Journal of Medical Entomology, 54(1), 2017, 178–186
doi: 10.1093/jme/tjw143
Advance Access Publication Date: 30 September 2016
Vector Control, Pest Management, Resistance, Repellents Research article

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Excito-Repellency of *Citrus hystrix* DC Leaf and Peel Essential Oils Against *Aedes aegypti* and *Anopheles minimus* (Diptera: Culicidae), Vectors of Human Pathogens

Jirod Nararak,[1] Sunaiyana Sathantriphop,[2] Monthathip Kongmee,[3] Michael J. Bangs,[1,4] and Theeraphap Chareonviriyaphap[1,5]

[1]Department of Entomology, Faculty of Agriculture, Kasetsart University, Bangkok 10900, Thailand (rgt532@gmail.com; michael_bangs@fmi.com; faasthc@ku.ac.th), [2]National Institute of Health, Department of Medical Sciences, Ministry of Public Health, Nonthaburi 11000, Thailand (sunaiyana@yahoo.com), [3]Department of Entomology, Faculty of Agriculture at Kamphaeng Saen, Kamphaeng Saen Campus, Kasetsart University, Nakhon Pathom 73140, Thailand (fagrmtk@ku.ac.th), [4]Public Health & Malaria Control Department, PT Freeport Indonesia, International SOS, Jl. Kertajasa, Kuala Kencana, Papua 99920, Indonesia, and [5]Corresponding author, e-mail: faasthc@ku.ac.th

Received 12 May 2016; Accepted 9 August 2016

Abstract

The essential oils of kaffir lime (*Citrus hystrix* DC.) at four different concentrations (0.5, 1.0, 2.5, and 5.0% v/v) were studied for their repellency, excitation, and knockdown properties against laboratory strains of *Aedes aegypti* (L.) and *Anopheles minimus* Theobald using an excito-repellency test system. Both contact and noncontact escape responses to leaf- and peel-derived kaffir lime oils were observed. Comparing unadjusted escape responses for *An. minimus*, leaf oil had strong combined irritant and repellent activity responses at 1–5% concentrations (90.0–96.4% escape) and the strongest spatial repellent activity at 1% and 2% (85.9% and 87.2% escape, respectively). The peel oil exhibited good excitation with repellency at concentrations of 2.5% (89.8% escape) and 5% (96.28% escape), while concentrations 1–5% showed more moderate repellent activity against *An. minimus*. For *Ae. aegypti*, 2.5% leaf oil produced the greatest response for both contact (56.1% escape) and noncontact (63.3% escape) trials, while 2.5% produced the strongest response among all concentrations of peel oil, with 46.5% escape. However, after adjusting the contact trial escape (a measure of combined excitation and repellency), the estimated escape due to contact alone was a much weaker response than spatial repellency for both species. Knockdown responses above 50% were only observed in *Ae. aegypti* exposed to 5% leaf oil. Kaffir lime oils were more active against *An. minimus* than *Ae. aegypti* mosquitoes. There were statistically significant differences between leaf (more active) and peel oils at each concentration against *An. minimus* in contact and noncontact trials, except at the highest (5%) concentration.

Key words: *Citrus hystrix* DC., essential oil, *Aedes aegypti*, *Anopheles minimus*, excito-repellency

Currently, >3,500 species of mosquitoes (Culicidae) have been described, with some species having cosmopolitan distributions inhabiting both urban and rural settings in close association with humans. None is more important than *Aedes aegypti* (L.), a vector of dengue, chikungunya, yellow fever, and Zika viruses (Marcondes and Ximenes 2015). This species is often found in and around human dwellings and preferentially feeds on humans during daylight hours. In contrast, *Anopheles minimus* Theobald, a forest and forest-fringe species with crepuscular and nocturnal biting activity, is responsible for transmitting malaria and lymphatic filariasis (*Wuchereria bancrofti*) agents throughout much of its native range in mainland Southeast Asia (Manguin et al. 2010, Sinka et al. 2011). Efficacious vaccine products against malaria and dengue are still under development and are likely years away from seeing broad application. Besides various personal protection measures, prevention and control of malaria and dengue in Thailand remains dependent on various vector control strategies to decrease transmission risk either by environmental manipulation or chemical means to eliminate mosquito larvae (e.g., use of temephos) and adult mosquitoes (mainly deltamethrin and permethrin space sprays (Chareonviriyaphap et al. 2013.

Numerous plant products have shown insect repellent activity and have found utility as active ingredients as natural repellent formulations and commercial products. Among these, kaffir lime, *Citrus hystrix* DC. (Rutaceae: Aurantioideae), displays promising insect repellent activity (Tawatsin et al. 2001, Maia and Moore 2011). The kaffir lime, sometimes referred to as the makrut lime or

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