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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, R^2=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, R^2=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. aegpyti* 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 cemetaries 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 (01º 27'N, 103º 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 Loganiaceae), *Prunus avuim* (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 ovitrap contained four *Tx. splendens* larvae ($p< 0.05$) (Table 1). There was a negative correlation ($r = -0.843$, $R^2 = 0.7112$) between *Tx. splendens* larval density and number of ovitrap positive with *Ae. albopictus* larval density.

The mean number of *Ae. albopictus* larvae per ovitrap were explained by the number of *Tx. splendens* larvae in an ovitrap (Table 2). A high corrrelation between the two parameter ($r=-0.987$, $R^2=0.9737$) was observed. As the number of *Tx. splendens* larvae in an ovitrap increased the occurence of *Ae. albopictus* larvae decreased.

Figures 1-5 show the parameters were inversely correlated. There was a medium correlation between larval population of *Tx. splendens* and *Ae. albopictus* ($r= -0.287$; impact size $R^2=0.0821$) (Figure 1). The correlation and impact size between *Ae. albopictus* and *Cx. fuscocephala* ($r= -0.0754$, $R^2=0.0057$) (Figure 2), between *Tx. splendens* and *Cx. fuscocephala* ($r= -0.084$, $R^2=0.007$) (Figure 3) were low and extremely low, respectively.

A medium correlation between weekly precipitation and larval population of *Tx. splendens* ($r= -0.5933$, $R^2=0.3521$) is shown in Figure 4. Figure 5 shows a weak correlation ($r= -0.159$, $R^2=0.0252$) between precipitation and number of *Ae. albopictus* larvae.

It was observed that cannibalism also occurred among *Tx. splendens* larvae. This happened when *Tx. splendens* larvae were 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).

Table 1. Number of ovitrap positive for *Ae. albopictus* larvae for June 2003 to January 2004

Number of *Tx. splendens* larvae/ ovitrap	Number of ovitrap positive for *Ae. albopictus* larvae
0	199
1	255
2	22
3	3
4	1
Total	480
p-value	P< 0.05

Table 2. Mean number of *Ae. albopictus* larvae in ovitrap

Number of *Tx. splendens* larvae per ovitrap	Mean number *Ae. albopictus* larvae per ovitrap ± SE
0	14.22 ± 1.664
1	9.19 ± 0.840
2	7.14 ± 2.475
3	2.67 ± 2.667
4	1.00
p-value	P < 0.05

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

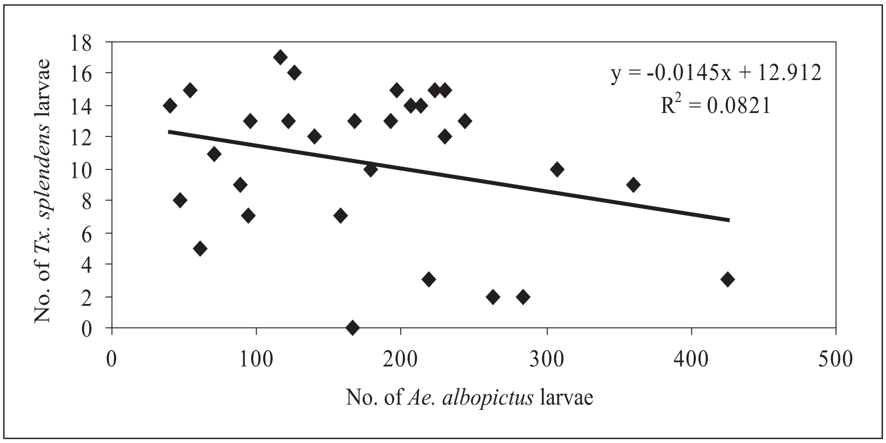

Figure 1. Correlation between *Tx. spendens* and *Ae. albopictus* larvae

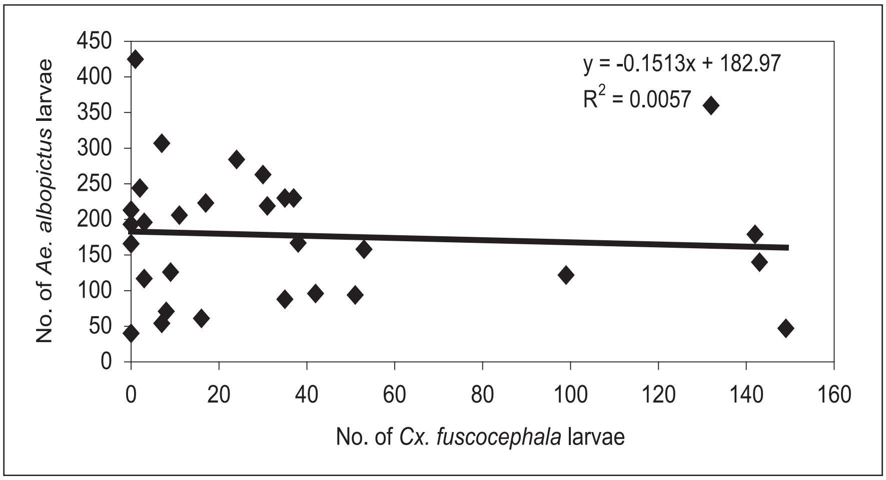

Figure 2. Correlation between *Cx. fuscocephala* and *Ae. albopictus* larvae

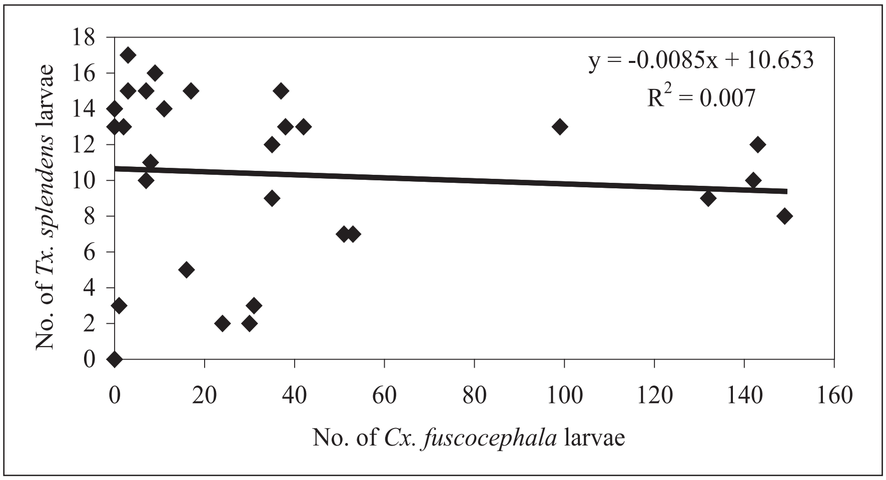

Figure 3. Correlation between *Tx. splendens* and *Cx. fuscocephala* larvae

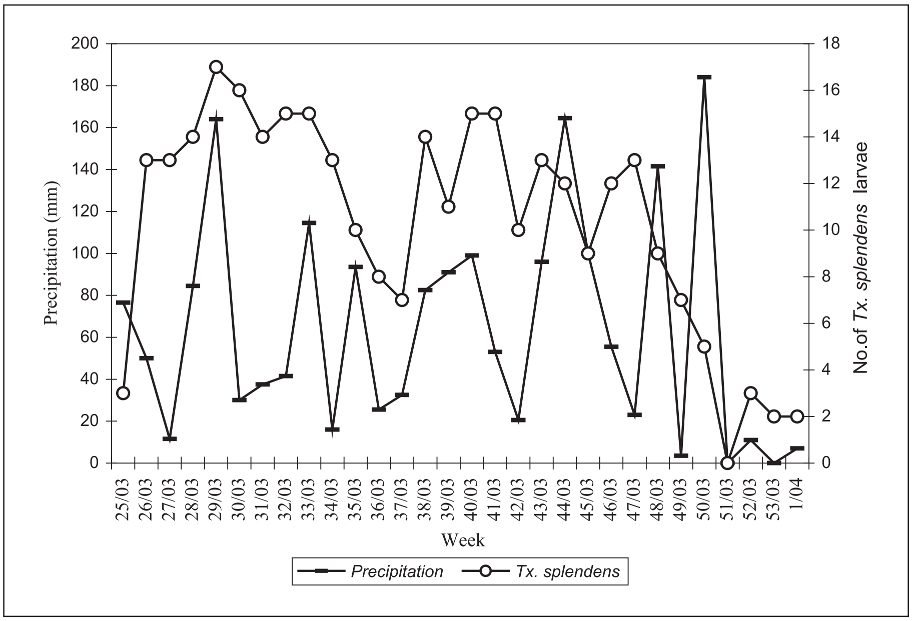

Figure 4. Correlation between precipitation and *Tx. splendens* larval population

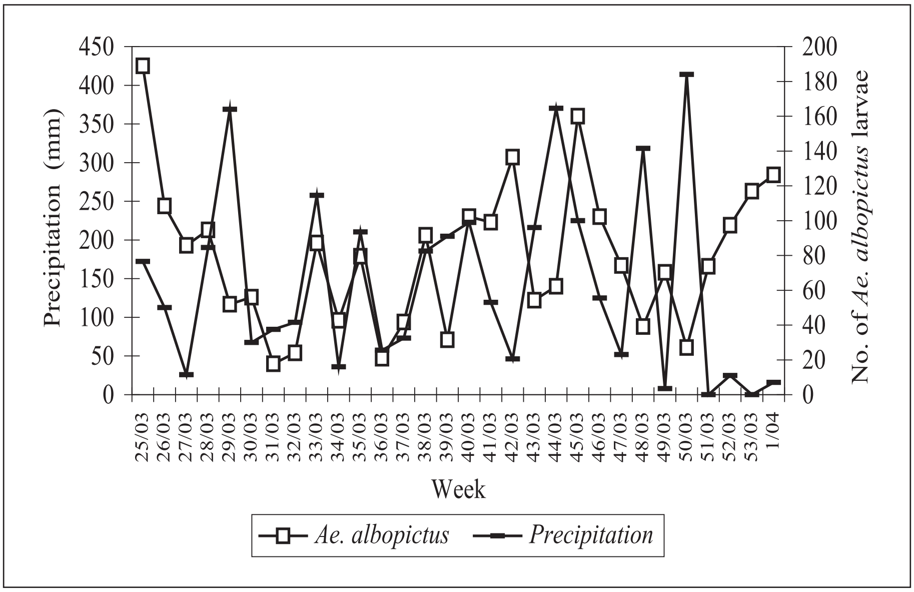

Figure 5. Correlation between precipitation and *Ae. albopictus* larval population

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 Mahmoodiah 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. Charateristics 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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REFERENCES

Aditya, G., Ash, A. & Saha, G.K. (2006). Predatory activity of *Rhantus sikkimensis* and larvae of *Toxorhynchites splendens* on mosquito larvae in Darjeeling, India. *Journal of Vector-Borne Diseases* **43**: 66-72. (online) www.mrcindia.org/journal/issues/432066.PDF. [7 April 2007]

Amalraj, D., Sivagnaname, N. & Das, P.K. (2005). Effect of food on immature development, consumption rate, and relative growth rate of *Toxorhynchites splendens* (Diptera: Culicidae), a predator of container breeding mosquitoes. *Memorias Instituto Oswaldo Cruz, Rio de Janerio* **100**(8): 893-902.

Annis, B., Nalim, S., Hadisuwasono, Wirdiarti & Boewono, D.T. (1990). *Toxorhynchites ambionensis* larvae released in domestic containers fail to control dengue vectors in a rural village in central Java. *Journal of American Mosquito Control Association* **6**(1): 75-78.

Chan, K.L. (1968). Observation on *Toxorhnychites splendens* (Wiedemann) (Diptera: Culicidae) in Singapore. *Mosquito News* **28**(1): 91-94.

Choochote, W., Jitpakdi, A., Suntaravitun, T., Jukum, A., Rongsriyam, K. & Chaithong, U. (2002). A note on laboratory colonization of *Toxorhnychites splendens* by using an artificial mating technique and autogenous *Aedes togoi* larva as prey. *Journal of Tropical Medicine and Parasitology* **25**: 47-50.

Chowanadisai, L., Benjaphong, N. & Phanthumachinda, B. (1983). A survey and biological study on aquatic insects, the natural enemies of mosquito. Division of Medical Entomology, Department of Medical Sciences, Bangkok, Thailand. 57pp.

Das, P.K., Sinagnanme, N. & Amalraj, D.D. (2006). Population interactions between *Culex vishnui* mosquitoes and their natural enemies in Pondicherry, India. *Journal of Vector Ecology* **31**(1): 84-88.

Fay, R.W. & Eliason, A. (1966). A preferred oviposition site as a surveillance method for A*edes aegypti*. *Mosuito News* **26**(4): 531-535.

Focks, D.A., Sackett, S.R. & Bailey, D.L. (1982). Field experiments on the control of *Aedes aegypti* and *Culex quinquefasciatus* by *Toxorhnychites rutilus rutilus* (Diptera: Culicidae). *Journal of Medical Entomology* **19**(3): 336-339.

Hutchings, R.S. (1994). Palm bract breeding sites and their exploitation by *Toxorhynchites* (Lynchiella) *haemorrhoidalis haemorrhoidalis* (Diptera: Culicidae) in an upland forest of the central Amazon. *Journal of Medical Entomology* **31**(2): 186-191.

Juliano, S.A. & Gravel, M.E. (2002). Predation and the evolution of prey behavior: an experiment with tree hole mosquitoes. *Behavioural Ecology* **13**(3): 301-311.

Kay, B.H., Cabral, C.P., Sleigh, A.C., Brown, M.D., Ribeiro, R.M. & Vasconcelos, A.W. (1992). Laboratory evaluation of Brazilian *Mesocyclops* (Copepoda: Cyclopidae) for mosquito control. *Journal of Medical Entomology* **29**(4): 599-602.

Kementerian Kesihatan Malaysia. (1986). Panduan pencegahan dan kawalan demam denggi/ demam denggi berdarah. 158 pp

Kesavaraju, B. & Juliano, S.A. (2004). Differential behavioural responses to water-borne cues to predation in two-container-dwelling mosquitoes. *Annals Entomology Society of America* **97**(1): 194-201.

Kloter, K.O., Bowman, D.D. & Carroll, M.K. (1983). Evaluation of some ovitrap materials used for *Aedes aegypti* surveillance. *Mosquito News* **43**: 438-441.

Kumar, R. & Hwang, J.S. (2006). Larvicidal efficiency of aquatic predators: A perspective for mosquito biocontrol. *Zoological Studies* **45**(4): 447-466.

Lee, H.L. (1991). Esterases activity and temephos susceptibility in *Aedes aegypti* (L.) larvae. *Mosquito-Borne Disease Bulletin* **8**(3): 91-94.

Lee, H.L., Nor Asikin, Nazni, W.A. & Sulaiman, S. (1998). Temporal variation of insecticide susceptibility status of field collected *Aedes albopictus* (Skuse) in Malaysia. *Tropical Biomedicine* **15**(2): 43-60.

Lima, J.B.P., Da-Cunha, M.P., Da Silva Junior, R.C., Galardo, A.K.R., Da Silva Soares, S., Braga, I.A., Ramos, R.M. & Valle, D. (2003). Resistance of *Aedes aegypti* to organophosphates in several municipalities in the state of Rio De Janeiro and Espirito Santo, Brazil. *American Journal of Tropical Hygiene* **68**(3): 329-333.

Miyagi, I., Toma, T. & Mogi, M. (1992). Biological control of container breeding mosquitoes, *Aedes albopictus* and *Culex quinquefasciatus*, in a Japanese Island by release of *Toxorhynchites splendens* adults. *Journal of Medical Veterinary Entomology* **6**: 290-300.

Paeporn, P., Supaphathom, K., Sathantriphop, S., Mukkhun, P. & Sangkitporn, S. (2005). Insecticide susceptibility of *Aedes aegypti* in tsunami-affected areas in Thailand. *Dengue Bulletin* **29**: 210-213

Panogadia-Reyes, C.M., Cruz, E.I. & Bautista, S.L. (2004). Phillipine species of *Mesocyclops* (Crustacea: Copepoda) as a biological control agent of *Aedes aegypti* (Linnaeus). *Dengue Bulletin* **28**:174-178. (online) http://www.searo.who.int/en/Section10/Section332/Section 1985-9815.htm. [2 December 2007]

Peters, T.M., Chevone, B.I. & Callahan, R.A. (1969). Interactions between larvae of *Aedes aegypti* (L.) and *Culex pipens* L. in mixed experimental population. *Mosquito News* **29**(3): 436-438.

Ping, L.T., Yatiman, R. & Lam, P.S.G. (2001). Susceptibility of adult field strains of *Aedes aegypti* and *Aedes albopictus* in Singapore to pirimiphos-methyl and permethrin. *Journal of American Mosquito Control Association* **17**(2): 144-146.

Ponlawat, A., Scott, J.G. & Harrington, L.C. (2005). Insecticide susceptibility of *Aedes aegypti* and *Aedes albopictus* across Thailand. *Journal of Medical Entomology* **42**(5): 821-825.

Santos, S.R.A., Melo-Santos, M.A.V., Regis, L. & Albuquerque, C.M.R. (2003). Field evaluation of ovitrap consociated with grass infusion and *Bacillus thuringiensis var israelensis* to determine oviposition rates of *Aedes aegypti*. *Dengue Bulletin* **27**: 156-162.

Tikasingh, E.S. & Eustace, A. (1992). Suppression of *Aedes aegypti* by predatory *Toxorhynchites moctezuma* in an island habitat. *Journal of Medical Veterinary Entomology* **6**(3): 272-80.

Trpis, M. (1973). Interaction between the predator *Toxorhynchites brevipalpis* and its prey *Aedes aegypti*. *Bulletin of the World Health Organization* **49**: 359-365.

Wesson, D.M. (1990). Susceptibility to organophosphate insecticides in larval *Aedes albopictus*. *Journal of American Mosquito Control Association* **6**(2): 258-264.

Vezzani, D. & Schweigamann, N. (2002). Suitability of containers from different sources as breeding sites of *Aedes aegypti* (L.) in a cemetery of Buenos Aires City, Argentina. *Memorias Instituto Oswaldo Cruz, Rio de Janerio*. **97**(6): 789-792.

Vu Duc Huong & Nguyen Thi Bach Ngoc. (1999). Susceptibility of *Aedes aegypti* to insecticides in South Vietnam. *Dengue Bulletin* **23**:85-88. (online) http://www.cdc.gov/ncidod/dvbid/dengue/map-distribution-2003. htm. [12 December 2006].

Vu Sihn Nam, Marchand, R., Tran Van Tien & Nguyen Van Bihn. (1997). Dengue vector control in Viet Nam using Mesocyclops through community participation. *Dengue Bulletin* **21**: 96-104.

Vu Sihn Nam, Yen, N.T., Kay, B.H., Marten, G.G. & Reid, J.W. (1998). Eradication of *Aedes aegypti* from a village in Vietnam, using copepods and community participation *American Journal of Tropical Medical Hygiene* **59**(4): 657-660.