ODONATA NYMPHS AS POTENTIAL BIOCONTROL AGENT OF MOSQUITO LARVAE IN MALAYSIA

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Abstract. Biocontrol has been proposed as an effective approach in controlling mosquito population. In this study, three Odonata (dragonfly) nymphs (*Neurothemis fluctuans, Orthetrum chrysis* and *O. sabina*) were investigated for their feasibility as biocontrol agents against dengue virus vectors *Aedes aegypti, Ae. albopictus* and *Culex quinquefasciatus.* Each Odonata nymph species was separately fed each of the mosquito species IV instar larvae maintained at a fixed level by replenishing every three hours for 24 hours under controlled laboratory conditions and 12-hour light-dark period. *N. fluctuans* and *O. sabina* nymphs preferred *Ae. aegypti* as their prey, while *O. chrysis* favored *Cx. quinquefasciatus.* Amount of larval consumption is significantly higher during light compared to dark period (p-value <0.05). However, overall there are no significant differences in consumption rates of the three dragonfly nymph species for the test mosquito larvae. Thus, Odonata nymphs are potential biocontrol agents against mosquito vectors of dengue disease.

Keywords: *Aedes aegypti, Aedes albopictus, Culex quinquefasciatus,* biocontrol, mosquito larva, Odonata nymph

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INTRODUCTION

Dengue continues to be the most prevalent mosquito-borne viral infection in humans, with an estimated 40% of the world population at risk and approximately 390 million infections annually; World Health Organization (WHO)'s Dengue Control Strategy aims to reduce deaths by 50% by 2020 (WHO, 2019). Currently, chemical insecticides are commonly used in control of human disease vectors, but this preventive measure has undesirable impacts on non-target populations (Kumar and Hwang, 2006) and, in addition, is harmful to the environment (Tok Gek Sun, 2013) and human health (Jaga and Dharmani, 2003; Damalas and Eleftherohorinos , 2011), costly (Halasa *et al*, 2012; WHO WPRO, 2013), and contributes to vector insecticide resistance (Hidayati *et al*, 2011; Liu, 2015). Malaysia is ranked among the top 30 countries with high prevalence of endemic dengue cases (WHO, 2019). Synthetic insecticides are commonly used to control adult and larval mosquito populations, but "fogging" has proven ineffective and failed to curb the spread of dengue fever in the country (Davis, 2009). Thus, there is an urgent need for an alternative solution to this predicament (Chareonviriyaphap *et al*, 1999; Benelli *et al*, 2016; Thomas, 2018: Prapanthadara *et al*, 2002; Hamden *et al*, 2005).

Biological control using predators and other biocontrol agents to control mosquito larvae is an environmentally-friendly and effective solution (Kumar and Hwang, 2006; Huang *et al*, 2017). Researchers are now focusing on biocontrol measures (WHO WPRO, 2003; Benelli *et al*, 2016) using biocontrol agents (predators) as a sustainable and eco-friendly alternative solution against the spread of dengue (Kumar and Hwang, 2006). For example, dragonflies (order Odonata) are predators with high propagation potential from juvenile to adulthood and Odonata nymphs prey on immature mosquitoes resulting in several studies over the years on the potential of dragonfly nymphs as biocontrol of mosquito larvae (Mathavan, 1976; Pandian *et al*, 1979; Quiroz-Martinez *et al*, 2005; Chandra *et al*, 2006; Chandra *et al*, 2008; Mandal *et al*, 2008; Saha *et al*, 2012). Sebastian *et al* (1990) and Singh *et al* (2003) reported *Brachythemis contaminata* (Family Libellulidae) larvae efficiently consumes dengue vectors *Aedes aegypti*, *Anopheles stephensi* and *Culex quinquefasciatus* larvae under laboratory conditions.

Here, we assessed the efficiency of Odonata nymph species, namely, *Neurothemis fluctuans*, *Orthetrum chrysis* and *O. sabina*, as biocontrol of *Aedes albopictus*, *Ae. aegypti* and *Culex quinquefasciatus* larvae under laboratory conditions. This study will give alternative method in controlling mosquito population especially when involved chemical control.

MATERIALS AND METHODS

Odonata nymphs and mosquito larvae collections

Odonata nymphs were collected from lakes, drains, and small streams in urban area (Putrajaya) and suburban (Kuala Selangor) and dominant species were identified based on taxonomic keys (Orr, 2005) for inclusion the study. Medium-size nymphs (15-20 mm) were selected and supplied with blood worms (*Glycera* sp). *Ae. aegypti*, *Ae. albopictus* and *Cx. quinquefasciatus* IV instar larvae were kindly provided by The Institute for Medical Research, Kuala Lumpur, Malaysia.

Feeding experiments

Feeding efficiency tests of the dominant predator dragonfly nymph species (*N. fluctuans*, *O. chrysis* and *O. sabina*) were carried out in an aquarium tank (22x13x11 cm) containing pond water at 24-29°C, pH 6.1-6.3, with dissolved oxygen of 5.2-6.3 mg/l, and under 12 hours day time and 12 hours night time. Each tank contained a species of Odonata nymphs (n = 1) and a mosquito species IV instar larvae (n = 100). Mosquito larva consumption by Odonata nymphs was measured every 3 hours for 24 hours by collecting larvae in a sieve and counting to allow observation of feeding behaviour under both light and dark conditions. Mosquito larvae and pond water were replenished every 3 hours to maintain a constant prey density. Each experiment was conducted in triplicate on three different days using a fresh set of Odonata nymphs and mosquito larvae for each experiment.

Data collection and analysis

At the end of each experimental trial, total number of each mosquito larva species consumed (mean ± standard error of the mean (SEM)), and feeding rate of each Odonata nymph species for each mosquito species under light and dark conditions were recorded. Statistical analysis, analysis of variance (ANOVA), was performed and a p-value <0.05 is considered significant.

RESULTS

Consumption rate of three Odonata nymph species, namely, *N. flactuans*, *O. chrysis* and *O. sabina*, were separately measured for each of the three mosquito IV instar larval species, *Ae. aegypti*, *Ae. albopictus* and *Cx. quinquefasciatus*, over a 24-hour period, as well as over a 12-hour light and dark duration, under a laboratory setting. Over 24 hours, *O. sabina* nymphs had the highest rate of consumption for *Ae. aegypti* larvae, and also had the lowest rate of consumption for *Cx. quinquefasciatus* (Table 1) and predation of mosquito larvae by Odonata nymphs during light (12 hours) and dark (12 hours) periods under laboratory setting showed all three Odonata nymphs were more active during the light than dark period (Table 2). Statistical analysis revealed that *N. fluctuans* and *O. sabina* favored *Ae. aegypti* larvae, while *O. chrysis* preferred those of *Cx. quinquefasciatus*, but overall, there are no significant differences in consumption rates of the three dragonfly nymph species for the test mosquito larvae (Table 3). Statistical analysis of rates of larvae consumption during light and dark periods showed

Table 1

Predation of mosquito larvae by Odonata nymphs over a 24-hour period under laboratory setting

Odonata nymph species	Mosquito larva consumed over a 24-hour period (mean ± SEM)		
	Aedes albopictus	*Ae. aegypti*	*Culex quinquefasciatus*
Neurothemis fluctuans	3.9 ± 1.1	8.3 ± 1.8	4.7 ± 1.1
Orthetrum chrysis	4.7 ± 1.1	4.9 ± 1.8	5.2 ± 1.1
O. sabina	6.5 ± 1.1	9.1 ± 1.8	3.3 ± 1.1

SEM: standard error of the mean

Table 2

Predation of mosquito larvae by Odonata nymphs during light (12 hours) and dark (12 hours) periods under laboratory setting

Odonata nymph species	Mosquito larva consumed during light (12 hours) and dark (12 hours) periods (mean ± SEM)	
	light (12 hours)	dark (12 hours)
Neurothemis fluctuans	56 ± 3.06	41.6 ± 2.02
Orthetrum sabina	63 ± 2.96	36.6 ± 4.4
Orthetrum chrysis	73.3 ± 4.40	26 ± 4.9

SEM: standard error of the mean

Table 3

Statistical analysis of three mosquito larval species predation rate by three species of Odonata nymphs

Parameter	Df	F	*p*-value*
Odonata nymph (D)	2	3.42	0.310
Mosquito larva (L)	2	5.35	<0.050
D × L	4	2.31	0.950

Calculations are based on data in Table1.

*Significant when $p<0.05$ using two-way analysis of variance

Df: degree of freedom; F: statistics based on ratio of mean squares all three Odonata nymphs were more active during the light than dark period, which indicates that predators used visual cues to identify their preys (Table 4).

DISCUSSION

Utilisation of biocontrol agents has gained more acceptance in control programs of insect-borne diseases (WHO SEARO, 2011). For example, studies on pre-emptive action towards mosquito larvae propagation using biocontrol agents are widely reported (WHO, 2011; McGregor and Connelly, 2021; Faithpraise *et al*, 2014). Examples of predators tested include *Rhantus sikkimensis* and larvae of *Toxorhynchites splendens* (Aditya *et al*, 2006; Aditya *et al*, 2007), *Diplonychus* spp and *Anisops* spp (Shaalan *et al*, 2007), Odonata nymphs (Chandra *et al*, 2006; Mandal *et al*, 2008), *Acilius sulcatus* (Order Coleoptera, Family Dytiscidae) (Chandra *et al*, 2008), *Mesocyclops* (Order Cyclopoida, Family Cyclopidae) (Marten *et al*, 1989; Marten, 1990; Marten *et al*, 1994; Soumare and Cilek, 2011), planaria (*Dugesia bengalensis*) (Kar and Aditya, 2003), guppy fish (*Poecilia reticulata*) (Seng *et al*, 2008); diving beetles (Ohba and Takagi, 2010), and *Laccotrephes greisus* (Ghosh and Chandra, 2011)

In Malaysia, common biocontrol agents are *Bacillus thuringiensis israelensis* and guppy fish (Nyamah *et al*, 2011; Ong, 2016; Saleeza *et al*, 2014). *Toxorhynchites* spp larvae have also been used but the impact of their application on communities and environment are still ongoing (Nyamah *et al*, 2011). Saleeza *et al* (2011) reported *Ae. albopictus*,

Table 4

Statistical analysis of predation rate of three mosquito larval species by three Odonata nymph species between light (12 hours) and dark (12 hours) period

Parameter	SS	Df	MF	F	*p*-value*
Light/dark period	640.667	1	640.667	14.089	<0.001
Odonata sp.	69.481	2	34.741	0.764	0.467
Light/dark period x Odonata sp.	109.778	2	54.889	1.207	0.301
Error	9,549.333	210	45.473		

Calculations are based on data in Table1.

Significant when $p<0.05$ using two-way analysis of variance

Df: degree of freedom; F: statistics based on ratio of mean squares; MF: mean square; SS: sum of squares

Ae. aegypti, and *Cx. quinquefasciatus* larvae are commonly found in urban and suburban residential areas. Predatory efficiency of Odonata nymphs on mosquito larvae and species preference vary among them: highest consumption rate of IV instar larvae is by *O. sabina* (170 larvae in 24 hours), followed by *N. fluctuans* (135 larvae in 24 hours), then *O. chrysis* (73 larvae in 24 hours). In our study, under laboratory conditions over a 24-hour period overall there are no significant differences in consumption rates among dragonfly nymph species (*N. flactuans*, *O. chrysis* and *O. sabina*) of test mosquito larvae (*Ae. aegypti*, *Ae. albopictus* and *Cx. quinquefasciatus*).

Chatterjee *et al* (2007) investigating the biocontrol efficiency of *Brachytron pratense* nymphs against *Anopheles subpictus* larvae reported that the predator is more active during the day than night. Similarly, Chandra *et al* (2006) found that *B. pratense* nymphs are active during the day and are daylight stalkers. However, Ghosh *et al* (2005) commented that behavioral responses of biocontrol agents to light and dark periods may not significantly influence a biocontrol program. We observed that *O. sabina* was more active compared to other two Odonata species and had highest preference for *Aedes* spp larvae. Overall, Odonata nymphs favored *Aedes* over *Culex* spp as larvae of the former spp are smaller and lighter than those of the latter spp. A smaller and lighter prey expedites the feeding process and lessens the need for handling by predators.

Aedes larvae are often found below the water surface, while *Culex* larvae remain at the water surface (Yee *et al*, 2004). Thus, *Aedes* larvae are more easily targeted by Odonata nymphs compared to *Culex* larvae as the predators spend most of their time stalking prey at the bottom of the body of water. In addition, Odonata nymph species prefer mosquito larvae instead of pupae because the former are freely distributed in the water while the latter tend to be near the water surface (Mathavan, 1976).

In summary, the daily feeding rate was assessed by exposing the 100 IV instar mosquito larvae of every species to a single predatory species. The overall feeding rates of *O. sabina* were significantly higher than the overall feeding rates of *O. chrysis* and *N. flactuans*. In terms of preying preferences, there was a significant difference in the number of preying species between *Ae. aegypti* and *Cx. quinquefasciatus* consumed by the predators. The Odonata predators showed specific preying preference; *N. fluctuans* and *O. sabina* consumed more *Ae. aegypti* larvae than *Ae. albopictus* larvae and *Cx. quinquefasciatus* larvae, while *O. chrysis* do not show any larvae preference as they consumed all types of mosquito larvae *Cx. quinquefasciatus* larvae, *Ae. aegypti* larvae and *Ae. albopictus* larvae.

There was no significant difference among the three species of odonata nymphs in terms of mosquito larvae consumption. However, there was a significant difference in terms of the mosquito species most preferred by the Odonata nymphs. It was observed that the Odonata nymphs consumed more on *Ae. aegypti*.

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