Scientific Note

An improved excito-repellency test chamber for mosquito behavioral tests

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Despite significant gains in its control, malaria remains a serious threat in Thailand especially in areas that border with neighboring countries (Chareonviriyaphap et al. 2000). The prevention of malaria transmission in Thailand relies on the effective treatment of infections and the reduction of contacts between vectors and humans. Understanding the behavioral responses of malaria vectors, especially avoidance behavior to residual insecticides, is of particular importance to any vector control program. There have been numerous attempts to accurately measure the behavioral responses of mosquitoes to insecticides using various types of excito-repellency test systems (Roberts et al. 1984, Rutledge et al. 1999, Sungvornyothin et al. 2001). However, no test system has been fully accepted as a standardized method of testing and analyzing avoidance responses (Roberts et al. 1984 and Evans 1993). Chareonviriyaphap et al. (1997), using an experimental escape chamber system (Roberts et al. 1997), provided information on both contact irritability and non-contact repellency for behavioral response tests on *Anopheles albimanus* under laboratory and field conditions. Unfortunately, this prototype test system was cumbersome and required extended time to attach the test papers onto the inner walls. To overcome this problem, a collapsible excito-repellency test chamber was developed (Chareonviriyaphap and Aum-Aong 2000). This test system evaluated the behavioral responses of a laboratory colony and field populations of *Anopheles minimus* to DDT, deltamethrin and lambdacyhalothrin (Chareonviriyaphap et al. 2001). Although the chamber system could be disassembled for transport and results were reproducible, this test system required much time to assemble. Moreover, test paper holders were affected by the assembly screws penetrating the inner chamber, complicating test set-up. To overcome these technical problems, an improved version of the excito-repellency test chamber design was developed as described in this report.

Keyword Index; Excito-repellency, improved system, behavioral test.

The improved version of the excito-repellency test system is shown in Figure 1. As in previous models, the outer chamber is constructed with four metal sides, each side wall measuring 33.5 x 33.5 cm^2. Walls are constructed of stainless steel (thickness 0.7 mm) with an aluminum sliding rib on each end. The screened inner chamber is a 4 side-box slightly smaller than the outer chamber walls, measuring 33.5 x 22.5 cm^2 each. The inner chamber functions as the test paper holder, each wall having 2 functional sides. Each side of the wall has a framed panel to hold the test paper in place. Depending upon the objective of the test, the impregnated papers can be placed on either side of the panel in a position to allow or prevent mosquitoes from making physical contact with the test paper surface. There is a 0.9 cm gap between the test paper and screen barrier to prevent mosquitoes making direct contact with the test paper surfaces during the non-contact repellency test. A Plexiglas™ holding frame is used to hold the Plexiglas panel in place and secure the whole system tightly without the use of metal screws. The panel has a rubber door of 15 cm diameter made of a split sheath of dental dam, allowing mosquitoes to be placed inside the chamber or to remove them after the testing period. A forward exit portal is a composed of a horizontal opening, 15 cm long and 2 cm wide, at the end of an outward projecting funnel. A stainless steel

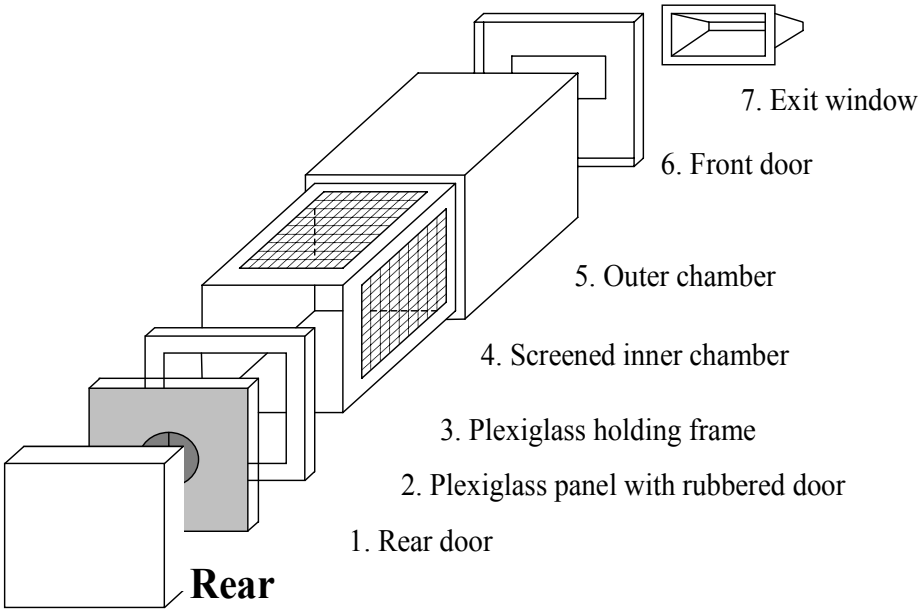

Figure 1. An improved excito-repellency test system for behavioral study.

cover secures the rear door tightly.

Insecticide treated surfaces (insecticide-impregnated or non-impregnated control papers) are attached to one side of each holding frame depending on the test objective (either contact and non-contact test design). There are four small spring clamp mechanisms on each corner to firmly secure the test papers. To assemble the excito-repellency chamber, the four inner walls are connected by sliding the appropriate aluminum tongue and groove elements together to construct the screened inner chamber. Each inner wall also serves as the test paper holder in either the contact or non-contact mode. A spring mechanism on each corner of the wall secures the test paper. The four outer walls are connected by sliding the appropriate corner tongue and groove elements together to form a box. The inner chamber with attached papers is then carefully inserted into the outer chamber so that no part of the inner chamber is exposed outside and the rear door cover can be attached. The front door is then attached to the chamber together with the front escape funnel. The rear metal door cover is attached. A Plexiglas holding frame is attached to secure the entire system. A receiving box, 6 x 6 x 6 cm^3, is constructed of stiff paper carton material with screen netting on top for observation of escaped mosquitoes. The box has a square hole the same size of the outward projection of the escape funnel and is attached to the exterior exit portal of the chamber. A hole, 5 cm diameter and sealed with a piece of split dental dam, is placed on the front face of the receiving cage to allow collection of the escaped mosquitoes with an aspirator.

Test methods and analysis have been described elsewhere (Sungvornyothrin et. al. 2001, Roberts et al. 1997). Only female specimens are used in excito-repellency tests. After a test is completed (30 or 60-min exposure times), the number of dead and live specimens is recorded separately in the exposure chamber and receiving box. Immediately following the test, all live specimens in control and treatment test chambers are maintained separately by lot (escaped or nonescaped) and given a 10% sucrose diet to observe post-exposure 24-h mortalities.

This recent version of the excito-repellency test system has been used to measure the behavioral responses of *An. minimus* and *Anopheles dirus* laboratory colonies exposed to paper surfaces impregnated with 2 g/m^2 DDT and 20 mg/m^2 deltamethrin. Results showed that female *An. minimus* and *An. dirus* demonstrated a dramatic escape response to DDT and deltamethrin compared to mosquitoes exposed to the untreated control chambers. A more rapid response in time to escape to both insecticides was observed with *An. minimus* compared to *An. dirus*. Results revealed that most specimens escaped the test chamber without acquiring a lethal dose at 24-h post-exposure. DDT and deltamethrin demonstrated a small degree of non-contact repellency with both species. Details from these excito-

repellency tests will be reported in a future study. This modified excito-repellency test system is a vast improvement, with more desirable operational attributes compared to previous designs regarding ease of use and reproducibility of test observations. The entire test system is easy to assemble and can be disassembled in minutes. It is also much easier to remove remaining mosquitoes from the test chambers after the test is completed compared to the previous versions. We have found the new test system can generate consistent and standardized results for measuring mosquito behavioral avoidance and separate contact irritancy and non-contact repellency responses.

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