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A study on the breeding patterns of *Toxorhynchites splendens* and *Aedes albopictus* in the natural environment

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Abstract

The study aimed to investigate the breeding patterns of *Toxorhynchites* and *Aedes* mosquitoes in Kesbawa in the District of Colombo. The number of *Ae. albopictus* larvae per tyre dropped markedly with increasing number of *Tx. splendens* larvae per tyre (Pearson correlation = -0.588, $P<0.05$). *Ae. albopictus* pupae per tyre with a weekly average of 6.7 was recorded in the presence of *Tx. splendens* larvae with a value of 1.7 weekly average. *Tx. splendens* larvae per tyre per visit showed positive linear correlation with temperature (Pearson correlation= 0.622, $P<0.05$), negative linear correlation with rainfall (Pearson correlation = -0.521, $P<0.05$) and there was no linear correlation with relative humidity (Pearson correlation= 0.020, $P>0.05$). Container Indices (CI) of 50.00% and 12.50% for cement tanks and tyres respectively were recorded for *Tx. splendens* while the respective values for *Ae. albopictus* amounted 50.00% and 62.50%. For discarded receptacles and others such as bird baths the values were respectively 2.45% and 0% for *Tx. splendens* and 27.87% and 50.00% for *Ae. albopictus*.

Key Words : *Texorhynchites Splendens, Aedes albopictus*

Introduction

A dengue vaccine is not available; transmission can only be reduced by measures against the vectors, *Ae. aegypti* and *Ae. albopictus*. In view of the expansion of geographic range of the vector, it is possible that the role of *Ae. albopictus* in the transmission of dengue (Gubler and Kuno, 2004) and chikungunya (Tsetsarkin *et. al*., 2007) will increase. Previous studies have indicated that *Ae. albopictus* occur in greater abundance than *Ae. aegypti* in several areas of Sri Lanka that have experienced out breaks of dengue in the past (Hapugoda *et al*., 2003).

The need for new methods of vector control is becoming increasingly evident, due to the phenomenon of pesticide resistance increasing environmental concern, and the widespread dependence on insecticides. Use of efficient biocontrol agents could provide solutions to these problems. One biological system reconsidered for mosquito control is to employ the predatory larvae of *Toxorhynchites* species (Toma and Miyagi, 1992). *Toxorhynchites* mosquitoes have been used as biological control agents against *Aedes* species in some countries as an additional control measure because the adult *Toxorhynchites* do not feed on blood and cannot act as vectors of disease. In addition female *Toxorhynchites* mosquitoes oviposit into pools of water that are not accessible to chemical control methods (Collins and Blackwell, 2000). Mated female *Toxorhynchites* releasing programs have an advantage in that they can disperse and lay eggs in places where source reduction is not possible (Weerasuriya *et. al*., 2003).

However, biological control using *Toxorhynchites spp* has not always been successful, often because the introduced *Toxorhynchites spp* populations have not always become established and, even if established *Toxorhynchites spp* populations have frequently failed to give an adequate level of control of pest mosquitoes (Service, 1983). Little effort has been spent on studying predation dynamics in natural habitats or evaluating their efficacies in reducing mosquito vector densities (Mercer *et al.*, 2005). The present study was carried out in order to determine the efficacy of *Tx. splendens* in reducing *Ae. albopictus* in the natural environment, the breeding site choices of *Tx. splendens* and *Ae. albopictus* and the correlations of *Tx. splendens* population with climatic factors.

Materials and Methods

The study was carried out at Gonamaditta in the Kesbawa Pradeshiyasaba area which is in the Colombo District of the Western Province of Sri Lanka. The selected site is approximately $2km^2$ in an area bounded by Piliyandala Horana main road on the North, Kesbewa wewa on the East and Paddy fields towards the South and West. The study site can generally be termed a rural setting with large trees and other vegetation surrounding human habitations.

A total of six motor car tyres filled with 500ml of water in the bottom side were placed in six selected outdoor stations 100m apart. Larval breeding in these were sampled and the number of *Aedes* larvae, pupae and *Toxorhynchites* larvae were recorded at the end of one week, after which the water was discarded and the tyres were refilled with water. *Aedes* larvae from each tyre were collected in separate containers and identified in the laboratory using standard keys (WHO, 1995). *Toxorhynchites* larvae collected were brought to the laboratory, reared and released to the same locality again. Rain fall, temperature and relative humidity data for the area were obtained from the Meteorological Department, Sri Lanka.

Additionally larval surveillance limited only for outdoor potential breeding habitats was also carried out in 100 randomly selected premises (including houses, temples, schools, building sites). All potential breeding habitats of *Aedes* and *Toxorhynchites* species were examined for their larval breeding and 10 mature *Aedes* larvae from each container were collected in separate containers and identified in the laboratory using standard keys (WHO, 1995). If a container had less than 10 larvae, all larvae were collected. (Kusumawathie and Fernando, 2003). Pipetting, siphoning and dipping techniques were used for collecting larvae depending on the nature of the breeding habitat. The percentage of positive containers for *Aedes Toxorhynchites* is termed as Container Index (CI).

Results

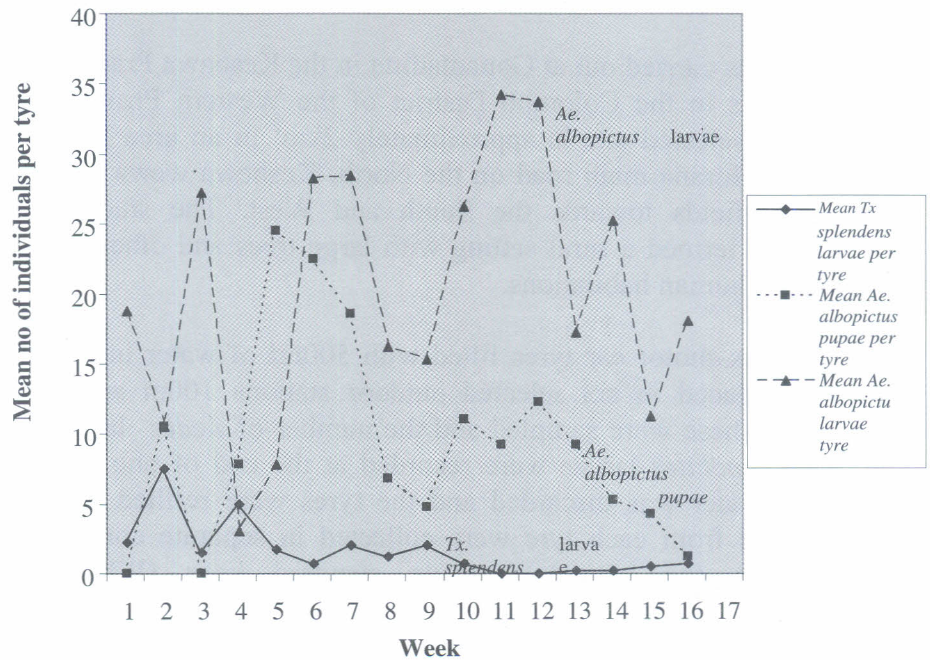

Figure 1. Mean number of *Ae. albopictus* larvae, pupae and *Tx. splendens* larvae per tyre per week

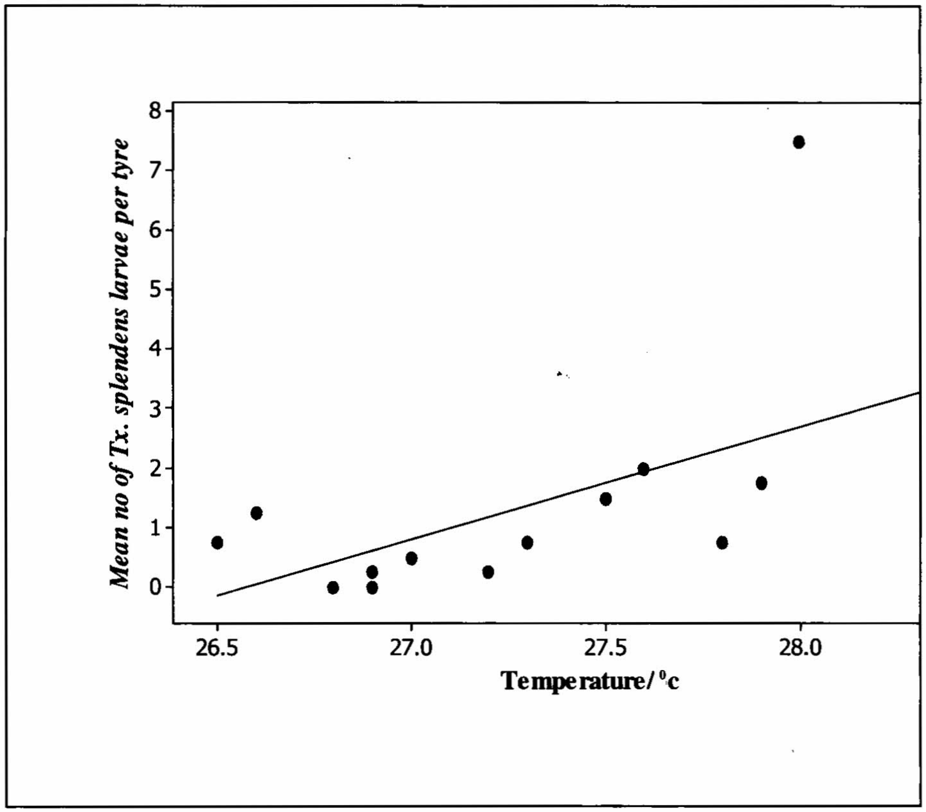

Figure 2. Mean number of *Tx. splendens* larvae per tyre with weekly temperature

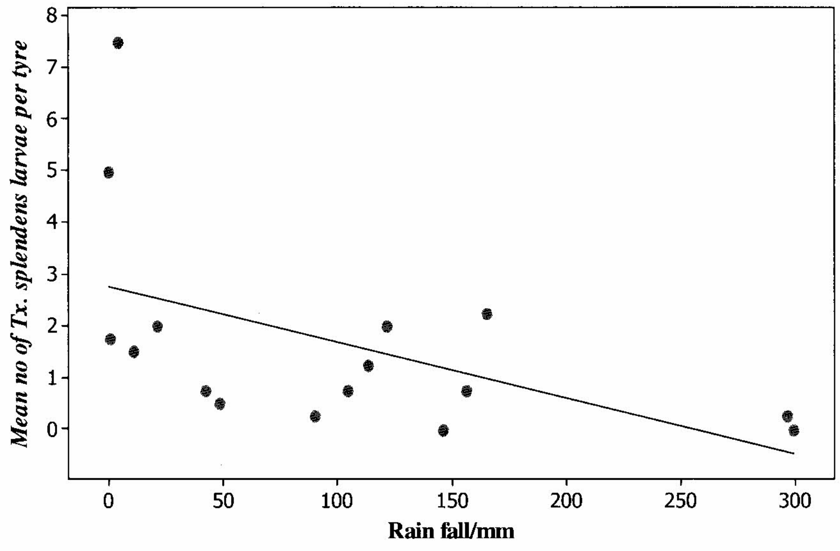

Figure 3. Mean number of *Tx. splendens* larvae per tyre with weekly rain fall

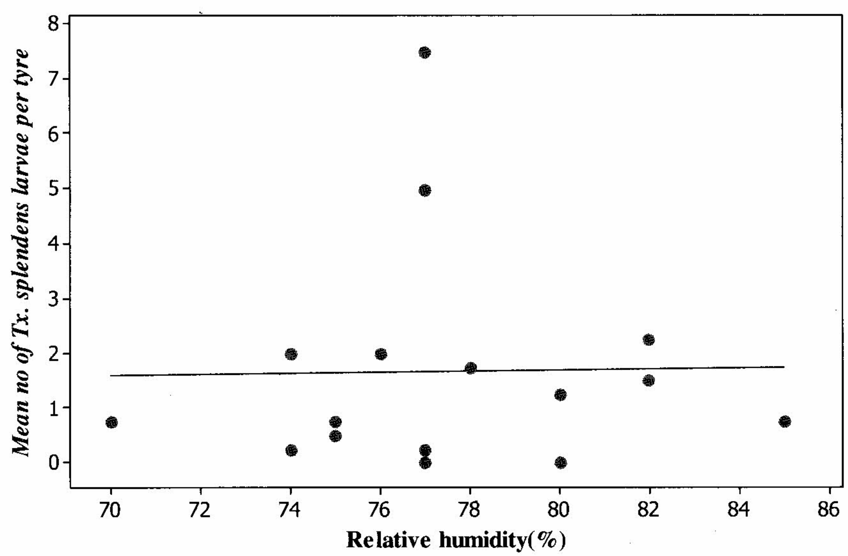

Figure 4. Mean number of *Tx. splendens* larvae per tyre with weekly relative humidity

Table 1. Breeding site choices and CI values of *Aedes albopictus* and *Toxorhynchites splendens* in Gonamaditta Area, Kesbewa

Container type	Potential containers	Total positive containers	Positive for *Ae. albopictus*	Positive for *Tx. splendens*	CI for *Ae. albopictus*	CI *Tx. splendens*
Cement tanks	4	3	2	2	50.00	50.00
Discarded receptacles	122	34	34	3	27.87	02.45
Tyres	8	5	5	1	62.50	12.50
Natural habitats	33	8	8	0	24.24	00.00
Others (Bird baths, etc)	16	8	8	0	50.00	00.00
Total	183	58	57	6	**	**

All the *Aedes* mosquito larvae found in the outdoor potential breeding habitats were *Ae. albopictus* while all the *Toxorhynchites* species were *Tx. splendens*. The number of *Ae. albopictus* larvae dropped markedly with increasing number of *Tx. splendens* larvae per tyre (Pearson correlation of mean *Ae. albopictus* larvae per tyre and mean *Tx. splendens* larvae per tyre = -0.588, $P<0.05$). A weekly average of 6.7 *Ae. albopictus* pupae per tyre was recorded in the presence of *Tx. splendens* larvae with a weekly average of 1.7 (Figure 1). *Tx. splendens* larvae per tyre per visit showed a positive linear correlation with temperature (Pearson correlation= 0.622, $P<0.05$) (Figure 2) and a negative linear correlation with rainfall (Pearson correlation = -0.521,

$P<0.05$) (Figure 3). No linear correlation was shown between relative humidity and the number of *Tx. splendens* larvae per tyre (Pearson correlation= 0.020, $P>0.05$) (Figure 4). Container indices of 50.00% and 12.50% were recorded for cement tanks and tyres respectively for *Tx. splendens* while the respective values for *Ae. albopictus* were 50.00% and 62.50%. For discarded receptacles, others and natural containers it was respectively 2.45%, 0% and 0% for *Tx. splendens* and 27.87%, 50.00% and 24.24% for *Ae. albopictus* (Table 1).

Discussion

All the *Aedes* species found in the outdoor breeding habitats were *Ae. albopictus* while all the *Toxorhynchites* species were *Tx. splendens*. It is mainly due to the rural setting with large trees and other vegetations surrounding human habitations in the study area, the known natural breeding habitats of *Toxorhynchites splendens* (Collins and Blackwell, 2000) and *Ae. albopictus* (Gubler and Kuno, 2004). Results of the present study indicate that the number of *Ae. albopictus* larvae per tyre dropped significantly with increasing mean number of *Tx. splendens* larvae per tyre (Pearson correlation = -0.588, $P<0.05$). *Ae. albopictus* pupae per tyre with a weekly average of 6.7 was recorded in the presence of *Tx. splendens* larvae with a weekly average of 1.7. This probably due to the prey – predator relationship of the two species. Also the higher value of *Ae. albopictus* pupae was likely due to early oviposition of *Ae. albopictus* and the lesser food preference for increased age classes of prey larvae by *Tx. splendens* as observed by Amalraj *et. al.* (2005).

Toma & Miyagi (1992) have reported that the oviposition of *Toxorhynchites* species was most prolific in sunny weather and much less in dull weather. The present study also indicated that the number of *Tx. splendens* larvae increased with increasing temperature (Pearson correlation =0.622, $P<0.05$) and range in between 27.5^0C – 28.5^0C was the most suitable temperature range. Bradshaw & Holzapfel (1984) cited that *Toxorhynchites* spp oviposit during the rainy season but not during the dry season. In the present study, a large number of *Tx. splendens* larvae were recorded at a rain fall of 170mm. However a lesser number of larvae were observed with higher rainfall more than 170mm. (Pearson correlation = -0.521, P<0.05). This situation can mainly cause due to the destruction of *Tx. splendens* breeding habitats through run off. An earlier study has shown breeding of *Tx. moctezuma* to be correlated strongly with ambient relative humidity with increased oviposition in response to falling ambient relative humidity (Jordan, 1992). But in the present study, *Tx. splendens* did not show linear correlation with relative humidity in the natural environment (P>0.05).

Container index values for *Tx. splendens* and *Ae. albopictus* indicated that the cement tanks and tyres were highly preferred by both these mosquito species. Discarded receptacles and others such as bird baths and water collections on floor were highly preferred by *Ae. albopictus* while *Tx. splendens* exhibited a very minimal preference for them. However a clay pot that produced a dark and cold environment was found positive for *Tx. splendens* larvae. Although a single larva of the predator mosquito was not found in natural containers such as tree holes, leaf axils etc., the possibility that these mosquitoes lay eggs in such places cannot be ruled out as these are known to be breeding habitats of Tx splendens.

Tx. splendens has been used successfully in Pudapet, a coastal village of India where there were significant reductions in the number of *Ae. aegypti* breeding in domestic water containers six months after treatment began (Collins and Blackwell, 2000). Second instar *Tx. splendens* larvae were also used successfully to suppress *Ae. aegypti* and *Ae. albopictus* breeding in domestic water containers in Malaysia (Chuah and Yap, 1984). The present study showed that both *Ae. albopictus* (CI=50%) and *Tx. splendens* (CI=50%) prefer cement tanks as their oviposition sites. These tanks were located in dark places covered with trees. Thus in areas where water storage tanks, specially cement tanks, have been identified as the most productive breeding sites of *Aedes* species, introduction of *Tx. splendens* is very likely to bring about effective suppression of *Aedes* breeding.

The use of *Toxorhynchites spp* is similar to other bio control techniques in that its use will involve the production of large numbers of the biotic agent. The few and usually brief accounts in the literature on mass production techniques for *Toxorhynchites* probably reflect the fact that it is not too difficult to rear species of this genus (Focks, 1982). In the present study a large number of fourth instars of *Tx. splendens* larvae were found in a cement tank in the presence of *Chironomid* larvae as prey. Therefore *Chironomid* larvae can be used as an alternative prey for the fulfillment of their dietary requirements to minimize the ethical problems which may arise in using *Aedes* larvae for the maintenance of *Tx. splendens* colonies.

The results of the present study are suggestive of the possibility of using *Tx. splendens* as an additional measure for the satisfactory control of *Ae. albopictus* breeding in man-made water collecting containers.

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