# The ability of plant essential oils to inhibit detoxifying enzymes in *Aedes aegypti*

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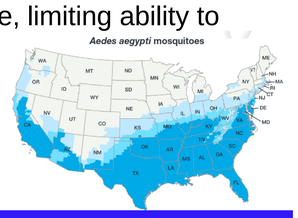
## *Aedes aegypti*

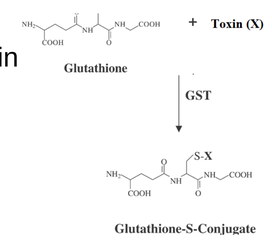
- Mosquitoes vector multiple debilitating pathogens including those that cause dengue fever, yellow fever, zika, chikungunya
- Often controlled by synthetic insecticides[1]
- Populations develop insecticide resistance, limiting ability to control range and spread of disease[1]




## Detoxification Enzymes

Glutathione S-transferase (GST)

Transfer a glutathione molecule onto a toxin

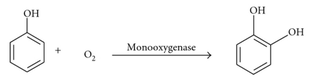



α-naphthyl esterase

Split esters into an acid and an alcohol

Cytochromes p450 monooxygenase

Add a hydroxyl group onto toxin




## Plant Essential Oils

- Plant-based distillate containing multiple active ingredients, providing various modes of action that can prevent resistance development

Basil

Geranium

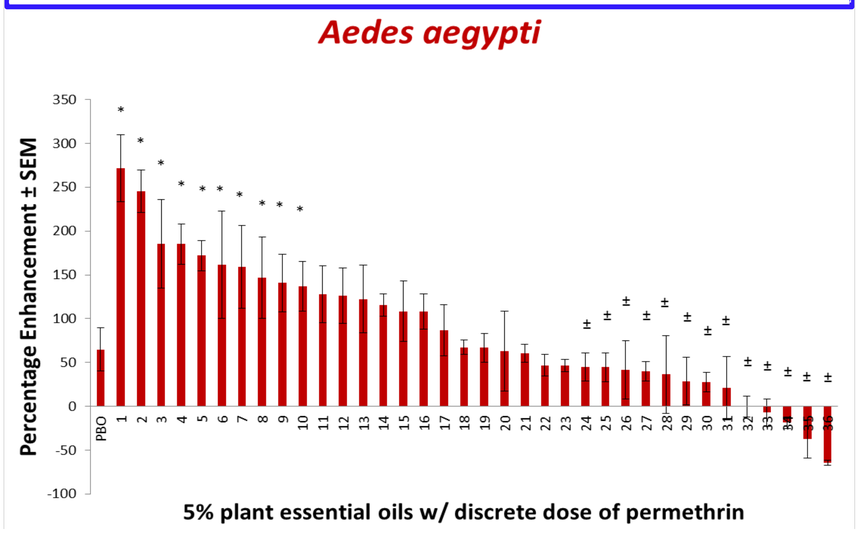
Cedarwood Texas

## Plant essential oil insecticide enhancement

**Modified World Health Organization Topical Application Protocol**

- Mixture applied to pronotum of adult female mosquitoes.
- $LD_{25}$ of insecticide applied in combination with 10µg of plant essential oil.
- Determine mortality 24-hr post-application.

$$\text{Enhanced/reduced mortality} = \frac{\text{(\% dead in mixture) – (\% dead in permethrin)}}{\text{(\% dead in permethrin)}}$$



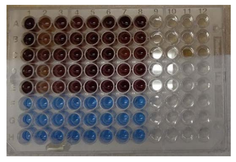

- Positive control of detoxification enzyme inhibitor (piperonyl butoxide) significantly enhanced insecticides
- A majority of plant essential oils enhanced permethrin as well or better than piperonyl butoxide

## Objectives

1. Determine which plant essential oils are capable of inhibiting detoxification enzymes
2. Explore each major enzyme-dependent detoxification pathway available to *Aedes aegypti* mosquitoes

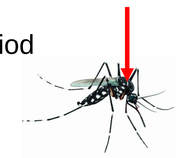
## Materials and Methods

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

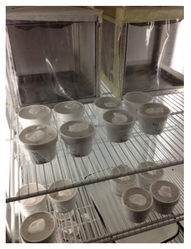


Cytochromes p450 monooxygenase[2]

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

Glutathione S-transferase and α-naphthyl esterase[3]

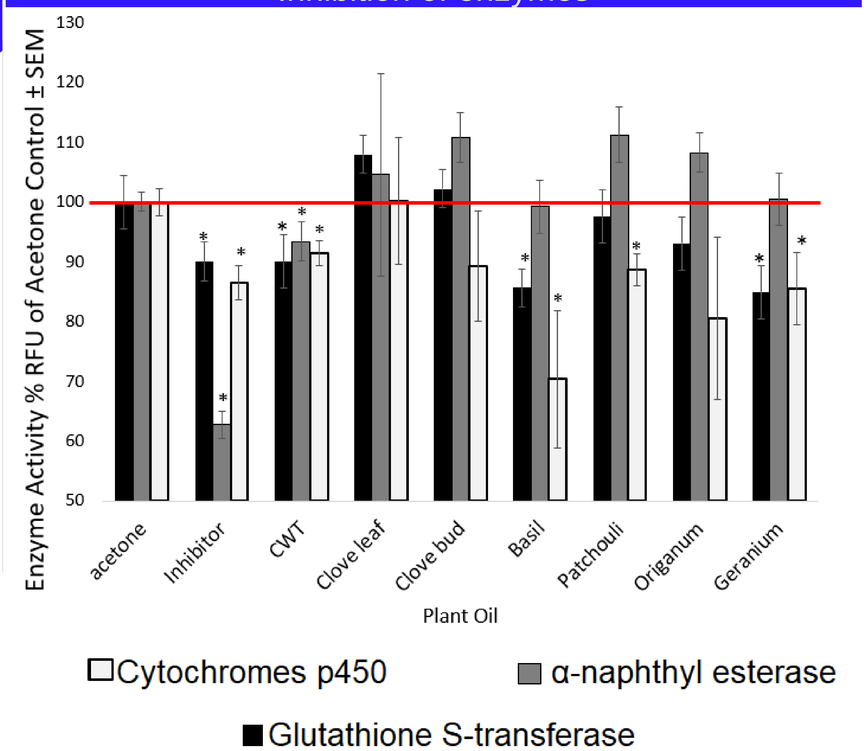
1. Treated mosquitoes incubated for 3 hours
2. Individually homogenize mosquitoes
3. Plate homogenate onto well plate
4. Introduce substrate
   - reduced glutathione or α-naphthyl acetate
5. Aliquot 40 µL of fast blue stain solution



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 $IC_{50}$ 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 oils in insecticide-resistant strains

## References

1. Insecticide resistance and vector control. 1970. Seventeenth report of the WHO Expert Committee on Insecticides. WHO Technical Report Series no. 443. World Health Organization,Geneva, Switzerland.
2. Anderson, Troy D., and Kun Yan Zhu. "Synergistic and antagonistic effects of atrazine on the toxicity of organophosphorodithioate and organophosphorothioate insecticides to Chironomus tentans (Diptera: Chironomidae)." Pesticide Biochemistry and Physiology 80.1 (2004): 54-64.
3. World Health Organization: Techniques to detect insecticide resistance mechanisms (field and laboratory manual). Geneva 1998

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