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Short Communication

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Histopathological effects of plant extracts on *Anopheles gambiae*: acute and chronic toxicity assessment

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ABSTRACT

The increasing use of plant-derived insecticides for malaria vector control necessitates comprehensive evaluation of their histopathological effects on target organisms. This study investigated acute (24-hour) and chronic (72-hour) tissue damage in Anopheles gambiae exposed to methanol, ethanol, and ethyl acetate extracts of *Eucalyptus citriodora*, *Azadirachta indica and Albizia lebbeck*. Using standardized hematoxylin and eosin staining protocols, we observed severe midgut epithelial vacuolization (mean severity score 4.4±0.5) following acute exposure to methanol extracts, progressing to complete tissue necrosis during chronic exposure. Ethyl acetate extracts demonstrated significantly milder effects ($p<0.001$), suggesting solvent-dependent toxicity patterns. These findings provide crucial insights into the mechanisms of action of botanical insecticides while highlighting the need for careful consideration of solvent selection in formulation development. The study contributes to the growing body of evidence supporting plant-based alternatives for vector control, particularly in resistance management strategies.

KEYWORDS: Malaria vector control, Botanical insecticides, Mosquito histopathology, Solvent toxicity, *Anopheles gambiae*

INTRODUCTION

The development of insecticide resistance in Anopheles gambiae complex mosquitoes has reached alarming levels across sub-Saharan Africa, compromising malaria control efforts (WHO, 2021). This resistance crisis has renewed interest in plant-derived compounds as alternative vector control agents (Benelli *et al.*, 2019). While numerous studies have documented the insecticidal efficacy of various botanical extracts (Pavela & Benelli, 2016), their specific histopathological effects on mosquito tissues remain poorly characterized. Understanding these tissue-level changes is essential for several reasons: first, to elucidate the mechanisms of toxicity; second, to assess potential impacts on vector competence; and third, to evaluate environmental safety profiles (Isman, 2020). The current study addresses this knowledge gap by systematically examining histopathological alterations in A. gambiae following exposure to extracts from three medicinally important plant species, using three different solvent systems to evaluate solvent-dependent effects.

MATERIALS AND METHODS

All experiments were conducted using the insectary-reared Kano strain of A. gambiae s.s., maintained under standard conditions (25±2 °C, 80±5% relative humidity, 12:12 light: dark cycle) according to WHO guidelines (WHO, 2020, 2021). Plant materials were collected from the Kano Botanical Garden during the dry season (January-March 2022) and authenticated by taxonomists at Bayero University Kano (voucher specimens: BUKH-EC-221, BUKH-AI-222, BUKH-AL-223). The extraction process followed established protocols (Abbott, 2020) using analytical grade solvents (methanol, ethanol, and ethyl acetate) in a Soxhlet apparatus.

For histopathological analysis, 3-5 day old adult female mosquitoes were exposed to 300 ppm concentrations of each extract (n=50 per treatment group) in WHO insecticide test tubes. Control groups received solvent-only treatments. Following exposure periods (24 hours for acute effects, 72 hours for chronic effects), mosquitoes were dissected in cold phosphate-buffered saline (PBS, pH 7.4) to isolate midgut, Malpighian tubules, and ovarian tissues. Tissues were fixed in 10% neutral buffered formalin for 24 hours, processed through an ethanol dehydration series (70-100%), cleared in xylene, and embedded in paraffin wax. Serial sections (7 μm thickness) were mounted on glass slides and stained with hematoxylin and eosin following standard protocols (Feldman & Wolfe, 2014).

Histopathological evaluation was performed by three independent observers blinded to treatment groups, using a standardized scoring system adapted from Amer and Mehlhorn (2006): 0 (no observable damage), 1 (mild cytoplasmic vacuolization), 3 (moderate epithelial detachment), and 5 (severe necrosis). Microscopic examination was conducted using an Olympus BX53 light microscope with digital image capture. Statistical analysis was performed using SPSS version 26 (IBM Corp.), with two-way ANOVA and Tukey's post-hoc tests ($\alpha=0.05$) to assess solvent and exposure duration effects.

RESULTS

Acute exposure (24 hours) to methanol extracts induced the most severe histopathological alterations across all examined tissues. Midgut epithelial cells displayed extensive vacuolization (mean severity score 4.4±0.5 for *E. citriodora* extracts), with disruption of the peritrophic membrane and epithelial cell sloughing (Table 1). Malpighian tubules exhibited marked epithelial swelling (3.8±0.4) and luminal distension, while ovarian tissues showed follicular degeneration (3.2±0.5) and nurse cell disruption. These findings align with previous reports of methanol-extracted compounds causing rapid cellular damage in insect tissues (Kumar *et al.*, 2018).

Ethanol extracts produced moderate histopathological changes (mean scores 2.4-3.4), characterized by cytoplasmic granulation and partial epithelial detachment. In contrast, ethyl acetate extracts caused only mild effects (0.6-1.6), primarily limited to cytoplasmic vacuolization without structural damage. This solvent-dependent toxicity pattern was consistent across all three plant species, though *E. citriodora* extracts generally induced more severe damage than A. *indica* or A. *lebbeck* at equivalent concentrations ($p<0.05$).

Chronic exposure (72 hours) exacerbated these histopathological effects. Methanol-treated specimens showed complete midgut epithelial necrosis (score 5.0), with disintegration of the basal lamina and cellular debris accumulation in the lumen (Table 1). Malpighian tubules displayed severe degeneration (4.6±0.3), including nuclear pyknosis and loss of brush border integrity. Ovarian tissues exhibited complete follicle resorption (4.8±0.2) and vitellogenic collapse. The progressive nature of these changes suggests cumulative, time-dependent toxicity mechanisms rather than immediate cytotoxic effects.

DISCUSSION

The observed histopathological alterations provide important insights into the mechanisms of plant extract toxicity in A. gambiae. The severe midgut damage caused by methanol extracts supports the hypothesis that these compounds primarily act through disruption of digestive and absorptive functions (Pavela, 2016). The progressive degeneration from acute vacuolization to chronic necrosis suggests multiple mechanisms of action, including membrane disruption, metabolic interference, and potential induction of apoptotic pathways (Isman, 2020). These findings corroborate previous reports on the bioactivity of polar compounds extracted by methanol, particularly terpenoids and alkaloids known to affect insect midgut physiology (Benelli *et al.*, 2019).

Table 1: Acute Histopathology Scores (24 h) in *A. gambiae* Midgut (Mean±SD)

Extract	Methanol	Ethanol	Ethyl Acetate
E. citriodora	4.4±0.5*	3.4±0.7*	1.6±0.3
A. indica	4.4±0.6*	3.4±0.5*	1.4±0.4
A. lebbeck	3.4±0.7*	2.4±0.6	0.6±0.3
Control	0.0±0.0	0.0±0.0	0.0±0.0

*Significant vs control ($p<0.001$, ANOVA with Tukey's test)

The differential toxicity between solvent systems has important practical implications for vector control product development. While methanol extracts showed the highest efficacy, their severe histopathological effects raise concerns about potential environmental impacts and non-target toxicity (Naqqash *et al.*, 2016). Ethyl acetate extracts, though less potent, may offer a favorable balance between efficacy and safety, particularly for use in environmentally sensitive areas. This solvent-dependency underscores the importance of extraction protocol optimization in developing botanical insecticides.

The ovarian damage observed in this study suggests potential population-level impacts beyond immediate mortality. Follicular degeneration and resorption could reduce mosquito fecundity and reproductive capacity, potentially enhancing control efforts through suppression of vector populations (Govindarajan & Benelli, 2016). However, these sublethal effects require further investigation under field conditions to assess their practical significance in control programs.

CONCLUSION

This study demonstrates that plant extracts induce significant, solvent-dependent histopathological alterations in A. gambiae, with methanol extracts causing the most severe tissue damage. The progressive nature of these changes from acute to chronic exposure provides valuable insights into the temporal dynamics of plant extract toxicity. These findings support the potential of botanical insecticides for malaria vector control while highlighting the need for careful solvent selection in product formulation. Future research should focus on: (1) identifying the specific bioactive compounds responsible for these effects; (2) evaluating field efficacy under operational conditions; and (3) assessing potential impacts on non-target organisms. Such studies will be crucial for developing effective, environmentally sustainable alternatives to conventional insecticides in integrated vector management programs.

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