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Evaluation of Mosquito Attractant Candidates Using a High-Throughput Screening System for *Aedes aegypti* (L.), *Culex quinquefasciatus* Say. and *Anopheles minimus* Theobald (Diptera: Culicidae)

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Simple Summary: Trapping mosquitoes can enhance its capture rate by adding attractants such as carbon dioxide or human hosts' odor-mimicking synthetic blends. Various olfactometers exist to test mosquitos' behavior, but high-throughput screening system (HITSS)—one of the diffusion assays—has not been applied to developing lures. In this study, six different newly prepared chemical lure candidates (Kasetsart University (KU)-lures) were tested for diurnal *Aedes aegypti*, nocturnal *Culex quinquefasciatus* and nocturnal *Anopheles minimus*, using the HITSS assay. Results showed species-specific different lure preferences; the diurnal species were attracted to KU-lure #1 (29.7%), while both of the nocturnal species preferred KU-lure #6 (68.3% and 74.3% for *Cx. quinquefasciatus* and *An. minimus*, respectively). In addition, the selected lure candidates clearly demonstrated dose-dependent reversal responses for each *Ae. aegypti* and *Cx. quinquefasciatus*. Our results indicate that the HITSS assay distinguishes potential species-specific lure candidates. In addition, the HITSS assay was equally effective in determining the host-seeking behavior in pyrethroid-resistant and -susceptible strains. Further studies are needed to determine the accuracy of the HITSS assay in large-scale semi-field screen house tests using commercial traps.

Abstract: Several types of olfactometers have been used to evaluate mosquito responses to agents that mimic natural volatiles that repel or attract. The Y-tube olfactometer has been widely used to study repellents and attractants, while the high-throughput screening system assay has only been used to study repellents. Whether the high-throughput screening system assay is suitable for evaluating attractants is unknown. We evaluated the responses to four lactic-acid-based mixtures and two non-lactic-acid-based chemical lure candidates using the high-throughput screening system (HITSS) for three mosquito species (laboratory strains and field populations of both *Aedes aegypti* (L.) and *Culex quinquefasciatus* Say.; laboratory strain of *Anopheles minimus* Theobald) under laboratory-controlled conditions. HITSS assay results showed that KU-lure #1 elicited the greatest percent attraction for pyrethroid-resistant and -susceptible *Ae. aegypti*. KU-lure #6 elicited the strongest attractive response for pyrethroid-susceptible and -resistant *Cx. quinquefasciatus* and pyrethroid-susceptible *An. minimus*. The response to the lures from each species was independent of the pyrethroid susceptibility status (*Ae. aegypti*, $p = 0.825$; *Cx. quinquefasciatus*, $p = 0.056$). However, a significant difference in attraction to KU-lure #6 was observed between diurnal and nocturnal mosquitoes (*Cx. quinquefasciatus* vs. *Ae. aegypti*, $p = 0.014$; *An. minimus* vs. *Ae. aegypti*, $p = 0.001$). The laboratory-level HITSS assay effectively selects potential lure candidates. Because the host-seeking behavior differs between mosquito species, further studies are needed to develop species-specific attractants. Additional studies in semi-field screen houses using commercial traps are necessary to evaluate the accuracy of these laboratory assay results.

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