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Full Length Article

Diurnal test periods influence behavioral responses of *Aedes aegypti* (Diptera: Culicidae) to repellents

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ABSTRACT

There is a broad understanding of the influence of environmental factors on various aspects of normal mosquito behavior. How these external factors influence responses to repellent compounds is far less clear. The objective of this study was to investigate the effect of different daytime periods combining the normal circadian activity of a laboratory colony of *Aedes aegypti* (L.) with behavioral responses of mosquitoes exposed to three different compounds possessing repellent properties. Using an excito-repellency test chamber with different test designs (contact irritancy + repellency and noncontact repellency), female mosquitoes were exposed to each chemical or matching blank control during four different 3-h time intervals beginning 0600 to 1800 h. Mosquitoes showed more significant avoidance responses (escape movement away from the chemical) when exposed to either DEET or hairy basil during the afternoon periods. With deltamethrin, there was no significant difference in repellent escape movement during any period of testing. Escape activity with deltamethrin was significantly greater during all diurnal periods in contact tests compared to DEET and hairy basil. From this study, it was shown that time of diurnal testing can significantly influence behavioral responses of *Ae. aegypti* exposed to chemical-based repellents. Therefore, the assessment of chemicals (toxins, repellents, attractants) and must carefully consider time-of-test as a potential confounding factor during evaluation and comparisons.

Introduction

Arthropod-borne viruses represent a public health threat globally due to their rapid geographical spread and increasing disease burden in humans (Dash et al., 2013). Important arboviruses transmitted by mosquitoes include yellow fever, West Nile, chikungunya, Japanese encephalitis, Zika and dengue, among others (Gubler and LeDuc, 1998; Rogesrs et al., 2006; Hayes, 2009; WHO, 2017). In particular, dengue, chikungunya, and more recently, Zika viruses have evolved a near cosmopolitan distribution, especially in the tropical/sub-tropical areas with suitable climatic conditions that allow for year-round transmission (Liang et al., 2015; Attar, 2016; Benelli et al., 2016; Benelli and Mehlhorn, 2016). Dengue is the most commonly reported arboviral disease in the world, including Thailand and much of the Asia-Pacific region (Gubler, 1997; Bhatt et al., 2013). Worldwide, annual morbidity due to dengue viruses has been estimated between 50 and 100 million infections and 12,500 deaths per year (WHO, 2017); while others have placed estimates of overall burden as high as 390 million infections, of which maybe only 96 million reach a level of clinical presentation (Bhatt et al., 2013). In Thailand, < 100,000 cases of dengue fever are reported annually while the number of symptomatic cases has continued to increase dramatically in the Asian region (Halstead, 2006; Limkittikul et al., 2014; BVBD, 2017; WHO, 2017).

In urban areas, dengue viruses are primarily transmitted by *Aedes aegypti* (L.), a day-active mosquito (Gould et al., 1968; Yasuno and Tonn, 1970). *Aedes albopictus* is also common in Thailand and capable of transmitting dengue viruses (Rodhain and Rosen, 1997; Bonizzoni et al., 2013). *Aedes aegypti* is also responsible for transmitting other viruses (e.g., yellow fever, chikungunya and Zika viruses), an ability making it arguably the most important arthropod vector species in the world (Higgs and Vanlandingham, 2015; Chouin-Carneiro et al., 2016; Paixao et al., 2016; Vasconcelos and Monath, 2016), thus making it a species of intense interest to vector biologists and operational control programs.

Behaviorally, *Ae. aegypti* preferentially feeds on humans during daylight hours (Powell and Tabachnick, 2013; Lima-Camara et al., 2014). It is an extremely difficult species to control because of its behavioral and biological traits, placing it in close association with the human environment. Currently, the most feasible methods of prevention and control of transmission relies on various forms of vector

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