Evaluation of a “fogging” canister for indoor elimination of adult Aedes aegypti

Pang Sook Chenga, Foo Siew Yoonga, Png Ah Bahb, Deng Luca, Lam-Phua Sai Geka, Tang Choon Siangb and Ng Lee Chinga#

aEnvironmental Health Institute, National Environment Agency, 11 Biopolis Way #06-05/08, Helios Block, Singapore 138667
bEnvironmental Health Department, National Environment Agency (EHD), 40 Scotts Road, Environment Building, #21-00, Singapore 228231

Abstract

Aedes aegypti is the primary vector for dengue transmission in Singapore. In response to dengue transmission, ultra-low volume (ULV) spraying of insecticides in apartments in transmission areas is used to supplement source-reduction effort. This study seeks to determine the efficacy of Mozzie Zap, a “fogger” in an aerosol canister, which contains 1.7% cypermethrin and 3.4% piperonyl butoxide (PBO, a pesticide synergist). The product, which has the unique continual release mechanism, was tested using two canisters in a two-bedroom apartment block. Caged female adult mosquitoes and magnesium oxide slides were positioned prior to the release of the chemicals. All bioassays and slides were exposed for 30 minutes before removal. Subsequent residual activity tests were carried out using WHO contact bioassay cones on horizontal (floor) and vertical (wall) surfaces. Mozzie Zap’s fog produced fog of effective droplet size (average VMD=24.32 µm), and achieved 100% mortality against Ae. aegypti. However, no residual activity on wall surfaces (3.1% on Day 2) was observed.

Keywords: Aedes aegypti; ULV spray; apartments; Singapore.

Introduction

Aedes aegypti is the primary vector of dengue transmission in Singapore. A programme based on source reduction through public education backed by law enforcement was set in place in 1970. This led to a period of low dengue incidence that lasted for more than a decade. However, from the 1990s, the incidence of dengue has increased despite the control measures. Possible contributory factors include increase in human population and their activities, lowered immunity levels and increased frequency of travel. To address the dengue challenge, Singapore’s dengue control programme has evolved into an integrated programme that focuses on inter-epidemic field and laboratory surveillance, coupled with intersectoral cooperation for source reduction. The programme is continuously evolving...
through operational research and exploration, and the application of innovative products which are accepted by the community and can be used easily.

In response to dengue transmission, ultra-low volume (ULV) spraying of insecticides in apartments where transmission takes place is used to supplement source-reduction efforts.[2] However, ULV spraying causes inconvenience. The equipment requires a power source as well as trained officers to operate. The inconvenience caused and the negative perceptions about fogging often lead to resistance by residents.

We thus explored the effectiveness of Mozzie Zap, which offers an alternative insecticide delivery mechanism: continuous release of chemicals through an aerosol canister. The canister contains 1.7% cypermethrin and 3.4% piperonyl butoxide (PBO) with citrus scent. PBO, a pesticide synergist, is typically added to synthetic pyrethroids and natural pyrethrins to negate the effect of resistance to the insecticides. It is a well-known inhibitor of enzymes that are necessary for detoxification of chemicals and transformation of hormones.[3] This study aimed to determine the feasibility of using Mozzie Zap, which has the unique design of continual release mechanism, in Singapore’s typical housing units for the control of dengue and chikungunya vector, *Ae. aegypti*.

**Materials and methods**

**Site**

Three two-bedroom, government-built apartments, each having an area of 71.27 sq. metre, were used as test sites for both efficacy and residual tests. The walls within each unit are made of painted plastered cement while floors are made of wooden parquet, ceramic tiles and marble tiles. Two units were used for Mozzie Zap spraying and one unit was left as control.

**Mosquitoes**

*Ae. aegypti* larvae, collected throughout the island of Singapore, were reared into adults in the insectary. Unfed, 3–5-day-old F2 progeny of the mosquitoes were used to test the efficacy and residual effect of Mozzie Zap.

**Efficacy test**

Mosquitoes were transferred into cylindrical mesh cages (5 cm diameter × 15 cm height) prior to the spraying day. Two cans of Mozzie Zap were used for each flat as recommended by the supplier (Trojan Hospitality Asia Pte Ltd). Cages were hung at 11 randomly selected points, approximately 1.2 m high from ground level, before the canister was triggered to release the chemicals. All windows and the main door leading to the corridor were shut during the entire exposure period. Thirty minutes after the release of the insecticide, mosquitoes were removed from the apartments and transferred instantaneously into clean cups with 10% sucrose as food source. Per cent mortalities at removal, 10 minutes, 1 hour and 24 hours were scored. Control was carried out in a similar manner except that no insecticide was released in the apartment.

**Residual test**

WHO guidelines for testing mosquito adulticides for indoor residual spraying and treatment of mosquito nets were adopted for this study with little modification.[4] The residual test was done two days after the spraying. Bioassay cones were attached to sixteen points identified for each apartment – 11 points on
Evaluation of a “fogging” canister for indoor elimination of adult *Aedes aegypti*

Ten mosquitoes were introduced into each bioassay cone and aspirated out after 30 minutes. Ten per cent sucrose was supplied as the food source and the mortality was scored 24 hours post-exposure. The test was discontinued if the mortality dropped below 70%.\(^5\)

**Droplet size analysis**

Insecticidal droplets were sampled at five points within a unit using magnesium oxide-coated slides. Distances from canister to slides ranged from 0.81 m to 6.58 m. Four slides were placed at each point, with three slides placed horizontally at 0 m (ground level), 1.2 m and 2.3 m, and one slide was placed parallel to the wall surface at a height of 1.2 m. At least 100 droplets were measured at a magnification of 100 times under a compound microscope. Droplets-size data were analysed by Sofield and Kent (1984) and expressed in terms of Volume Median Diameter (VMD), Number Median Diameter (NMD) and ratio of VMD and NMD.

**Results and discussion**

Mozzie Zap attained a high efficacy of 100% mortality at 24 hours post-exposure in the typical Singapore apartment. Of 220 mosquitoes exposed to Mozzie Zap aerosols, 201 mosquitoes were found moribund or dead after 30 minutes of exposure (Table 1). Knockdown effect is a unique characteristic of pyrethriods.\(^6\) A subsequent observation of the mortality was made to ensure that the actual mortality was recorded. The mortality was observed to increase to 99.5% after one hour of post-exposure. As the cages of mosquitoes were placed as far away as 6.04 m from the canister, the total kill of mosquitoes in the room showed that aerosols from the canister were able to reach out to at least 6.04 m to kill *Ae. aegypti* adults.

Magnesium oxide slides were placed at allocated points, with some points in close proximity to the mosquito cages. Slides were adhered vertically (wall surfaces) and horizontally (floor surfaces) prior to spraying to capture insecticidal droplets. The results indicated that the slides placed vertically captured only a few droplets, averaging 0.4 droplets per optical field. In contrast, there were more than 200 droplets per optical field impinged on the slides that were placed horizontally at a distance between 1–2 m. Table 2 shows the inverse relation between the number of droplets and the distance from the dispensing canister. The droplet size of the aerosols ranged from 18.84 to 30.16 µm, with an average VMD value of 24.32 µm. They fall within the range considered to be the most effective droplet size for killing

**Table 1: Efficacy results of Mozzie Zap against *Ae. aegypti***

<table>
<thead>
<tr>
<th>Treatment</th>
<th>N</th>
<th>No. of mosquitoes knocked down upon removal (% knockdown)</th>
<th>No. of mosquitoes knocked down at 1 hr post exposure (% knockdown)</th>
<th>No. of mosquitoes moribund or dead (24-hrs % mortality)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mozzie Zap treated</td>
<td>220</td>
<td>201 (91.4%)</td>
<td>219 (99.5%)</td>
<td>220 (100%)</td>
</tr>
<tr>
<td>Control</td>
<td>110</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
</tbody>
</table>
adult mosquitoes.\textsuperscript{[7]} It is apparent that the droplet size upon dispensing is critical for an effective coverage of sprayed areas as large insecticidal droplets are uneconomical and have the tendency to deposit on the ground near the dispenser while droplets that are too small contain insufficient dosage to kill a mosquito.\textsuperscript{[7,8]}

It is interesting to note that Mozzie Zap was very effective despite a previous finding that \textit{Ae. aegypti} from Singapore, in general, has developed resistance against cypermethrin with an average RR50 and RR95 values of 53.8 and 62.3 respectively (unpublished data). This could be explained by the synergist effect of the mixture of cypermethrin and PBO included in Mozzie Zap, thus increasing the mortality rate. A significant increase in mortality rate (Synergist Ratio= 21.22) was reported after the addition of four parts of PBO into one part of cypermethrin.\textsuperscript{[6]} However, the study also showed that the level of synergism of PBO with synthetic pyrethroids depends greatly on the types of insecticide resistance mechanisms for individual insecticides, and multiple resistance mechanisms in mosquitoes increased the complexity further.

A study is underway to further understand the resistance and the effect of PBO on local mosquitoes. Nevertheless, the mixture, continuously dispensed through the novel nozzle, was found to be effective in killing local mosquitoes. Being handy, user-friendly and less intimidating, Mozzie Zap’s design may increase the acceptability of insecticide treatment of homes by residents. It will also reduce manpower needs. Approximately 80% of Singapore residents live in similarly built

\textbf{Table 2:} Comparison between droplet size and residual activities for floor and wall surfaces

<table>
<thead>
<tr>
<th>Distance from canister (m)</th>
<th>Orientation</th>
<th>24 hours % mortality</th>
<th>VMD (µm)</th>
<th>VMD/NMD</th>
<th>No. of droplets/ optical field</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Day 2</td>
<td>Day 3</td>
<td>Wk 1</td>
<td>Wk 2</td>
</tr>
<tr>
<td>0.81</td>
<td>Floor</td>
<td>90</td>
<td>100</td>
<td>100</td>
<td>95</td>
</tr>
<tr>
<td></td>
<td>Wall</td>
<td>___</td>
<td>___</td>
<td>___</td>
<td>___</td>
</tr>
<tr>
<td>2.00</td>
<td>Floor</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>85</td>
</tr>
<tr>
<td></td>
<td>Wall</td>
<td>0</td>
<td>___</td>
<td>___</td>
<td>___</td>
</tr>
<tr>
<td>3.10</td>
<td>Floor</td>
<td>90</td>
<td>80</td>
<td>50</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>Wall</td>
<td>0</td>
<td>___</td>
<td>___</td>
<td>___</td>
</tr>
<tr>
<td>4.70</td>
<td>Floor</td>
<td>100</td>
<td>85</td>
<td>75</td>
<td>15.6</td>
</tr>
<tr>
<td></td>
<td>Wall</td>
<td>0</td>
<td>___</td>
<td>___</td>
<td>___</td>
</tr>
<tr>
<td>6.58</td>
<td>Floor</td>
<td>70</td>
<td>37.1</td>
<td>12.5</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Wall</td>
<td>___</td>
<td>___</td>
<td>___</td>
<td>___</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Average number of droplets per optical field on floor surfaces is 195.
\textsuperscript{b} Average number of droplets per optical field on wall surfaces is 0.4.
\textsuperscript{c} No droplet was collected on slide.
\textsuperscript{d} No bioassay done at these distances.
government apartments, and the protocol for its usage in these apartments would be simple
and straightforward.

The results of the residual activities of the insecticides on horizontal and vertical surfaces are presented in Table 3. Residual effects on vertical surfaces attained only 1.1% mortality after a two-day post-spraying for all 11 bioassay cones in both apartments. Residual test for vertical bioassay cones was stopped after Day 2 since the results were consistently low between the two apartments. Bioassays on floor surfaces showed residual activity of the insecticide three days post-spraying, with 75.4% mortality. The mortality was found to increase with a decrease in the distance from the canister (see Figure).

The behaviour of the mosquitoes in the WHO contact bioassay cones was observed during the exposure periods. No significant sign of avoidance of insecticide-treated surface

<table>
<thead>
<tr>
<th>Surfaces</th>
<th>Days post-spraying</th>
<th>Average % mortality at 24 hours (± standard deviation)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wall</td>
<td>D2</td>
<td>1.6 (± 2.25)</td>
</tr>
<tr>
<td>Floor</td>
<td>D2</td>
<td>91.7 (± 7.07)</td>
</tr>
<tr>
<td></td>
<td>D3</td>
<td>75.4 (± 25.42)</td>
</tr>
<tr>
<td></td>
<td>Wk1</td>
<td>64.6 (± 10.01)</td>
</tr>
<tr>
<td></td>
<td>Wk2</td>
<td>39.9 (± 9.00)</td>
</tr>
</tbody>
</table>

was noticed. However, rapid-flight escape to areas or surfaces without insecticides was observed, demonstrating significant contact irritancy of *Ae. aegypti*. The observations were consistent with other studies on *Aedes* response to synthetic pyrethroids.[9,10]

**Figure**: Residual activities of Mozzie Zap on different floor surfaces against *Ae. aegypti*
Despite good residual activities shown on the floor surface, the feasibility of using Mozzie Zap’s continuous release mechanism for residual spray is uncertain, as frequent cleaning of floors and horizontal surfaces is commonly practised by most residents. Nevertheless, Mozzie Zap has the application potential for eliminating indoor Ae. aegypti in an apartment, which is especially important for dengue outbreak areas.

Acknowledgements

We would like to thank Mr Paranjothy Charles Joseph from M/s Trojan Hospitality Asia Pte. Ltd. for generously providing us with the supply of insecticides for this study. Also, we wish to extend our gratitude to the South-West Regional Office of the National Environment Agency for their assistance, and the Housing Development Board (HDB) for providing the facilities for this study.

References


