

## Synergistic repellent and irritant effect of combined essential oils on *Aedes aegypti* (L.) mosquitoes

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**ABSTRACT:** This study was designed to compare the behavioral responses of *Aedes aegypti* to a single essential oil and to a mixture of two or three essential oils using an excito-repellency test chamber. Mixtures were prepared from essential oils extracted from *Litsea cubeba* (LC), *Litsea salicifolia* (LS), and *Melaleuca leucadendron* (ML). In general, the mixture of essential oils produced a much stronger escape response by *Ae. aegypti*, regardless of the test conditions. No significant difference in escape responses was seen when the mixture of oils was compared with a standard commercial product containing DEET. Greater contact irritancy was seen from mixed oils of LC and LS than with other mixed oils. Mixtures of LC and LS at 0.075% showed the highest synergistic action (65.5% escaped) compared to that with unmixed oil alone at the same concentration (LC=20% and LS=32.2%). In addition, mixtures of LC and LS at 0.075% demonstrated the highest non-contact repellency (62.7%) and showed a greater effect than the use of LC (20%) or LS (20.3%) alone. We conclude that mixtures of two essential oils show potential as active ingredients for mosquito repellents. *Journal of Vector Ecology* 39 (2): 298-305. 2014.

**Keyword Index:** Excito-repellency, irritancy, essential oils, *Melaleuca leucadendron*, *Litsea cubeba*, *Litsea salicifolia*, *Aedes aegypti*, DEET.

### INTRODUCTION

For decades, synthetic insecticidal compounds within different chemical classes have been used by the private and public health sectors. Synthetic pyrethroids have become ubiquitous due to their relatively low mammalian toxicity and ability to rapidly immobilize invertebrates at low levels (Jirakanjanakit et al. 2007, Thanispong et al. 2008). Because of the frequent use of pyrethroids, selection pressure for insecticide resistance has become a major concern (Roberts et al. 1997, Jirakanjanakit et al. 2007, Thanispong et al. 2008). In addition to toxicants, effective repellents such as DEET (*N, N*-diethyl-*meta*-toluamide) are readily available and commonly used (Fradin and Day 2002). DEET is used extensively to protect against biting mosquitoes, however it has been shown to have significant adverse or toxic effects to humans (Brown and Hebert 1997, Odalo et al. 2005). Consequently, there is interest in the development of insect repellents from natural plant extracts that might be less toxic to humans, such as thyme (Park et al. 2005) and citronella (Yang and Ma 2005).

Recently, several plant species were evaluated as potential repellents or toxicants against blood-feeding invertebrates and pestiferous flies in Lao PDR (De Boer et al. 2010). Although several essential oils have exhibited significant repellent activity against target insects (Noosidum et al. 2008, Kim et al. 2012, Suwansirisilp et al. 2013), comparatively little has been done to identify the degree of behavioral responses using the mixtures of at least two essential oils. In this study, we compared the behavioral responses of *Aedes aegypti* (L.) to a single essential oil and mixtures of essential oils from *Litsea cubeba* (Lour.) Persoon (LC), *Litsea salicifolia* (Roxburgh ex Nees) Hook. f. (LS), and *Melaleuca leucadendron* L. (ML)

using an excito-repellency test chamber.

### MATERIALS AND METHODS

#### Mosquitoes

*Aedes aegypti* populations were collected from Pu Teuy Village, Sai Yok District, Kanchanaburi Province, Thailand (14°17'N, 99°1'E). All female mosquitoes were identified to species and colonies were established using modified methods from Kongmee et al. (2004). Two days post-emergence, mosquitoes were blood-fed on live guinea pigs held for three days until oviposition occurred on filter paper contained in dishes with tap water. Egg and larval stages were reared in plastic pans and pupae were maintained in plastic cups, which were held at 25 ± 5° C, 12:12 (L:D) photoperiod, and 80 ± 10% relative humidity. Adults were held in 30×30×30 cm screened holding cages and provided with cotton pads soaked with 10% sugar solution. Only three to five day-old female *Ae. aegypti* were used and were sugar-starved (water only) for 12 h before testing.

#### Oil extraction

Fresh ML leaves were collected from Chanthaburi Province, Thailand, in October, 2010 (13°98'N, 48°18'E). Mature fruits of LC and LS were collected from Doi Angkhang, Fang District, Chiang Mai Province in November, 2010 (19°54'N, 99°2'E). Essential oils from the plants were extracted over 6 h by water distillation using a Clevenger-type apparatus. The superior phase was collected from the condenser, dried over anhydrous sodium sulfate, and stored in amber-colored vials at 0° C until testing (Noosidum et al. 2008).