Field observation on the efficacy of Toxorhynchites splendens (Wiedemann) as a biocontrol agent against Aedes albopictus (Skuse) larvae in a cemetery

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Abstract. This study explored the efficacy of Toxorhynchites splendens, predator of Aedes albopictus as a biocontrol agent. There was a negative correlation between Ae. albopictus larval population and Tx. splendens larval population in ovitraps (r= -0.287, R²=0.0821). The correlation is higher between the mean number of Ae. albopictus larvae per ovitrap and the number of Tx. splendens larvae in an ovitrap (r=-0.987, R²=0.9737). Larvae of Tx. splendens were observed to co-exist with larvae of Ae. albopictus and Culex fuscocephala in the ovitraps placed in the study area. The existence of Tx. splendens larvae in the study area coincides with their habit, preferring to breed in bamboo stumps. A total of 480 ovitraps were inspected for 30-week study period and 281 ovitraps were positive with Ae. albopictus larvae respectively. There was a significant difference between numbers of ovitrap positive for Ae. albopictus larvae with number of Tx. splendens larvae in the ovitraps (ANOVA, F (4,475) 2.655, p< 0.05). Of 281 ovitraps positive with Ae. albopictus larvae, 255 ovitraps contained only one Tx. splendens larva each. Only one ovitrap contained four, the most number of Tx. splendens larvae (p< 0.05). Thus, Tx. splendens could be utilised as an alternative for dengue vector control programme.

INTRODUCTION

Dengue fever and dengue haemorrhagic fever (DF/DHF) vector control strategies consist of Aedes survey, source reduction, law enforcement, health promotion and chemical control (KKM, 1986). Frequent usage of insecticides in vector control as well as in the agricultural sector has created tolerant, resistant and cross resistant problems in insects (Kumar & Hwang, 2006).

Aedes aegypti is reported to be tolerant to temephos in Malaysia (Lee, 1991), resistant to permethrin, lambda-cyhalothrin and deltamethrin in South Vietnam (Vu & Nguyen, 1999). Ae. aegypti is also resistant to permethrin even though permethrin is no longer used in vector control in Singapore since nine years ago (Ping et al., 2001). Aedes aegypti larval resistance to temephos was reported in Brazil (Lima et al., 2003) and in Thailand (Ponlawat et al., 2005).

Two Aedes albopictus strains in Thailand were resistant to chlorpyrifos, fenithrothion, malathion, fenthion and temephos (Wesson, 1990). All strains of Ae. albopictus larvae studied in Thailand were reportedly resistant from low to moderate levels to permethrin and resistant at low levels to temephos, permethrin and malathion (Ponlawat et al., 2005). Cyfluthrin was recently introduced in Thailand and Ae. albopictus was found to be tolerant due to cross resistance to permethrin and deltamethrin that have the same mechanism of action (Paeporn et al., 2005). In Malaysia, Ae. albopictus adults were
reportedly resistant to DDT and permethrin (Lee et al., 1998). DDT was used for many years in malaria control while permethrin was only introduced in 1995 to treat mosquito nets in DF/DHF vector control.

Biological control through the usage of predators and other biological agents is gaining interest as a suite of alternative control measures. Introduction of predators which can breed in the environment may provide a continuous control. Vector-borne disease control strategies which were emphasized on eliminating preimaginal stages are more effective as compared to adult control which is not very effective and environmental friendly (Kumar & Hwang, 2006). Among natural predators of Aedes spp. larvae studied were Toxorhynchites in the Amazon basin (Hutchings, 1994), and in the Philippines (Panogadia-Reyes et al., 2004); and Mesocyclops in Brazil (Kay et al., 1992), and in Vietnam (Vu et al., 1997, 1998).

Studies on Toxorhynchites spp. as biological agents for mosquito larval control conducted in several countries showed promising results. Toxorhynchites splendens life table studies were carried out in Singapore (Chan, 1968) and in Thailand (Chowanadisai et al., 1983). Mass production and mass release of Tx. splendens for Ae. albopictus larval control was carried out in Japan (Miyagi et al., 1992) and Toxorhynchites ambionensis in Indonesia (Annis et al., 1990). Colonization and mass production of Tx. splendens in small cages was explored by Choochote et al., 2002 in Thailand. Other species of Toxorhynchites studied were Toxorhynchites rutilus (Coquillett) on Ae. aegypti larvae (Focks et al., 1982) and Toxorhynchites moctezuma (Tikasingh & Eustace, 1992).

In this study, a cemetery was chosen due to high Aedes population in this area. High Aedes populations in cemeteries were also reported by Schlutz (1989) in the Philippines and Vezzani & Schweigamann (2002) in Buenos Aires. The objective of this study was to evaluate the correlation between populations of Tx. splendens larvae with population of Ae. albopictus larvae.

MATERIALS AND METHODS

The study was carried out at a muslim cemetery (01° 27’N, 103° 45’E) which is situated approximately five km from Johor Bahru City center, Johor, Malaysia. Vegetation found in this area included Fagraea fragrans Roxb. (family Loganiaeae), Prunus avium (family Rosaceae), Codiaeum variegatum (Lod.) (family Euphorbiaceae), Cananga odorata (Lam.) (family Annonaceae), Jasminum officinale L. (Family Oleaceae), Michelia champaca Linn (family Magnoliaceae) and Mangifera indica L. (family Anacardiaceae). Sixteen ovitraps were placed at four randomly chosen plots. The study lasted for 30 weeks from June 2003 to January 2004. Precipitation data for the 30-week study period was provided by the Department of Irrigation and Drainage, Johor.

Ovitraps were made of 1000 ml Nescafe® bottles measuring 8.1 x 8.1 x 19.2 cm. Nescafe® bottles were cleaned and painted black on the outside, left to dry and then washed to get rid of paint fumes. The advantage of using 1000 ml Nescafe® bottles as ovitraps are 1) they could hold a larger volume of water than the conventional ovitraps and 2) water would not dry up easily between weekly inspections. Conventional ovitraps used by several researchers were made of 500 ml black glass jars or black plastic pots (Fay & Eliason, 1966; Kloter et al., 1983; Santos et al., 2003).

Ovitraps containing 1000 ml water were placed in the open and inspected weekly. Data recorded were number of larvae and pupae for Tx. splendens, Ae. albopictus and Cx. fuscocephala. All larvae and pupae were then returned to the respective ovitraps. These ovitraps served as breeding sites for Ae. albopictus, Tx. splendens and Culex fuscocephala. Tx. splendens occur naturally in the study site. Female Tx. splendens and other mosquito spp. laid eggs in the ovitraps placed in the study site. Eggs hatched and developed into larvae.

Statistical analysis were carried out using SPSS programme version 11.5 (ANOVA and Correlation).
RESULTS

Larvae of *Tx. splendens* were observed to co-exist with larvae of *Ae. albopictus* and *Cx. fuscocephala* in the ovitraps placed in the study area. The existence of *Tx. splendens* larvae in the study area coincides with their habit, preferring to breed in bamboo stumps. A total of 480 ovitraps were inspected during the 30-week study period and 281 ovitraps were positive with *Ae. albopictus*. The Ovitrap Index (OI) for *Ae. albopictus* was 58.5. Of 281 ovitraps positive with *Ae. albopictus* larvae, 255 ovitraps contained only one *Tx. splendens* larva each. The most number of *Tx. splendens* larvae found in ovitraps were four and only one ovitraps contained four *Tx. splendens* larvae each. The number of *Tx. splendens* larvae per ovitrap increased the occurrence of *Ae. albopictus* larvae decreased. Similar results were obtained by Trpis (1973) and Focks et al. (1982). Trpis (1973) in Tanzania recorded the frequency of receptacles containing one *Tx. brevipalpis* larva in one receptacle was placed in a small container without *Ae. albopictus* larvae as prey. *Tx. splendens* larvae were reported to survive without food (Amalraj et al., 2005) for a month (Chan, 1968).

DISCUSSION

Even though the correlation between larval population of *Tx. splendens* and *Ae. albopictus* was weak, the correlation between the number of *Tx. splendens* larvae per ovitrap and the number of ovitrap positive with *Ae. albopictus* larvae was high. As the number of *Tx. splendens* larvae per ovitrap increased, the number of ovitrap positive with *Ae. albopictus* larvae decreased. Similar results were obtained by Trpis (1973) and Focks et al. (1982). Trpis (1973) in Tanzania recorded the frequency of receptacles containing one *Tx. brevipalpis* larva in one receptacle was

<table>
<thead>
<tr>
<th>Number of <em>Tx. splendens</em> larvae/ovitrap</th>
<th>Number of ovitrap positive for <em>Ae. albopictus</em> larvae</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>199</td>
</tr>
<tr>
<td>1</td>
<td>255</td>
</tr>
<tr>
<td>2</td>
<td>22</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>480</td>
</tr>
<tr>
<td>p-value</td>
<td>P &lt; 0.05</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Number of <em>Tx. splendens</em> larvae per ovitrap</th>
<th>Mean number <em>Ae. albopictus</em> larvae per ovitrap ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>14.22 ± 1.664</td>
</tr>
<tr>
<td>1</td>
<td>9.19 ± 0.840</td>
</tr>
<tr>
<td>2</td>
<td>7.14 ± 2.475</td>
</tr>
<tr>
<td>3</td>
<td>2.67 ± 2.667</td>
</tr>
<tr>
<td>4</td>
<td>1.00</td>
</tr>
<tr>
<td>p-value</td>
<td>P &lt; 0.05</td>
</tr>
</tbody>
</table>
Figure 1. Correlation between *Tx. spendens* and *Ae. albopictus* larvae

![Graph showing correlation between *Tx. spendens* and *Ae. albopictus* larvae with the equation \( y = -0.0145x + 12.912 \) and \( R^2 = 0.0821 \).]

Figure 2. Correlation between *Cx. fuscocephala* and *Ae. albopictus* larvae

![Graph showing correlation between *Cx. fuscocephala* and *Ae. albopictus* larvae with the equation \( y = -0.1513x + 182.97 \) and \( R^2 = 0.0057 \).]

Figure 3. Correlation between *Tx. splendens* and *Cx. fuscocephala* larvae

![Graph showing correlation between *Tx. splendens* and *Cx. fuscocephala* larvae with the equation \( y = -0.0085x + 10.653 \) and \( R^2 = 0.007 \).]
the most as compared to two or more Tx. brevipalpis larvae per receptacle. Focks et al. (1982) in the study carried out in Algiers reported that reduction in number of Ae. aegypti and Cx. quinquefasciatus exuviae as the number of Tx. rutilus larvae increased in receptacles.

In this study, larvae of Tx. splendens, Cx. fuscocephala and Ae. albopictus cohabitated in ovitraps laid. The presence of Tx. splendens in ovitraps affected Ae. albopictus larval population but not that of Cx. fuscocephala. It was observed that Tx. splendens preference is for Ae. albopictus...
larvae rather than Cx. fuscocephala eventhough the latter population was higher. It was observed that Ae. albopictus larvae moved actively compared to Cx. fuscocephala and this characteristic attracted Tx. splendens to feed on them. Culex fuscocephala larvae restricted their movement and became less active. This habit was also observed in Ochlerotatus triseriatus (Say) in the presence of Tx. rutilus (Coquillett) in the study conducted by Kesavaraju & Juliano (2004). Ochlerotatus triseriatus took less food, restricted their movement and opted to be resting. Juliano & Gravel (2002) reported that Aedes triseriatus larvae restricted their movement and preferred to be near the water surface in order to avoid predation. Also observed in this study that in the absence of Ae. albopictus in the ovitraps, only then did Tx. splendens feed on Cx. fuscocephala. Aditya et al. (2006) in laboratory studies in India also reported that Tx. splendens fed on Cx. quinquefasciatus larvae when no other prey were provided.

Another finding was that in the absence of Tx. splendens, there was a negative correlation between Ae. albopictus larval population and Cx. fuscocephala larval population. This could be due to the possibility of metabolites produced by Ae. albopictus larvae that caused the water to become unsuitable for Cx. fuscocephala larvae to survive. Peters et al. (1969) reported that metabolites produced by Ae. aegypti larvae have significant effect on Culex pipiens larval mortality.

Correlation between precipitation and Tx. splendens larval population and between precipitation and Ae. albopictus larval population were both negative. Similar finding in a study in paddy field was reported by Das et al. (2006) that there was no positive correlation between predators of Culex vishnui (such as Notonectidae, Hydrophilidae and Gerridae) and precipitation in Pondicherry, India.

In urban areas, Tx. splendens breed in densely vegetated areas such as cemeteries, botanical gardens and other areas where there were less human activities which contradicted the finding by Chan (1968). In this study, Tx. splendens bred naturally in Mahmoodyah Cemetery and no adult serial releases were carried out. According to Kumar & Hwang (2006), predators found naturally in the environment are safe to people and economical in their propagation. Characteristics of Tx. splendens which proved to be good habits are ability to survive without food (up to one month) and ability to consume up to three or four Ae. albopictus generations throughout its life span (Chan, 1968).

In conclusion, use of Toxorhynchites spp. should be promoted as an alternative for vector control in certain situations to alleviate insecticide resistant problems as reported in many countries. Toxorhynchites spp. are environmental friendly and attack larval stages which could provide more effective control.

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