (F1) generation of *C. maculatus* was recorded after 44 days (Radha and Susheela, 2014).

Evaluation of repellency activity of botanical insecticides on *C. maculatus*

Filter papers were divided into two halves by drawing a straight line across the center with a marker and placed at the bottom of Petri dishes. One-half of each filter paper, labeled as the control (C), was treated with 0.1 mL of fatty acid methyl ester (used as the solvent carrier). The other half was treated with the test insecticide at graded concentrations of 100, 50, 25, 12.5, 6.25, 3.1, 1.5, 0.7, 0.3, and 0.1 μg/mL. The treated papers were allowed to air-dry at room temperature. After drying, twenty (20) pairs of adult *C. maculatus* were introduced into each petridish, which was then covered with a perforated lid to allow air exchange. The number of insects present on each half of the filter paper was recorded at 2-hour intervals over a 6-hour exposure period. This was performed in triplicates. Percentage repellency (PR) was calculated using equation 1 below:

$$PR = \frac{nc-nt}{nc+nt} \times 100 \qquad (1)$$

Where nc = number of insects on the control area and nt = number of insects on the treated area (Nerio *et al*., 2009)

Insect mortality test

Insect mortality tests were conducted by exposing adult *C. maculatus* to 1 mL of each botanical insecticide azadirachtin, α-humulene, and myristicin at concentrations of 0.1953, 0.0976, 0.0488, 0.0244, 0.0122, 0.0061, 0.0030, 0.0015, 0.0007, and 0.0003 μg/mL, following the procedure described by Ogbonnaya *et al*. (2024). Mortality was assessed at 2-hour intervals over a 96-hour exposure period. An insect was considered dead when it failed to respond to a gentle pin prick at the thorax region, indicating loss of muscular activity and absence of life, as described by Birah *et al*. (2008). Percentage mortality was calculated using equation 2, and the median lethal concentration (LC_{50}) at 96 hours was determined through probit regression analysis, as outlined by Finney (1971).

$$\%\ Adult\ mortality = \frac{No.of\ dead\ adults}{No.of\ adults\ introduced} \times 100 \qquad (2)$$

Statistical analysis

The data generated were analysed using SPSS (Version 23) and results were expressed as Mean ± Standard Deviation. Also, data were analysed using one-way Analysis of Variance (ANOVA) and differences in compared using Tukey's post hoc test. P-values less than or equal to 0.05 were considered statistically significant.

RESULTS

The repellency activity of azadirachtin-based insecticide against *C. maculatus* was evaluated at concentrations ranging from 0.1 to 100 μg/mL over a 6-hour exposure period, with observations recorded at 2-hour intervals (Figure 1). Results showed that optimal repellency was achieved at the 6th hour, with complete repellency (100.00 ± 0.00%) observed at the highest tested concentration of 100 μg/mL.

Figure 2 illustrates the repellency activity of α-humulene-based insecticide against *C. maculatus* across the same concentration range (0.1–100 μg/mL) and exposure duration (0-6 hours), with data recorded at 2-hour intervals. The optimal repellency activity of the α-humulene-based insecticide was progressively achieved by the 6th hour of *Callosobruchus maculatus* exposure. The highest repellency (100.00 ± 0.00%) was recorded at a concentration of 100 μg/mL

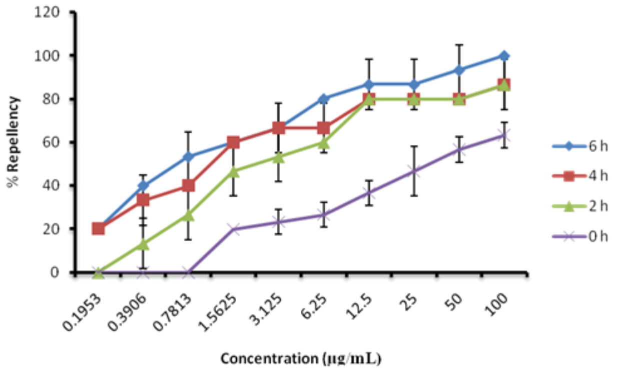

Figure 1: Repellency Activity of Azadirachtin-based Insecticide on *C. maculatus* (n=3)

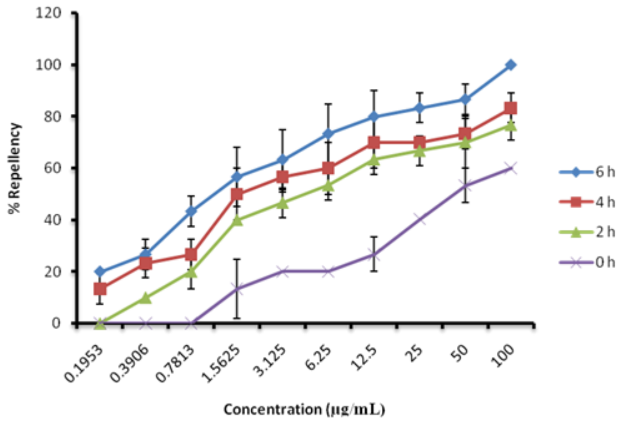

Figure 2: **Repellency Activity of alpha-Humulene-based Insecticide on** *C. maculatus* (n=3)

The repellency activity of the myristicin-based insecticide against *C. maculatus* across a concentration range of 0.1–100 µg/mL and exposure intervals of 2 hours over 6 hours is presented in Figure 3. The optimal repellency (100.00 ± 0.00%) was observed at 100 µg/mL by the 6th hour.

Figure 4 presents the percentage mortality of *C. maculatus* exposed to lower concentrations of azadirachtin-based insecticide. Notably, complete mortality (100.00 ± 0.00%) was observed at the 96th hour of exposure to 0.0015 µg/mL.

Similarly, Figure 5 shows that exposure to 0.0122 µg/mL of α-humulene-based insecticide resulted in 100.00 ± 0.00% mortality at the 96th hour.

As illustrated in Figure 6, the myristicin-based insecticide achieved full insect mortality (100.00 ± 0.00%) at a concentration of 0.0061 µg/mL after 96 hours of exposure. The lethal concentration 50 (LC_{50}) values derived from the 24-hour post-treatment mortality data are presented in Figure 7. Among the test insecticides, azadirachtin exhibited the highest potency with an LC_{50} of 0.1 ng/mL, followed by α-humulene (LC_{50} = 2.8 ng/mL) and myristicin (LC_{50} = 4.4 ng/mL).

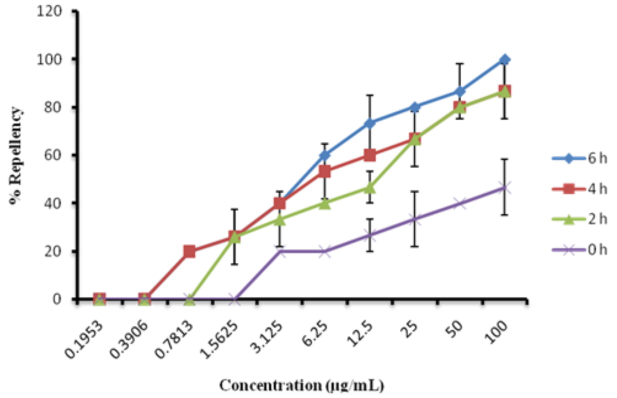

Figure 3: Repellency Activity of Myristicin-based Insecticide on *C. maculatus* (n=3)

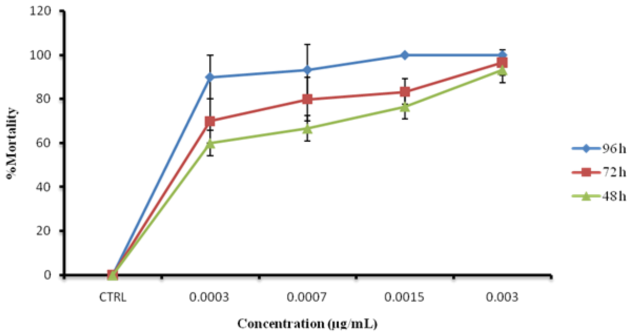

Figure 4: Percentage Mortality of *C. maculatus* Exposed to Lower Concentrations of Azadirachtin-based Insecticide (n=3)

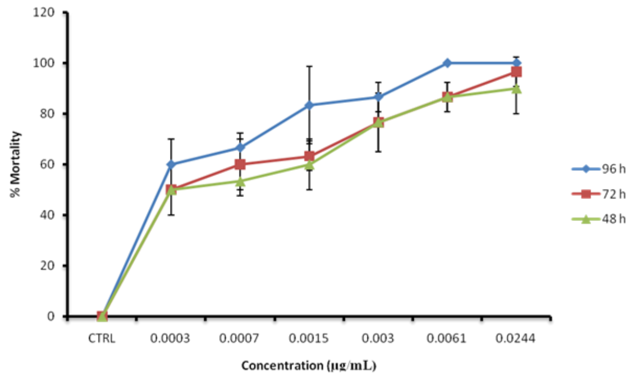

Figure 5: Percentage Mortality of *C. maculatus* Exposed to Lower Concentrations of alpha-humulene-based Insecticide (n=3)

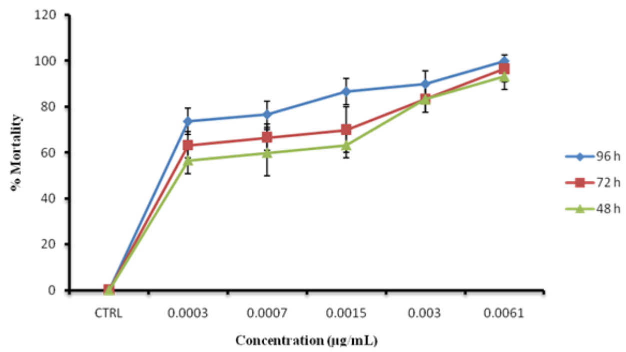

Figure 6: Percentage Mortality of *C. maculatus* Exposed to Lower Concentrations of Myristicin-based Insecticide (n=3)

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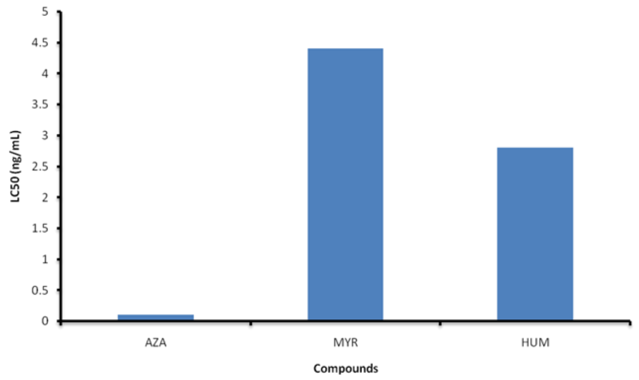

Figure 7: Lethal Concentration 50 (LC_{50}) of Azadirachtin, Myristicin and alpha-Humulene based Insecticides (n=3)

Aza (Azadirachtin), Myr (Myristicin), Hum (alpha-Humulene)

DISCUSSION

Numerous reports have highlighted the residual toxicity associated with the use of synthetic insecticides on stored seeds. Research has shown that the mode of action of a pesticide significantly influences its method of application and potential to leave harmful residues. Consequently, modern insect control strategies should prioritize options that minimize or eliminate residual toxicity. The repellency activity as well as the insect mortality observed with azadirachtin-based insecticide align with the findings of Ibrahim *et al*. (2019), which demonstrated that n-hexane fraction of Azadirachta indica (neem) seed extract exhibited excellent dose-dependent repellency against mosquitoes. Similarly, Kiplang'at and Mwangi (2013) reported that a cream formulation containing 5% neem oil effectively repelled *Aedes aegypti* mosquitoes, while the reported activity on α-humulene is consistent with the findings of Osei-Owusu *et al*. (2023), which reported that essential oils and their headspace volatiles containing a-humulene, among other constituents, exhibited potent repellency against *Anopheles gambiae*. Similarly, Rayane *et al*. (2015) demonstrated that oil extracted from *Commiphora leptolepis* leaves, rich in α-humulene, effectively deterred *Aedes aegypti* oviposition. The observed activity on myristicin-based insecticide corroborates the findings of Bhoopong *et al*. (2022), who established the strong repellency effect of extracts from *Myristica fragrans* (nutmeg) seeds, an abundant natural source of myristicin. The repellency activities of azadirachtin, myristicin, and α-humulene may be attributed to the disruption of insect olfactory receptor neurons, interference of antennal receptivity by the strong aromatic odour, and masking of seed kairomones that attract *C. maculatus*, respectively.

The increasing repellency with time may be attributed to the cumulative effects of the test insecticides, governed by biochemical interactions within the insects. These processes are both time and concentration-dependent, reflecting the dynamic nature of the insecticides' mode of action and the physiological responses of the insects. These differences in efficacy may be attributed to the distinct modes of action employed by each compound, including the disruption of vital metabolic processes such as protein synthesis.

These findings are consistent with the report of Ogbonnaya *et al*. (2024), which established that concentrations of 0.0061 μg/mL (azadirachtin), 0.0122 μg/mL (myristicin), and 0.0244 μg/mL (α-humulene) effectively protected cowpea seeds from *C. maculatus* infestation, indicating their potential as sustainable bio-insecticidal agents for stored product protection.

CONCLUSION

Azadirachtin-, myristicin-, and α-humulene-based insecticides exhibited optimal repellency at a concentration of 100 μg/mL, which was notably higher than those at which these compounds demonstrated peak insecticidal (mortality-inducing) activity against Callosobruchus maculatus. The azadirachtin-based formulation proved to be the most effective among the three tested insecticides.

A notable limitation of the study was its relatively short duration. For research aimed at evaluating the efficacy of insecticides for protecting stored products in short-, medium-, or long-term storage, a longer observation period would provide more comprehensive insights into long-term protective capabilities.

AUTHORS' CONTRIBUTIONS

EO: conceptualized the research problem, designed the experiment and drafted the manuscript. AKM: expanded the design, SMB: supervised the write-up and effected necessary corrections on the manuscript. AM: supervised the experimental set-up while LJB: statistically analyzed raw data generated, IUO performed the data analysis, and EP revised the manuscript before submission Basically, AKM, SMB, AM and LJB took part in supervising the research. All authors read and approved the manuscript

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CONFLICT OF INTEREST

The authors declare that they have no competing interests in this section

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