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Insecticide Susceptibility Status of Dengue Vector *Aedes aegypti* against Temephos in Hinthada Township, Ayeyarwady Region

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Dengue virus is transmitted by *Aedes* female mosquitoes mainly of the species *Aedes aegypti* (*Ae. aegypti*). Temephos is mainly used as a larvicide to control mosquitoes in dengue endemic countries including Myanmar. In 2022, Hinthada township reported the highest number of dengue cases in Ayeyarwady Region. Therefore, the main object of the study was to assess the insecticide susceptibility status of dengue vector *Ae. aegypti* larval population against temephos in selected areas, Hinthada Township. The present study was laboratory based study and conducted from June to November, 2022. The *Aedes* larvae were collected from three urban areas and three rural areas of Hinthada township. Larvicide susceptibility test was conducted in Department of Medical Research (Pyin Oo Lwin) and WHO (2016) larval bioassays method was applied in the present study. Two dosages of temephos; diagnostic dosage (0.02 mg/l) and operational dosage (1 mg/l) were used in larval susceptibility test. The 50% mortality at lethal time (LT_{50}) for all strains of *Ae. aegypti* larvae against diagnostic dosage and operational dosage of temephos showed range from 83.35 to 326.57minutes and 34.05 to 43.49 minutes, respectively. The highest resistance ratios (RR_{50}) of *Ae. aegypti* larvae at diagnostic dosage and operational dosage were 3.92 and1.28 that found in Ka Naung Su ward. Mortality rates of *Ae. aegypti* larvae from Ka Naung Su, Tar Ka Lay, U Yin (South) wards, and Yone Tha Lin, Sit Kone, Duya villages and Laboratory strain were 63.75%, 74.25%, 83.25%, 88.50%, 95.00%, 98.75% and 100%, respectively, after 24 hours exposure with diagnostic dosage of temephos. However, 100% mortality was found in all study areas of the township after 24 hours exposure with operational dosage of temephos. *Ae. aegypti* larvae from the present study areas in Hinthada township were still susceptible to existing operational dosage of temephos which serves as an effective tool to control *Ae. aegypti* population.

Keywords: Ae. aegypti, Temephos, Susceptibility, Hinthada Township

INTRODUCTION

Dengue is a mosquito-borne viral disease that has rapidly spread in all regions of World Health Organization (WHO) in recent years. Dengue virus is transmitted by *Aedes* female mosquitoes mainly *Aedes aegypti* (*Ae. aegypti*) species. *Aedes* mosquitoes are also the vectors of chikungunya, yellow fever and zika viruses. The number of dengue cases reported to WHO increased over 8 fold over the last two decades, from 505,430 cases in 2000, to over 2.4 million in 2010, and 4.2 million in 2019.[1]

From 2015 to 2019, dengue cases in the South-East Asia Region increased by 46%

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(from 451,442 cases in 2015 to 658,301 cases in 2019) and deaths decreased by 2%, (from 1584 in 2015 to 1555 in 2019).[2] In Myanmar, dengue is one of the principal epidemic diseases and sporadic cases of dengue have been reported since 1960. Between 2011 and 2015, a total of 89,832 dengue cases and 393 deaths were reported in hospitals and among them 97% of cases were children.[3] Within the first six months of 2022, Ayeyarwady Region was the highest dengue reported region with 2717 cases among states and regions of Myanmar and Hinthada Township was the highest in the region with 371 dengue cases. (VBDC, personal communication, 14 July 2022).

In vector control, one of the major problems is the development of resistance to existing insecticides in the vectors. The extensive usage of insecticide has led to the development of insecticide resistance in vector mosquitoes and become the crucial problems to prevent disease control.The early detection of insecticide resistance can raise the effectiveness of vector control by applying the implementation of alternative control strategies.[4] According to the National Strategic Plan for Dengue Prevention and Control (2016-2020) of Ministry of Health, monitoring of mosquito vectors insecticide resistance is needed to conduct regularly to create the effective vector control strategy Myanmar.[5] Previous study conducted in 2014 in Myanmar also pointed out that the long-term use of larvicide should be managed carefully to control *Ae. aegypti* and regular monitoring of insecticide resistance is necessary.[6]

Temephos is a non-systemic organophos-phorus insecticide, mainly used as a larvicide to control mosquitoes.[7] The formula of temephos is $C_{16}H_{20}O_6P_2S_3$ and Abate is a trade name. Abate (temephos 1% sand granule) is registered and sold in Australia and many countries in Central and South America, Asia, Europe and Africa.[8] World Health Organization (WHO) suggested two dosages of temephos: diagnostic and operational dosages for the vector mosquito control. Diagnostic dosage is used because it permits discrimination of insect response and dead larvae after exposure are labeled as susceptible and larvae who survive as resistant.[9] Temephos is recommended by WHO for addition to potable water as a larvicide treatment at an application rate not exceeding 1 mg/l which is utilized as the operational dosage in Myanmar and many other countries.[7]

Many control interventions of dengue vector pointed the larval stages of the *Aedes* mosquito that prefer to breed in artificial containers near human dwellings. Temephos is widely used to control immature dengue vectors because of its cost-effectiveness and community acceptance.[10] As a con-sequence of its widespread use, resistance to temephos in *Ae. aegypti* has been reported in many Latin American countries, including Brazil, Cuba, El Salvador, Argentina, Bolivia, Venezuela, Peru and Colombia.[11-18]

Temephos resistance of *Ae. aegypti* has also been recorded in Asia including Cambodia, Thailand, and Malaysia.[19-22] Previous study in Monywa Township, Myanmar reported the detection of the *Ace 1* gene mutation which is the target gene of organophosphate in *Ae. aegypti* population as a first time.[23] However, insecticide susceptibility study of *Ae. aegypti* against temephos has not been conducted in Hinthada Township. Therefore, the present study was to assess the insect-cide susceptibility status of dengue vector *Ae. aegypti* larval population against temephos in selected areas, Hinthada Township.

MATERIAL AND METHODS

The present study was the laboratory based experimental study and conducted from June to November, 2022 in Hinthada Township which is located on the Irrawaddy River bank and in the northern portion of Ayeyarwady Region (N 17° 26′ and 17° 48′, E 95° 11′ and 95° 33′). In Hinthada Township, urban population was 24.7 % and rural population was 75.3% of total popu-lation. There are 21 wards in the urban area and 103 villages in the rural area in Hinthada Township.[24] According to the recommendation of Public

Health Department in Hinthada District, mosquito collection for the laboratory experiments were conducted in three urban and three rural areas selected based on the 2022 reported dengue fever cases. Among the study areas, Tar Ka Lay, Ka Naung Su and U Yin (South) wards are urban areas, and Yone Tha Lin, Duya and Sit Kone villages are rural areas.

Mosquito collection and rearing

The larvae were collected from *Aedes* mosquito breeding sites from selected areas. For each study area, larvae from indoor and outdoor water containers were collected from random 50 houses. A total of six times (one time per month in each study area) was visited with the collaboration of Vector Born Disease Control (VBDC) team of Ayeyarwady Region. Collected larvae were stored and labeled in the plastic containers, and then transferred to insectarium of Medical Entomology Research Division, Department of Medical Research (Pyin Oo Lwin Branch) for rearing to the adult stage.

Adult stage of *Ae. aegypti* were identified by using the pictorial keys of Leopoldo M. Rueda.[25] *Ae. aegypti* mosquitoes from each study area were transferred into each mosquito cage. Mosquitoes were provided with 5% sugar solution and female mosquitoes were blood fed by using laboratory reared white mice 2-3 days after adult emergence.

After blood fed, container in which filter paper was pasted in the inner surface with water was filled up to the middle of the filter paper was provided for oviposition. When eggs found on filter paper, it was transferred to a plastic tray filled with the tab water for larval hatching to produce F1 progeny.The larvae were fed daily with the fish food. The third or fourth instar larvae of F1 generation from each study area were used for insecticide larval susceptibility test. The laboratory strain of *Ae. aegypti* was colonized from Salingyi Township, Sagaing Region and has been maintained for F_{10} generations.

Insecticide

Two dosages of temephos were recommended by WHO for larval bioassay testing, diagnostic dosage, 0.02 mg/l of temephos was prepared from technical grade of temephos with 96.9% (PESTANAL™, Sigma-Aldrich), while for operational dosage, 1 mg/l of temephos was prepared from Abate® containing temephos 1% sand granule.[7, 26]

Larval bioassays

Larval bioassays were conducted by using WHO (2016) method. Twenty five late third or early fourth instar larvae were transferred by the droppers to disposable test cups, each containing 250 ml of tap water and the standard insecticide (diagnostic or operational dosage). Small, unhealthy or damaged larvae were removed and replaced. As for control group, only 1 ml of ethanol was added in the water. The same procedure was conducted for each study area and laboratory strain mosquito. Experiments were done under maintain room temperature (27°C±2°C). Mortalities were assessed within two hours and after 24 hours of insecticide exposure. The whole procedure was replicated for three times.[27]

Statistical analysis

Lethal time (LT_{50}) was analyzed using probit analysis by SPSS statistics software (version 23) based on the result of mortality rate. The mortality rates of *Ae. aegypti* larvae were recorded at 10 minutes intervals within the first two hours of larvicide exposure and recorded again at 24^{th} hrs. of exposure for all field and laboratory strains. The resistance ratio (RR) was calculated by dividing the LT_{50} of the field strain by the LT_{50} of the laboratory strain.[4]

$$\text{RR of } LT_{50} = \frac{LT_{50} \text{ of field strain}}{LT_{50} \text{ of laboratory strain}}$$

RESULTS

In diagnostic dosage (0.02 mg/l) of temephos, no 100% mortality was observed in all strains of *Ae. aegypti* within120 minutes (Fig 1). However, *Ae. aegypti* larval mortalities in operational dosage (1 mg/l) of temephos were found that laboratory strain was 100% and field strains from Hinthada Township were over 97%, respectively (Fig. 2).

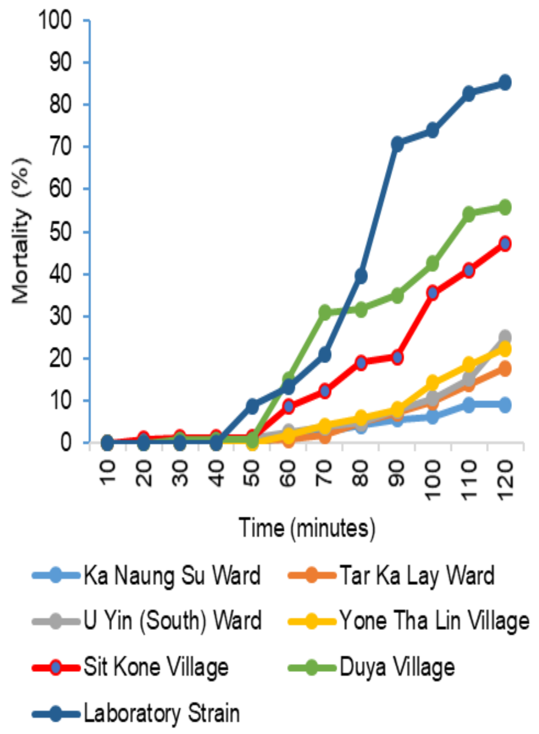

Fig. 1. Percent mortalities of *Aedes aegypti* larvae at diagnostic dosage (0.02 mg/l) temephos within 120 minutes exposure

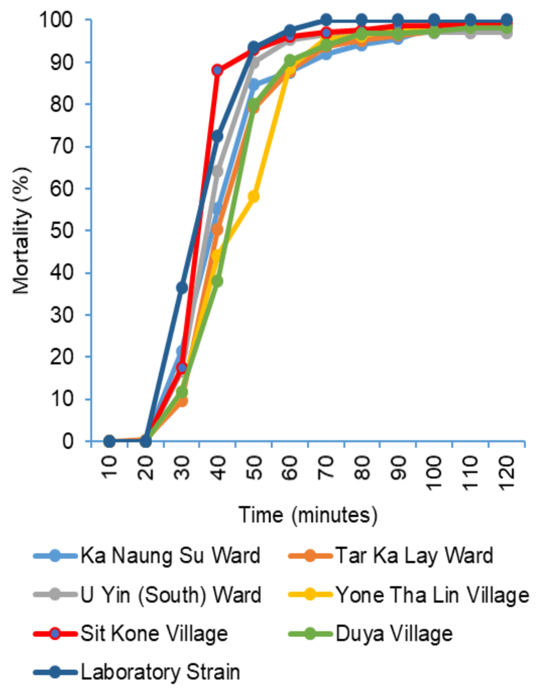

Fig. 2. Percent mortalities of *Aedes aegypti* larvae at operational dosage (1mg/l) temephos within 120 minutes exposure

For the diagnostic dosage, LT_{50} values ranging from 83.35 to 326.57 minutes and resistance ratios (RR_{50}) ranging from 1.27 to 3.92 for all field and laboratory *Ae. aegypti* strains were detected.

Meanwhile, LT_{50} of the operational dosage against *Ae. aegypti* larvae ranged from 34.05 minutes to 43.49 minutes and resistance rations (RR_{50}) were between 1.28 and 1.03 (Table 1).

Table 1. Lethal times (LT_{50}) and resistance ratios (RR_{50}) of *Aedes aegypti* against diagnostic dosage and operational dosage of temephos

Study area	Diagnostic dosage (0.02 mg/l)		Operational dosage (1 mg/l)	
	LT_{50} (minute) (95% CL)	RR_{50}	LT_{50} (minute) (95% CL)	RR_{50}
Ka Naung Su Ward	326.57 (258.82 – 47 2.92)	3.92	43.49 (34.51 – 50.69)	1.28
Tar Ka Lay Ward	265.32 (206.65 – 409.91)	3.18	42.55 (34.86 – 49.22)	1.25
U Yin (South) Ward	234.26 (185.88 – 360.23)	2.81	41.86 (36.97 – 46.22)	1.23
Yone Tha Lin Village	227.02 (177.35 – 344.53)	2.72	39.56 (34.41 – 44.31)	1.16
Sit Kone Village	123.29 (117.55 – 130.90)	1.48	37.88 (26.20 – 48.40)	1.11
Duya Village	105.78 (97.69 – 117.72)	1.27	35.11 (21. 41 – 47.07)	1.03
Laboratory strain	83.35 (75.77 – 88.880)	0.00	34.05 (32.73 – 35.31)	0.00

LT_{50}=50% lethal time, RR_{50}=50% resistance ratio, 95% CL=95% confidence limit

Table 2. Percent mortalities of *Aedes aegypti* larvae against diagnostic dosage and operational dosage of temephos after 24 hours exposure

	0.02 mg/l		1 mg/l	
	Mortality (%) of test	Mortality (%) of control	Mortality (%) of test	Mortality (%) of control
Ka Naung Su Ward	63.75	0	100	0
Tar Ka Lay Ward	74.25	0	100	0
U Yin (South) Ward	83.25	0	100	0
Yone Tha Lin Village	88.50	0	100	0
Sit Kone Village	95.00	0	100	0
Duya Village	98.75	0	100	0
Laboratory strain	100.00	0	100	0

The percent mortalities of *Ae. aegypti* larvae from field and laboratory strains against diagnostic and operational dosages of temephos for 24 hours exposure were showed in table 2. In diagnostic dosage, the mortality rates of field strains had ranged from 63.75% to 98.75% although laboratory strain of *Ae. aegypti* larvae described 100% mortality. Importantly, field and laboratory strains in operational dosage were 100% mortalities after 24 hours test. No mortality was recorded in control groups of both operational and diagnostic dosages.

DISCUSSION

Dengue Hemorrhagic Fever (DHF) is accepted as a serious vector borne disease worldwide. As vector control is the primary method to control dengue, control programs need the limiting of the population of vectors to prevent the dengue outbreak.[28] Temephos is the most common used larvicide not only in Myanmar but also in other dengue endemic countries for the suppression of *Ae. aegypti* larvae. However, continuous usage of temephos for many years can lead to the possible insecticide resistance. Very few studies have been done in Myanmar regarding to *Ae. aegypti* susceptibility status against temephos is a confined factor to be the success of control programs.[10]

In the present study, the highest resistance ratio of *Ae. aegypti* against diagnostic and operational dosages of temephos was found in Ka Naung Su Ward and followed by Tar Ka Lay Ward, U Yin (South) Ward, Yone Tha Lin Village, Sit Kone Village and Duya Village. It indicated that *Ae. aegypti* larvae from urban areas were appeared to be more resistance to temephos than from rural areas in Hinthada Township, as compared to a susceptible laboratory strain. It may be due to the more frequent vector control intervention activities in urban areas. However, the resistance ratios (RR) of all field strains in the present study were found less than 4 in diagnostic dosage and less than 2 in operational dosage. The category of resistance ratios (RR) recommended by WHO (2016) is: RR <5 the field population is considered susceptible, RR between 5 and 10 is considered moderate resistance, RR>10 is highly resistant.[27] Therefore, *Ae. aegypti* larvae from all study areas in Hinthada Township were considered susceptible to temephos.

Previous study conducted in Indonesia (2010) noted that the highest resistance ratio (RR) of *Ae. aegypti* larvae from Surabaya District were ranging from 2.8 to 8.5. The resistance ratio (RR) value of larvae from Sawahan indicated to moderate resistance. Knowing the fact that temephos has been widely used as the principle larvicide for controlling *Ae. aegypti* in Indonesia since 1970.[29] The study in Peru reported that the resistance to temephos has reached very high levels as RR was 39.4 and temephos had been used for more than 25 years.[30]

In the present study, an average mortality of *Ae. aegypti* larvae from six study areas was 83.92% after 24 hours exposure with diagnostic dosage of temephos. However, 100% mortality rates were found in operational dosage for all field strains. Andiarsa D, (2020) described that the average mortality of *Ae. aegypti* larvae with temephos 0.02 mg/l (diagnostic dosage) was 27.66% and the results were concluded as resistant.[31] Yuniyanti MM, (2021) reported that the mean percentage of *Ae. aegypti* larval mortality was 97% in the treatment group of temephos 0.02 mg/l (diagnostic dosage) and these results cannot be said to be susceptible or resistant to temephos. It is necessary to carry out additional tests such as biological test and molecular test to know the resistant mechanisms. If the results of the two additional tests show mortality below 98%, it can be said that the resistance is confirmed.[32]

In conclusion, the present study showed the resistance levels of *Ae. aegypti* larvae were less than five and mortality rates for 24 hours were 100% in operational dosage (1mg/l) of temephos. Therefore, the results of the present study confirmed that *Ae. aegypti* larvae from six study areas were susceptible to existing applied dosage or operational dosage of temephos. For controlling *Ae. aegypti* larvae in these areas, temephos is still the effective insect-cide. However, previous study conducted in Myanmar reported that the mortality of *Ae. aegypti* larvae was 92.19% against temephos diagnostic dosage for 24 hours and the mutation of organophosphate insecticide target gene which was suspected for temephos resistance was found in Monywa Township.[23] Thus, further studies are needed to verify for diagnostic dosage (0.02 mg/l) of temephos. The valuable findings of the present study can support to Vector Borne Disease Control Program in Hinthada Township. Regular evaluation of the insecticide susceptibility status of the dengue vector is necessary in each dengue endemic area to detect the development of temephos insecticide resistance in order to consider the alternate insecticides or vector control activities.

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