Aedes aegypti
- Mosquitoes vector multiple debilitating pathogens including those that cause dengue fever, yellow fever, zika, chikungunya
- Often controlled by synthetic insecticides
- Populations develop insecticide resistance, limiting ability to control range and spread of disease

Plant Essential Oils
- Plant-based distillate containing multiple active ingredients, providing various modes of action that can prevent resistance development
- L50 of insecticide applied in combination with 10μg of plant essential oil.
- Determine mortality 24-hr post-application.

Plant essential oil insecticide enhancement

Modified World Health Organization Topical Application Protocol
- Mixture applied to pronotum of adult female mosquitoes.
- LD50 of insecticide applied in combination with 10μg of plant essential oil.

Detoxification Enzymes
- Glutathione S-transferase (GST)
- Cytochromes p450 monoxygenase

Materials and Methods
- Mosquito detoxification enzymes available in homogenate are exposed to a substrate.
- Enzymes break down the substrate into different products.
- Enzyme inhibition directly related to quantity of breakdown products (assessed by fluorescence).
- Inhibitors decrease the activity of enzymes and decrease the amount of breakdown products.

Treatments
1. Apply 10μg acetone (control) or plant essential oil solution to pronotum of adult female mosquito.
2. Incubate live mosquitoes for necessary time period.

Glutathione S-transferase and α-naphthyl esterase
1. Treated mosquitoes incubated for 24 hours.
2. Homogenize mosquitoes into single container for each treatment.
3. Plate homogenate onto well plate.
4. Introduce substrate (7-ethoxycoumarin).
5. Removal of byproducts via human liver reductase.
6. Addition of oxidized glutathione as substrate for reductase.

Data Analysis
Data was normalized by correcting for the amount of protein in each sample and presented as total percentage of control activity.

Inhibition of enzymes

Results
- Each enzyme inhibitor (positive controls) successfully inhibited enzyme activity in all detoxification enzyme systems.
- Broad majority of oils capable of inhibiting one or multiple detoxification enzyme systems.
- Basil (Egyptian type) was the most potent cytochromes p450 inhibitor.
- Geranium (bourbon type) was the most potent GST inhibitor.
- Cedarwood Texas (CWT) was the most potent Esterase inhibitor.
- Multiple oils seemingly increased enzyme activity, especially in the esterase system.

Conclusions
- Plant essential oils are capable of inhibiting all three major detoxification enzyme systems in Aedes aegypti.
- Interaction between oils and enzymes provides insight into mechanism of enhancement in Aedes aegypti.
- This study highlights the importance of considering detoxification pathways in insecticide resistance.
- Oils such as basil (Egyptian type) or geranium (bourbon type) may have strong effects on some, but not all detoxification enzyme systems.
- Plant essential oils have the potential to act synergistically with synthetic insecticides to counter insecticide-resistant mosquito populations.
- Oils that increase enzyme activity may suggest enzyme induction, and may be relevant in enhancing other insecticidal active ingredients.

Future Studies
- Continue screening plant essential oils for inhibition of detoxification enzymes.
- Assess IC50 of plant essential oils in various detoxification enzyme systems.
- Investigate individual plant essential oil constituent chemicals for inhibitory properties.
- Compare enzyme inhibition by plant essential oil constituent chemicals for inhibitory properties.

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References