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A Laboratory Investigation on Oviposition Response of *Aedes aegypti* to Larvicide Treated Domestic Water

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Investigation on oviposition response of dengue vector *Aedes aegypti* to larvicides was conducted in the laboratory. Larvicides used in ovitraps were Abate (temophos 1% sand granules), Bti (*Bacillus thuringensis israelensis*) and pyriproxyfen (insect growth regulator). Laboratory-reared *Aedes aegypti* female gravid mosquitoes were exposed to larvicide-treated ovitraps and equal numbers of control ovitraps in order to lay eggs. Ovipostion response was measured by oviposition activity index (OAI) in terms of counting the number of eggs from each treated ovitrap and compared with non-treated control group. A total of four hundred female mosquitoes were used in this experiment and the mean number of eggs laid per mosquito was 52.6±19.18 eggs and there were no survival of larvae and pupae from treated ovitraps. OAI for Abate, Bti and pyriproxyfen were observed as -0.03, +0.23 and -0.21, respectively. There were no significant differences detected among larvicides treatments (p=0.08 to 0.85). According to oviposition activity index, OAI of +0.3 and above are considered as attractants and -0.3 and below were considered as deterrents. The number of eggs laid varied depending on types of water as mosquito has visual and olfactory cues to assess water before laying eggs. In conclusion, commercially available and currently used larvicides Abate, Bti and pyriproxyfen have neither oviposition attractant nor oviposition deterrent property. Hence, all these tested larvicides had no influence on egg laying behavior of dengue vector *Aedes aegypti* and can be used for DHF vector control programme.

INTRODUCTION

Dengue fever is one of the most prevalent vector-borne diseases in the world and poses a heavy economic cost to health systems and societies. Depending on geographical and climatic conditions, spread and incidence as well as severity of dengue fever (DF) and dengue hemorrhagic fever (DHF) are increasing in tropical and subtropical regions including Southeast Asia.[1] In Myanmar, dengue is one of the major public health problems causing high morbidity and mortality especially in children. The first case of DHF was reported in 1969 and the first epidemic followed in 1970 in the capital Yangon where 1651 cases with 90 deaths were reported.[2] The morbidity and mortality of DHF in Myanmar 2009 were 24285 and 181, respectively.

The main vector, *Aedes aegypti*, usually breeds in domestic water containers around human dwellings. Oviposition (laying eggs) is an important component of most mosquito-borne diseases and selection of oviposition sites by gravid female mosquitoes is a crucial event for the survival of the species.[3] This container inhabiting mosquito species is known to follow visual or olfactory cues to appropriate water containers and then use both chemical and physical factors in the water before making decision to lay their eggs and then selecting it for oviposition.[4] Depending on the quality and infusion substances of water, oviposition attractants or deterrents (avoidance) can be present at breeding sites. Over many years, dengue control has mainly been based on the reduction of breeding sites and chemical control targeting on immature stages and adult stage due to the lack of vaccine for dengue.[5]

In Myanmar, especially in Yangon and Ayeyawady Regions, wide-scale applications of larvicide (especially Abate) have been carried out after Cyclone Nargis in 2008.[6] Currently available and used larvicides in Myanmar are:

(i) Chemical larvicide Abate (temephos 1% sand granules)

(ii) Bio-larvicide Bti (*Bacillus thuringiensis israelensis)* and,

(iii) Insect growth regulator pyriproxyfen

This study aimed to learn about the possible influence of these larvicides on the egg laying behavior of dengue vector *Aedes aegypti* and to support the effectiveness of further vector control activities in the programme.

MATERIALS AND METHODS

Study site

This study was conducted in the laboratory of Medical Entomology Research Division, Department of Medical Research (Lower Myanmar).

Mosquito

Aedes aegypti mosquitoes required for this experiment were obtained from the Insectary, Medical Entomology Research Division. *Ae. aegypti* colonies were maintained under controlled temperature and relative humidity at 25ºC to 30ºC and 80% to 85%, respectively. Mosquitoes were fed on 10% glucose-soaked cotton pads and later females were fed on laboratory white mice for blood meal and allowed to develop eggs and to become gravid.

Oviposition activity bioassay

Laboratory glass containers (250 ml) were used as ovitraps to attract female mosquitoes for laying eggs and inner surface of the container was covered by filter paper to collect eggs. Two hundred milliliters of domestic water was filled in each ovitrap. Then, Abate (temephos 1% sand granules), Bti (*Bacillus thuringiensis israelensis*) and pyriproxyfen (insect growth regulator) were added in concentration of 1 gm temephos/ 10 L, 2 gm Bti/L and 2 mg pyriproxyfen/ 10 L according to the WHO standard doses in each respective ovitrap.[7, 8]

For control group, only domestic water (water source from DMR-LM) was provided. These different larvicide-treated ovitraps and equal number of control ovitraps were placed in the rounded mosquito bed net by placing randomly to avoid positional bias. Thereafter, blood-fed gravid female mosquitoes were released in the bed net and exposed to ovitraps in order to lay eggs.

All ovitraps were collected one day after exposure to gravid female mosquitoes in the bed net. Then, filter papers from all ovitraps were removed and the number of eggs from the filter paper of each ovitrap was counted. And then, all the filter papers were placed back to the respective ovitraps and numbers of adult mosquitoes emerged from each larvicide-treated ovitrap and control ovitrap were observed and recorded. For one experiment, 16 ovitraps and 40 gravid female mosquitoes were used at the same time and this experiment was replicated ten times. Hence, in total, 400 gravid female mosquitoes and 160 ovitraps were used throughout the experiment.

Data and Statistical Analysis

Oviposition Activity Index (OAI) and Student ‘t’ test were used to analyze and determine the data. OAI means in terms of counting the number of eggs from each treated ovitrap and compared with non-treated control group.[9] According to these authors, OAI of +0.3 and above are considered as attractants and -0.3 and below are considered as deterrents. Microsoft Excel was applied for data entry.

Oviposition Activity Index (OAI) = $\frac{NT-NC}{NT+NC}$

NT=Number of eggs laid in treated water container

NC=Number of eggs laid in control water container

RESULT AND DISCUSSION

This experiment was conducted from April to October 2011 and a total of 400 gravid female mosquitoes were used to lay eggs in the laboratory controlled temperature and relative humidity. Number of eggs laid per each experiment was not concerned with different seasons and mean numbers of eggs per mosquito was 52.6±19.18 (Table 1).

Table 1. Total number of mosquitoes used, total number of eggs and mean number of eggs laid per mosquito

Total no. of mosquitoes used	Total no. of eggs	Mean no. of eggs per mosquito
400	21059	52.6±19.18

This experiment also showed the larvicidal action of all three larvicides according to their WHO recommended doses. There were no survival of larvae and pupae from all larvicides treated ovitraps but there was 95% survival rate from non-treated ovitraps (Table 2). It shows the doses used for this experiment were totally effective to all larva and pupae.

Table 2. Total number of survived mosquitoes from different treatments and different total number of eggs per ovitrap

Treatment	Total no. of eggs per ovitrap	Total no. of survived mosquitoes
Abate	4763	0
Bti	7974	0
Pyriproxyfen	3289	0
Control	5033	4781(95%)

Differential oviposition activities of *Aedes aegypti* mosquito for three larvicides were measured by comparing the mean numbers of eggs per each treated ovitrap and control ovitrap.[9] The comparison of different mean number of eggs per each treatment is shown in Table 3.

Table 3. Determination of oviposition response of *Aedes aegypti* to Abate, Bti and pyriproxyfen with Student 't' test and OAI

Treatment	Mean no. of eggs per ovitrap	P value (student t test)	OAI (Oviposition Activity Index)
Abate	119±82	0.85	-0.03
Bti	217±130	0.08	0.23
Pyriproxyfen	82.2±48.2	0.18	-0.21
Control	126±87		

Mean±SD of number of eggs for Abate, Bti and pyriproxyfen were 119±82, 217±130 and 82.2±48.2, respectively, and the control mean±SD was 126±87. Mean number of eggs in Abate treated ovitrap was close to the mean number of eggs in control ovitrap. On the other hand, mean numbers of eggs in Bti and pyriproxyfen oviraps varied from the mean number of eggs in control ovitrap. However, there were no significant differences of mean number of eggs between larvicide-treated ovitraps and control ovitrap (p value ranged from 0.08 to 0.85).

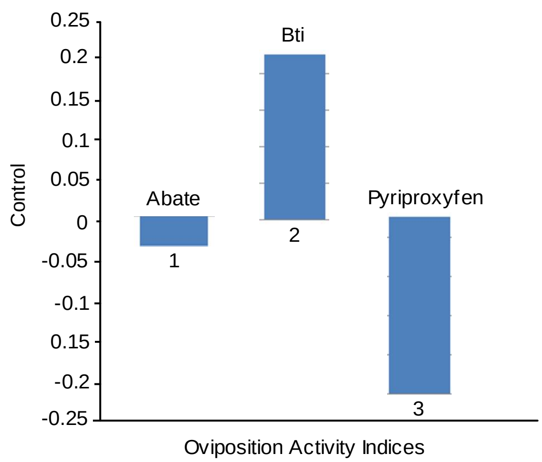

Fig. 1. Oviposition Activity Indices (OAI) of Abate, Bti and pyriproxyfen deviating from control

Furthermore, according to oviposition activity index,[9, 10] there were no OAI of less than -0.3 and greater than +0.3 (Fig. 1). Therefore, all these larvicides have neither oviposition deterrent nor attractant property.

If oviposition attractant and deterrent properties of *Aedes* mosquito exist in all these tested larvicides, vector control activities using these larvicides should be concerned about their properties. If attractant larvicides are used, doses of larvicides should be applied according to the manufacturers' field recommended doses in order to prevent the occurrence of tolerance and resistance of mosquitoes to these larvicides. If deterrent larvicides are used, every possible breeding source for mosquitoes should be applied. If not, mosquitoes can avoid that larvicides-treated water and can choose the non-treated sources. Therefore, detection of oviposition response of mosquitoes is important for successive vector control programme.

In summary, this study showed that there were no evidences that the oviposition (eggs laying behavior) of *Aedes aegypti* female mosquitoes are influenced by the currently available and commercially used larvicides. Therefore, all these tested larvicides can be safely used in vector control programme as all these larvicides do not affect the bionomics of the mosquitoes.

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