MOSQUITO LARVICIDAL AND RESIDUAL ACTIVITY OF CAPSULAR TEMEPHOS

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Abstract. Temephos sand granule formulation is an organophosphorus larvicide used effectively in dengue control program to control container-breeding vector *Aedes* mosquitoes. However, conventional sand granule formulation is difficult to apply to hard-to-reach breeding sites, such as roof gutter, flat roof and awning. In order to overcome this problem, a water-soluble capsular preparation of temephos in a two-part capsule (TEMEBATE® C) was tested during 2016 - 2018 for efficacy and residual activity in indoor and outdoor conditions, and for efficacy in hard-to-reach breeding sites. In indoor plastic containers, a temephos capsule (1 mg ai/l water) induced complete larval mortality in three field strains of *Ae. aegypti* for up to 28 weeks and up to 48 weeks in one field strain. In outdoor plastic containers and flower pot trays, complete larva mortality against *Ae. aegypti* field strains was achieved for up to 28 weeks. In outdoor rain gutter trials, complete mortality of *Ae. albopictus* and *Culex quinquefasciatus* larvae in a porous container was achieved after 48-hours of exposure to a temephos capsule (0.67 or 2.00 mg/l water), and this was maintained for up to six weeks, with placement of a container of new larvae every week. In conclusion, capsular temephos proved to be as effective as conventional sand granule formulation in terms of bioefficacy and residual activity, and could be disseminated into difficult-to-reach mosquito breeding sites.

Keywords: *Aedes aegypti*, *Aedes albopictus*, dengue vector, temephos capsule

INTRODUCTION

Dengue fever and dengue hemorrhagic fever (severe dengue) are serious viral diseases transmitted by *Aedes aegypti* and *Ae. albopictus* (Lee, 2000). Both dengue fever and dengue hemorrhagic fever are among the major public health problems in Southeast Asian countries. In Malaysia, up to the 52nd epidemiological week in 2018, 80,615 cases of dengue were reported with 147 deaths (Ministry of Health Malaysia, 2018). In addition, another arbovirus, Zika virus (ZIKV) was detected by RT-PCR in field caught *Culex gelidus and Cx. quinquefasciatus* at different developmental stages (larva, male and female adult) in Thailand, indicating these mosquitoes could possibly be potential vectors (Phumee *et al*, 2019).

No effective tetravalent vaccines or specific treatment is available for prevention and treatment of dengue.

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Control methods employed by the Vector Borne Disease Control Program (VBDCP) in Malaysia and other countries are fogging with chemical insecticides and source reduction in affected areas (Lee *et al*, 2008). Larvicides are especially useful in disrupting mosquito life cycle in their aquatic habitat, thereby preventing emergence of new generations of vector mosquitoes and reducing spread of these vector-borne diseases. Larvicide temephos, an organophosphorus compound with very low mammalian toxicity, has been recommended by WHO since the early 1970s for control of container-breeding *Aedes* mosquitoes (WHO, 1985). In the past 45 years temephos is extensively used for control of *Ae. aegypti* and *Ae. albopictus* larvae in Malaysia. However, Chen *et al* (2013) and Mohiddin *et al* (2016) reported field *Ae. albopictus* larvae exhibiting resistance ratio (RR) of 0.75-1.45 and 1.39-1.87 respectively to temephos, and Ishak *et al* (2015) observed an RR of 1.5-2.0 in three strains of *Ae. aegypti* larvae collected from urban cities. In all of these studies, RR is <5, indicating *Aedes* larvae are still considered susceptible to temephos (WHO, 2016) and no failure in *Aedes* larvicidal activity was reported at an operational temephos dosage of 1 mg/l, a concentration recommended by WHO (2017) for addition to drinking and potable water.

Sand granule formulation, packed in 10 or 20 g sachet, is the most widely used temephos formulation in vector control programs (WHO SEARO, 2011). Recently, a new capsular formulation, TEMEBATE® C (Imaspro Resources Sdn Bhd, Klang, Selangor, Malaysia) containing 1.0 g temephos/capsule, has been developed. The present study was conducted to study efficacy and residual activity of TEMEBATE® C in indoor and outdoor conditions, and efficacy in inaccessible or hard-to-reach breeding sites.

MATERIALS AND METHODS

Study site

A series of five trials were conducted in Malaysia between 2016 and 2018. Trial 1 and Trial 2 were carried out indoor (interior of a building) in February 2016, Trial 3 and Trial 4 outdoor (outside of a building but confined to the immediate vicinity, *eg* along corridor with shaded area) in March, 2017. Trials 1-4 were held at the Medical Entomology Unit, Institute for Medical Research (IMR), Jalan Pahang, Kuala Lumpur, and Trial 5 was conducted at North Port, Port Klang, Selangor on January 2018.

TEMEBATE® C temephos concentration

TEMEBATE® C contains temephos granules (0.5, 1 g and 3 g) in a two-part capsule and is soluble in water.

Mosquito source

Ae. aegypti larvae, collected in ovitraps from three dengue endemic areas in Selangor (Sg Jelok, Kajang; Seksyen 8, Bandar Baru Bangi and Ridzuan Condominium, Bandar Sunway) and bred in the laboratory of the IMR Medical Entomology Unit, *Ae. albopictus* (F78) and *Cx. quinquefasciatus* (F1047) maintained in the IMR insectarium without exposure to chemical and biological agents were used. Larvae at L3/L4 instar were employed in the larvicide assays.

Treatment schedule

Trial 1 (indoor stagnant water)

Plastic containers, 19 cm (width) x 32 cm (length) x 10.5 cm (height), were divided into three equal compartments to separately accommodate larvae of *Ae. aegypti* strains from the three collection sites. Three containers were for larvicide assays and one for untreated control. A temephos (0.5 g) capsule was introduced into each container containing 5 l of water and batches of 25 L3/L4 instar larvae were placed into each compartment. After 24 hours of exposure, larva mortality and/or adult emergence was recorded. Moribund (incapable of rising to the surface or not showing characteristic diving reaction when the water is disturbed) were included among dead (could not be induced to move when probed with a needle in siphon or cervical region) larvae in calculating percent mortality. Three replicates were conducted for each strain tested.

Trial 2 (indoor stagnant water)

Trial 2 was conducted as in Trial 1, but larvae of field *Ae. aegypti* from a dengue endemic region at Bandar Sri Damansara, Petaling Jaya, Selangor were used and the assay was carried out for a period of 48 weeks.

Trial 3 (outdoor stagnant water)

The trial was conducted in plastic containers (same size as described above) as well as round flower-pot trays of 43 cm (diameter) x 3 cm (depth). A batch of 25 field *Ae. aegypti* L3/L4 instar larvae from the same three dengue endemic regions as described above was placed into each plastic container and flower pot tray containing 5 l of water together with fish food pellet as larva food and a temephos (0.5 g) capsule. Each container was covered with netting to prevent other mosquitoes or insects from laying eggs and to prevent successfully emerged adults from escaping. Percent dead/moribund larvae at 24 hours or 48 hours post-treatment were recorded. The trial was repeated by introducing a new batch of 25 L3/L4 instar mosquito larvae into existing treated flower-pot trays and plastic containers on a weekly basis for four weeks. Observations on larvae were recorded as described above. Untreated controls were handled in the same way. Three replicates were conducted for each experiment.

Trial 4 (outdoor stagnant water)

Trial 4 was similar to Trial 3, except that field strains of *Ae. aegypti* larvae from dengue endemic region of Bandar Sri Damansara, Petaling Jaya and Ridzuan Condominium, Bandar Sunway were used in the tests.

Trial 5 (outdoor gutter)

The trial was conducted outdoor in a shaded area. Gutters, 3 m (length) x 15 cm (width) x 16 cm (height), were placed horizontally on ground and 15 l of tap water were added. Two gutters were each treated with a temephos (1 g) capsule, while two other gutters were each treated with a temephos (3 g) capsule. Two untreated gutters were used as controls. Plastic transparent containers, 102 mm (width) x 102 mm (length) x 45 mm (height), with pores were employed as floating cages to contain test larvae. Twenty laboratory reared *Ae. albopictus* (F78) and *Cx. quinquefasciatus* (F1047) L3/L4 larvae in the floating cages were introduced into each test and negative control gutters. Percent larva mortality was observed 24 or 48 hours post-exposure, and the trial was repeated by placing a new batch of 20 L3/L4 instar mosquito larvae of each species into the test gutters on a weekly basis for eight weeks.

Data analysis

Morbidity and mortality data were subjected to analysis of variance at 5% level of probability. Difference between treatment means was evaluated using Duncan's multiple range test (DMRT).

Only mortality data collected from 24 hours was reported. If mortality was >20% in untreated controls, the whole trial was rejected. If mortality in untreated controls was less than 5%, no correction of mortality data is required. If the control mortality was greater than 5% but not exceeding 20%, results from treated samples were corrected using Abbott's formula as follows:

$$\text{Corrected mortality} = \frac{\%\ \text{test mortality} - \%\ \text{control mortality}}{100 - \%\ \text{control mortality}}$$

If there was no dead larvae recorded in untreated control throughout the trial, percent mortality was calculated as follows:

$$\%\ \text{Mortality} = \frac{\text{Number of dead larvae}}{\text{Number of test larvae}} \times 100$$

The desired level of effective control should be indicated by 80-90% of larva mortality (WHO, 2005).

RESULTS

In indoor stagnant water trials using plastic containers, at 24 hours post-treatment with temephos (0.5 g) capsule in 5 l of water (1 mg ai/l), percent mortality of the three *Ae. aegypti* L3/L4 instar larvae collected from Sg Jelok, Kajang; Seksyen 8, Bandar Baru Bangi and Ridzuan Condominium, Bandar Sunway, Selangor was 99 (larvae from Sg Jelok, Kajang) and 100% (larvae from the other two locations), and 100% mortality was observed for up to 48 weeks (using larvae from Bandar Sri Damansara, Petaling Jaya, Selangor). No mortality in untreated controls were detected in all trials.

In outdoor water stagnant water trials using plastic containers and round flower-pot trays, tests were conducted as in the indoor trials for 24 and 48 hours using larvae from all four locations, but in a 4-week trial, fresh batches of larvae were added once a week and larvae were maintained on fish food pellet. Mortality was 100% in all trials, with no mortality in untreated controls. These results not only confirmed the indoor trials but demonstrated the potent residual larvicidal effect of temephos capsule for a period of (at least) four weeks.

In order to demonstrate the potential of applying temephos capsules to hard-to-reach mosquito breeding sites in homes, tests were conducted in gutters containing either a temephos (1g or 3g) capsule in 15 l of tap water. Laboratory reared *Ae. albopictus* (F78) and *Cx. quinquefasciatus* (F1047) L3/L4 larvae contained in porous cages were placed in treated and untreated gutters and mortality observed after 24 and 48 hours exposure, and residual effects were evaluated as described above for a period of eight weeks. Using temephos (1g or 3g) capsule (0.67 ai/l or 2.00 mg ai/l), 100% 24-hour mortality of *Ae. albopictus* larvae was observed in the first week, dropped to 80% on the 2nd week only for the lower temephos dosage, then rose to 100% for two weeks, before dropping to 65% and 50% at week 5 and 6 respectively (Fig 1A). In the 48-hour mortality tests, for both temephos dosages 100% mortality were observed up to 6 weeks (Fig 1B). Unfortunately, after week 6 a heavy downpour occurred, flushing away most of the temephos in the test gutters resulting in the artefactually low mortality results of the 8th week. Similar results were obtained in tests carried out concurrently with *Cx. quinquefasciatus* (Figs 1C and D). The drop in mortality on week 2 in tests involving the low temephos dosage was also an artefact due to an unexpected evaporation of water in the

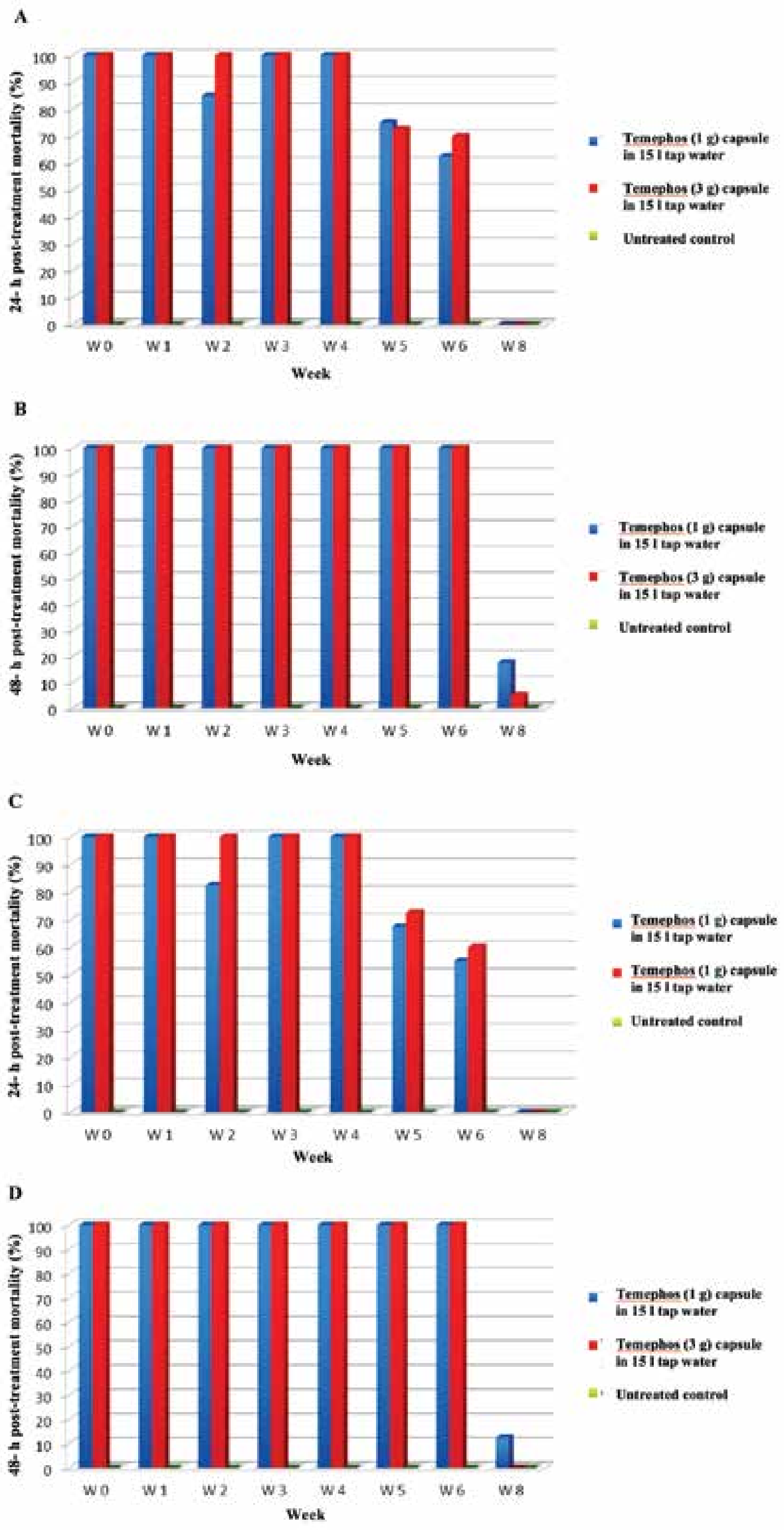

Fig 1-Mortality of laboratory reared *Aedes albopictus* (F78) (A, B) and *Culex quinquefasciatus* (F1047) (C, D) L3/L4 larvae exposed to temephos capsule (TEMEBATE® C) in a water-containing gutter.

A gutter, 3 m (length) x 15 cm (width) x 16 cm (height), placed on the ground and open to the environment, contained a temephos capsule in 15 l of water and larvae (*n* = 20) in a porous plastic transparent container, 102 mm (width) x 102 mm (length) x 45 mm (height). After 24 or 48 hours of exposure, percent mortality of the larvae were recorded. The procedure was repeated by placing a new container of L3/L4 instar mosquito larvae of each species into the test gutter on a weekly basis for eight weeks. Untreated control contained no temephos capsule.

test gutter. Nonetheless, it was possible to conclude a minimum of a temephos (1 g) capsule in 15 l (0.67 mg ai/l) of water in an receptacle mimicking a hard-to-reach breeding location had persistent lethal mosquito larvicidal activity for at least six weeks when exposed for 24 hours.

DISCUSSION

Previous studies have shown use of temephos in potable water is very effective against *Aedes* spp. Bang and Pant (1972) reported applying 1% (w/v) temephos sand granules in houses in Bangkok reduced the Breteau index, *ie* number of positive containers per 100 houses inspected (WHO SEARO, 2011), from 330 to 3.9 after the first application, concluding that control of dengue vectors can be achieved by applying with 1% (w/v) temephos sand granules just before the rainy season and repeating within two months. Geevarghese *et al* (1977) treated in a single application 127 houses in Poona, India with 1% (w/v) temephos granules, which allowed control of *Ae. aegypti* population for a period of eight weeks in the dry season and for 12 weeks in the wet season. More recently, Chadee (2009) in Trinidad, West Indies treated 9,403 water containers with 1% (w/v) temephos sand granules for 25 weeks, which resulted in a significant decline in *Ae. aegypti* from a Breteau index of 19 to 6 and pupa index (number of pupae per house) from 1.23 to 0.35 within a month, while in control untreated sites Breteau index increased from 23 to 38 and pupa index from 0.96 to 2.00; *Ae. aegypti* population did not return to pre-treatment levels until 9-11 weeks post-treatment.

Thavara *et al* (2004) noted such factors as temperature, organic debris and ultraviolet light from sunlight cause degradation of temephos applied in water receptacles, which in conjunction with rapid water exchange and refilling of containers often shorten residual effectiveness of temephos. In order to overcome these limitations, alternative methods of delivering temephos include application of temephos using permeable zip-lock bags, plastic spoon, granules placed in a well-perforated plastic micro-centrifuge tube and corncob grit granules (George *et al*, 2015). Although temephos applied inside perforated zip-lock bags provided longer residual activity as compared to spoon-based application, these modes of temephos application, in contrast to temephos capsule, would be difficult to be deliver to hard-to-access breeding sites of *Aedes*, such as in gutters. Other factors, such as regular supply and budget allocation for the purchase of temephos, are major factors affecting the success of a dengue control program.

Lau *et al* (2015) found complete mosquito larvicidal mortality for up to 26 weeks in indoor plastic containers treated with temephos capsule (1 mg ai/l). The long residual activity of temephos was probably enhanced by the fairly clean water in the containers and the slow-release effect of the capsule. Mulla *et al* (2003) observed glazed clay water storage jars treated with 1% (w/v) temephos sand granules and 1% (w/v) temephos zeolite granules yielded almost 100% mortality for more than 24 weeks. Chen and Lee (2006) reported residual effect of 1 mg/l temephos in earthen jar lasting 15 weeks under laboratory condition.

In summary, temephos capsule (Temebate® C) containing 1% w/w temephos in indoor non-disturbed stagnant water had lethal mosquito larvicidal within a 48 hour exposure and residual activity lasting for at least 48 weeks and up to 28 weeks in an outside (shaded) non-disturbed stagnant water, while in an exposed water environment (gutter), residual activity of temephos capsule lasted for at least six weeks, but re-treatment would be required if the water content is disturbed by rainfall or evaporation. Capsular formulation of temephos provides a simple alternative mode of application of this effective mosquito larvicide in drains, ponds and difficult-to-access places where long-term control is required.

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