Journal of Experimental Sciences 2025, 16: 30-31
doi: 10.25081/jes.2025.v16.9656
https://updatepublishing.com/journal/index.php/jes

Short Communication

ISSN: 2218-1768

Genotoxic effects of plant extracts on *Anopheles gambiae*: a comet assay study

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Received: July 16, 2025
Revised: August 08, 2025
Accepted: August 09, 2025
Published: August 19, 2025

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ABSTRACT

The increasing resistance of *Anopheles gambiae* to synthetic insecticides necessitates alternative vector control strategies, including plant-derived compounds. While previous studies have focused on the larvicidal and adulticidal effects of botanical extracts, their genotoxic potential remains poorly understood. This study evaluated DNA damage in A. *gambiae* exposed to methanol, ethanol, and ethyl acetate extracts of *Eucalyptus citriodora*, *Azadirachta indica*, and *Albizia lebbeck* using the alkaline comet assay. Adult female mosquitoes were exposed to 300 ppm of each extract for 24 hours, after which midgut cells were isolated and analyzed for DNA fragmentation. Results indicated significant solvent-dependent genotoxicity, with methanol extracts causing the highest DNA damage (78.6±3.8% tail DNA for *E. citriodora*, $^*p^*<0.001$). Ethyl acetate extracts exhibited the least genotoxicity (18-25% tail DNA). These findings suggest that plant-derived insecticides can induce substantial DNA alterations in A. *gambiae*, with implications for vector control strategies and resistance management.

KEYWORDS: *Anopheles gambiae*, Comet assay, DNA damage, Botanical Insecticides, Malaria Vector Control

INTRODUCTION

Malaria remains a major public health challenge in sub-Saharan Africa, with *Anopheles gambiae* being the primary vector (Ayele *et al.*, 2016). The widespread use of synthetic insecticides has led to resistance, prompting research into alternative control methods, including plant-derived compounds (Aktar *et al.*, 2009). While studies have demonstrated the larvicidal and repellent properties of botanical extracts (Conti *et al.*, 2010), their genotoxic effects on mosquitoes remain underexplored.

DNA damage in mosquitoes could influence vector competence, reproductive fitness, and resistance development (Collins *et al.*, 2004). The comet assay is a sensitive tool for detecting DNA strand breaks, making it ideal for assessing genotoxicity in insect vectors (Tice *et al.*, 2000). This study aimed to evaluate the DNA-damaging effects of *E. citriodora*, *A. indica*, and *A. lebbeck* extracts on *A. gambiae*, providing insights into their mechanisms of action and potential ecological impacts.

MATERIALS AND METHODS

Mosquito Rearing and Plant Extract Preparation

Anopheles gambiae colonies were maintained under standard insectary conditions (25±2 °C, 80% RH) (WHO, 2005). Leaves of *E. citriodora*, *A. indica*, and *A. lebbeck* were collected, dried, and extracted using methanol, ethanol, and ethyl acetate via maceration (Frank *et al.*, 2020). Stock solutions (100 mg/mL) were diluted to 300 ppm for bioassays.

Exposure and Comet Assay

Adult female mosquitoes were exposed to extracts for 24 hours in WHO insecticide test tubes (Sharma *et al.*, 2020). Midgut cells were isolated, embedded in agarose, and subjected to alkaline electrophoresis (pH13) (Singh *et al.*, 2017). DNA damage was quantified as percentage tail DNA using fluorescence microscopy and OpenComet software (Collins *et al.*, 2004).

Statistical Analysis

Data were analyzed using one-way ANOVA followed by Tukey's post-hoc test ($^*p^*<0.05$). Correlations between DNA damage and histopathology scores were assessed using Pearson's coefficient.

RESULTS

DNA Damage Induced by Plant Extracts

Methanol extracts caused the highest DNA fragmentation, with *E. citriodora* inducing 78.6±3.8% tail DNA ($^*p^*<0.001$ vs.

Table 1: DNA Damage (% Tail DNA) in *A. gambiae* Exposed to Plant Extracts (Mean±SD)

Extract	Methanol	Ethanol	Ethyl Acetate
E. citriodora	78.6±3.8*	62.4±3.2*	25.0±2.1
A. indica	70.8±3.5*	52.0±2.9*	20.0±1.9
A. lebbeck	45.0±2.7*	30.0±2.2	18.0±1.8
Control	4.4±1.1	4.0±1.0	3.6±1.0

Significant vs. control (p*<0.001, ANOVA)

control). A. *indica* and A. *lebbeck* methanol extracts resulted in 70.8±3.5% and 45.0±2.7% tail DNA, respectively. Ethanol extracts showed moderate genotoxicity (30-62.4%), while ethyl acetate extracts exhibited the least damage (18-25%) (Table 1).

Correlation with Histopathology

A strong positive correlation was observed between DNA damage and midgut histopathology scores (*r*=0.85, *p*<0.001), suggesting that genotoxicity contributes to mosquito mortality.

DISCUSSION

Solvent-Dependent Genotoxicity

The high genotoxicity of methanol extracts may be attributed to polar phytochemicals such as terpenoids and alkaloids, which can intercalate DNA (Tuetun *et al.*, 2004). Ethyl acetate's lower genotoxicity suggests it extracts non-polar compounds (e.g., fatty acids) that target neural or metabolic pathways instead (Singh *et al.*, 2019).

Implications for Vector Control

Plant extracts with high genotoxicity (e.g., *E. citriodora* methanol) could disrupt mosquito reproduction and vector competence. However, their ecological impact on non-target organisms requires further study (Aktar *et al.*, 2009).

Study Limitations

- Chronic exposure effects were not assessed.
- Specific DNA repair mechanisms in mosquitoes remain uncharacterized.

CONCLUSION

This study demonstrates that *E. citriodora*, A. *indica*, and A. *lebbeck* extracts induce significant DNA damage in A. *gambiae*, with methanol being the most genotoxic solvent. These findings support the potential of plant-based insecticides while highlighting the need for solvent-specific toxicity profiling.

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