

# Mosquito attractants/repellents from essential oils of plants of French Polynesia

 VAHIRUA-LECHAT I.<sup>1</sup>, MITERITE Y.<sup>1</sup>, ADAM F.<sup>1,2</sup>, DESLANDES E.<sup>2</sup>, BERNIER U.<sup>3</sup>, MENUT C.<sup>4</sup>
<sup>1</sup> Laboratoire de Recherche sur les Substances Naturelles, Institut Louis Malardé, BP 30, Papeete 98713, Tahiti, Polynésie française

<sup>2</sup> Laboratoire d'Ecophysiologie et Biotechnologie des Halophytes et des Algues Marines, LEBHAM.IUEM.UBO; Technopole Brest Iroise, 29280 Plouzane, France

<sup>3</sup> Mosquito & Fly Research Unit, USDA-ARS-CMAVE, 1600 SW 23rd Drive, Gainesville, FL 32608, USA

<sup>4</sup> Equipe Glycochimie, Institut des Biomolécules Max Mousseron, UMR 5247 CNRS-UMI-UM2, Ecole Nationale Supérieure de Chimie de Montpellier, CC453, 34296 Montpellier Cedex 5, France

## Introduction

Filarisis and Dengue Fever are two mosquito-borne diseases threatening French Polynesia. The potential vectors for transmission of these illnesses are *Aedes aegypti* (L.), the Yellow Fever mosquito, and *Ae. polynesiensis* Marks. A research program financed by the Ministry of Health of French Polynesia, the Establishment for Prevention and the Laboratory of Ecophysiologie and Biotechnology of Halophytes and Marine Algae was undertaken with the ultimate goal of development of improved insect repellents and lures for insect traps. This goal is made more urgent by the threat of mosquito-borne diseases.

## Material & Methods

Several natural products from terrestrial plants of French Polynesia were evaluated as spatial attractants against *Ae. Aegypti* mosquitoes using a triple cage-dual port olfactometer and as topical repellents using a "cloth patch assay" test with DEET as the positive control.



The Olfactometer test



The Cloth patch test

### TEST PROCEDURE

A dual-port olfactometer system with 3 test chambers was used to evaluate mosquito response by upwind flight to the source of a chemical attractant dispensed in glass vials, Petri dishes or polymer pads. A standard, L-lactic acid/Acetone / Dimethylsulfide mixture is usually included in studies with other candidate attractants. Test population of 75 female of same age are captured with the Airflow system and transferred to each of the 3 olfactometer chambers.

To bioassay, the standard lactic acid mixture is pipeted into the vial and placed inside and to the front of the sleeve of the A or B test port. The port door is opened, allowing conditioned air to pass over the attractant and into the test chamber containing the mosquitoes. Bioassays are normally run for 3 min. During this time, non responding mosquitoes remain at rest, while mosquitoes responding to the attractant take flight and orient to the air stream emitting from the port containing the attractant substance. The mosquitoes entering the port and passing through the screen funnel opening into the trap are counting by an observer.

### TEST PROCEDURE

50 mg of the test material is placed in a 2-dram vial to which 1 mL of acetone is added. When the chemical is thoroughly dissolved, a 50 cm square (5X10 cm) piece of Muslin cloth is rolled, paced in the vial and sealed until the cloth roll is completely saturated and ready to be bio-assayed. The rate treatment is 1mg/cm square.

The subject testing the treatments pulls a nylon knee-high hose over their arm and rubber gloved hand. The card and attached cloth are then taped over the nylon-covered forearm so that only the treated cloth allows the mosquitoes access to the skin. The arm is exposed in a cage of mosquitoes (approximately 1500) for 1 min. More than 3 bites through the treated cloth in 1 min denotes failure of the chemical. If the chemical does not fail, it is hung on a rack at room temperature and retested in 24hr and daily thereafter until the treatment becomes ineffective. A DEET standard is tested with each group candidate repellents.

The Minimum Dose Effective (MED) is to be determined. The method of testing is the same, except that the rate of treatment is reduced by half until more than 3 bites per min are received at the lowest dose. The MED's are determined only on the fresh treatments and the 1-day-old treatments.



Ocimum gratissimum



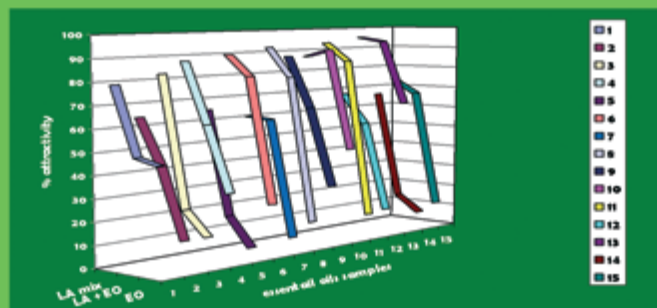
Ocimum basilicum

**Table 1. List of the species studied**

Plants studied	Samples	Major compounds identified in the oils
1. <i>Eucalyptus citriodora</i>	EO of leaves	citronellal, citronello
2. <i>Etligeria cevuga</i>	EO of leaves	methyl eugenol E methyl isoeugenol
3. <i>Lantana camara</i>	EO of leaves	$\alpha$ -humulene b-caryophyllen
4. <i>Plecthrantus amboinicus</i>	EO of leaves	camphor, carvacrol
5. <i>Ocimum basilicum</i>	EO of leaves	E methyl cinnamate Z methyl cinnamate
6. <i>Ocimum gratissimum</i>	EO of leaves	eugenol, Z b-ocimene
7. <i>Citrus sp.</i>	EO of peels	limonene, g-terpinene
8. <i>Citrus grandis</i>	EO of peels	limonene, g-terpinene
9. <i>Citrus grandis</i>	EO of fruit juice	limonene, g-terpinene
10. <i>Cananga odorata</i> (2007)	EO of flowers	germacrene-d EE farnesene
11. <i>Psidium guajava</i>	EO of leaves	b-caryophyllene eucalyptol
12. <i>Psidium cattleianum</i>	EO of leaves	b-caryophyllene $\alpha$ -humulene
<b>Samples of another origins</b>		
13. <i>Santalum sp.</i> (New Caledonia)	EO of wood	ND
14. <i>Hedychium coronarium</i> (Madagascar)	EO of rhizomes	ND
15. <i>Hedychium coronarium</i> (Madagascar)	EO of leaves	ND

## Results & Discussion

### The olfactometer test results



3 oils mixed with Lactic acid mixture reduced the attractiveness of the LA mix : *Lantana camara* (23.8%), *Ocimum basilicum* (19%) and *Hedychium coronarium* rhizomes (15.4%). Among the 15 pure oils tested 9 oils have spatial repellency's activity : *Lantana camara* (13.3%), *Ocimum basilicum* (5.2%), *Hedychium coronarium* rhizomes (7.8%), *Etligeria cevuga* (13.4%), *Citrus sp.* (6.5%), *Citrus grandis* (12.1%), *Cananga odorata* (19.8%), *Psidium guajava* (11%), *Psidium cattleianum* (12%) and *Hedychium coronarium* leaves (11%).

## Conclusion

The spatial repellency/attractivity of 15 essential oils was examined using a triple cage olfactometer. None of the oils attracted *Aedes aegypti*.

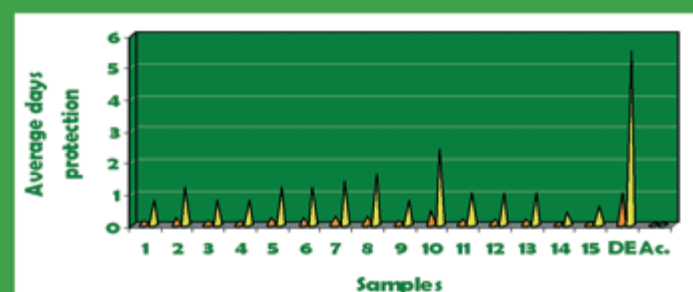
The topical repellency of 15 oils was observed using the cloth patch assay. Among the 15 samples tested, the oil of *Cananga odorata* was the most repellent (2.4 days average protection). Chemical analysis of the oils were performed using Gas Chromatography coupled with Mass Spectrometry leading to the identification of more than hundred of compounds. The main compounds identified in the oils are described here. The 15 oils are to be tested against *Aedes polynesiensis* in the near future.

## Acknowledgement

This work was financed by the Etablissement Public Administratif pour la Prévention (EPAP) and the Ministry of Health of French Polynesia.

## Results & Discussion

### The Cloth patch test results



All the samples tested were less repellent than the synthetic standard DEET (5,5 days of protection). The oil of *Cananga odorata* was the more active sample tested with an average 2.4 days protection. The oils from peels of two citrus samples were repellent : *Citrus sp.* (1.4 days protection) and *Citrus grandis* (1.6 days protection). The oils obtained from the *Ocimum* species were also relatively active *Ocimum basilicum* and *Ocimum gratissimum* (both samples allowing 1.2 days protection) such as the *Etligeria cevuga* oil (1.2 days protection).

## References

- [1] Posey K.H., Barnard D.R., Schreck C.E., Triple cage olfactometer for evaluating mosquito (Diptera: Culicidae) attraction responses, *J Med Entomol*, 35, 3, 330-334 (1998)
- [2] Techniques for the evaluation of the insect repellents : a critical review, *Ann Rev Entomol*, 22, 101-119 (1977)
- [3] Subumar S., Perich M.J., Perich J. and Boobar L.R., Botanical derivatives in mosquito control: A review, *J Am Mosq Control Assoc*, 7, 2, 210-237 (1991)
- [4] Lechat-Vahirua I., François P., Menut C., Lamaty G., Bessière J.-M. Aromatic plants of French Polynesia I. Constituents of the essential oils of three Zingiberaceae : *Zingiber zerumbet* Smith, *Hedychium coronarium* Koenig and *Etligeria cevuga* Smith, *JEOR*, 1993, 5, 55-59