Combination of *Mesocyclops thermocyclopoides* and *Bacillus thuringiensis* var. *israelensis*: A better approach for the control of *Aedes aegypti* larvae in water containers

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ABSTRACT: The efficacy of a local Thai-strain of the copepod, *Mesocyclops thermocyclopoides* and the larvicide, *Bacillus thuringiensis* var. *israelensis* (Bti), used jointly and singly, was studied against *Aedes aegypti* in water containers. In a laboratory test, copepods alone produced mortality of 98-100% in 1st instar larvae of *Ae. aegypti* at copepod:larvae ratios ranging from 1:1 to 1:4. In an outdoor field simulated experiment that ran for 16 wk, after a single inoculation, the treatment of copepods and Bti combined yielded the better, more sustainable results than the agents used individually. Numbers of mosquito larvae per sample in the combined treatment were zero during the first 8 wk; larval numbers then increased but were maintained at a very low level for the next 4 wk after which the larvae numbers increased moderately but still remained below numbers in the control. Bti alone kept the larvae at the zero level for the first 4 wk after which their numbers increased slightly and were at low levels up to 12 wk. Copepods alone maintained larval numbers at a low level as compared with those of the control. During the course of the experiment the larval numbers in the control were greater than 20 per sample. Statistically significant differences were noted among treatment means over the total period of the study. The number of copepods in the joint treatment was significantly higher than in the copepod alone treatment for the first 8 wk. The density of copepods, however, for the whole 16-wk period was not significantly different in these two treatments. *Journal of Vector Ecology* 29 (2): 218-226. 2004.

Keyword Index: *Aedes aegypti*, Bti, copepods, dengue, vector control, integrated control.

INTRODUCTION

Dengue and dengue haemorrhagic fever (DHF) are some of the most common infectious diseases which cause economic loss, morbidity, and mortality in most countries of tropical and sub-tropical regions. In Thailand the first epidemic of dengue was recorded in 1958 (Yasuno et al. 1969), and since then the disease has become widely known as one of the major public health problems of the country. Over the past 40 y, since the first outbreak, major epidemics of dengue have occurred every two to four y. The control of dengue in Thailand has focused primarily on the control of the mosquito vector, *Aedes aegypti* (L.), that develops in residential water storage and other artificial containers. From the initial control program in the 1960s until now, the control programs for dengue vectors by the Ministry of Public Health have concentrated on insecticide sprays and reduction of *Ae. aegypti* larvae. However, despite the intensive control program all over the country, the disease still appears in epidemic proportions in some parts of the country. This is indicated by the large number of reported cases in Thailand exceeding 30,000 per y for the past 15 y. The largest epidemic occurred in 1987 with 174,825 cases and 1,007 deaths (Sucharit 1993). The general lack of efficacy of ULV and thermal fog applications have led to a reevaluation of recommended strategies for prevention and control of dengue, focusing attention on the development of an integrated approach to *Ae. aegypti* control (Gubler and Clark 1996). Other consequences of the intensive use of insecticides are the problems of insecticide resistance that have occurred in many mosquito species and of insecticide residues retained in the environment and food chain which affect some components of the non-target biota. For these reasons, emphasis has been placed on using source
reduction, developmental site management, and searching for effective biological control agents.

Copepods are small crustaceans in the class Copepoda, which are commonly found in a variety of natural aquatic and semi-aquatic habitats. Most species are omnivorous (Williamson 1991). Some species of copepods, especially in the genus Mesocyclops, have been studied extensively because of their predatory nature on mosquito larvae (Riviere et al. 1987, Brown et al. 1991, Kay et al. 1992, Marten et al. 1994). In India, *M. thermocyclopoides* Harada was noted to show the greatest predation activity in laboratory assays against *Ae. aegypti*, followed by *Anopheles stephensi* Liston and *Culex quinquefasciatus* Say (Mittal et al. 1997) while in Costa Rica, this copepod was reported as the most successful species in reducing the number of *Ae. aegypti* larvae in laboratory evaluation (Schaper 1999). Another copepod species, *M. guangxiensis* Reid and Kay, was chosen for the *Ae. aegypti* control program in Lao People’s Democratic Republic and was generally accepted by the community (Jennings et al. 1995). In Australia, *M. aspericornis* (Daday) was shown to be the most effective predator of *Ae. aegypti* larvae (Brown et al. 1991). Another species, *M. longisetus* (Thiebaud), was found to kill 100% of *Ae. aegypti* at larval density of 200/L (Kay et al. 1992). In Thailand, a preliminary survey of copepods showed the presence of two species, *M. aspericornis* and *M. thermocyclopoides*, in artesian wells and artificial containers (Vihokto unpublished data). In this study, *M. aspericornis* was found to be an effective predator of *Ae. aegypti* larvae both in laboratory experiments and field trials. In the present study we wanted to know how effective the other local species is in controlling mosquito larvae.

The entomopathogenic bacteria *Bacillus thuringiensis* var. *israelensis* (Bti) has high larvicidal activity against mosquitoes and black flies. At sporulation, each bacterial cell produces proteinaceous parasporal crystalline proteins lethal to mosquito larvae upon ingestion (Gill et al. 1992). Bti has been found to be non-toxic to important non-target organisms, even to chemically sensitive taxa such as mayflies and others (Mulla et al. 1982) and could therefore control mosquitoes with minimal or no impact on beneficial organisms (Hershey et al. 1995). In Thailand, laboratory bioassays showed a high level of activity of liquid Bti (Skeetal) against *Ae. aegypti* larvae from the northeast region (Pipitgool et al. 1991).

Control methodologies that integrate Bti with other biological control agents or insecticides have been investigated. The combined strategies have yielded satisfactory results in terms of both potency and long-term efficacy of integrated strategies (Riviere et al. 1987, de Andrade and Modolo 1991, Tietze et al. 1994, Nerbabosa et al. 1997). In Thailand, major breeding sites of *Ae. aegypti* are water storage jars of various sizes, cement basins, and ant traps (Kittayapong and Strickman 1993). The present investigation was carried out to study the efficacy and compatibility of combining two biological control agents, *Bacillus thuringiensis* var. *israelensis* (Bti) and a local Thai-strain of the copepod, *M. thermocyclopoides*, for the control of *Ae. aegypti* larvae in laboratory and in earthen water storage jars under simulated natural conditions.

**MATERIALS AND METHODS**

**Liquid Bti bioassay**

Liquid Bti used in the experiment was produced at the Department of Biotechnology, Faculty of Science, Mahidol University, Bangkok, Thailand. In order to evaluate its activity and efficacy and to determine its potency in International Toxic Units (ITU), bioassays were carried out against *Ae. aegypti* larvae and compared with that of IPS 82 (Bti) a standard product with 15,000 ITU/mg as determined against *Ae. aegypti* larvae (Bora-Bora strain). Concentration-response relationship between our Bti product against *Ae. aegypti* was determined using WHO standards testing methods (WHO 1981). All assays were conducted in the laboratory at a temperature of 25 ± 2°C and 75 ± 10% RH using a light-dark cycle of 12:12 h. The test larvae were not fed during the 24-h exposure period.

Bioassay data were analyzed using log-probit analysis. Lethal concentrations were determined at 50th and 90th percentiles (LC50 and LC90). The potency of the Thai Bti product was obtained by using the formula,

\[
\text{ITU of Bti product} = \frac{15,000 \text{ ITU} \times \text{LC50 of IPS 82} / \text{Aedes aegypti}}{\text{LC50 of Thai liquid Bti product}}
\]

A concentration 10 times the LC90 was calculated and was used in the simulated field experiment.

**Predatory copepods**

The copepod species used in this study was *M. thermocyclopoides*, originally collected from natural breeding habitats (artesian wells) at Hua Sam Rong Subdistrict, Plaeng Yao District, Chachoengsao Province. Several isofemale lines of this species were established and have been maintained at the Department of Biology, Faculty of Science, Mahidol University, since 1990. Species identification was confirmed by Dr. Janet Reid, National Museum of Natural History, Smithsonian Institution, Washington, D.C. (Vihokto unpublished data). The copepod was mass-reared at 27 ± 2°C and 75
The copepod colony for the proposed studies was started by inoculation of 20 gravid female copepods into a plastic container filled with 3 L of culturing medium consisting of *Paramecium* sp. and commercial powdered fish food. Dechlorinated tap water with pH 7 and kept at 25°C was used for mass-rearing. The *Paramecium* sp. provided the protein source necessary for growth and survival of copepodids (preadults) and for successful reproduction of adult copepods. The powdered fish food was used for the nauplii stage. A stock culture of *Paramecium* sp. was also mass-reared by using boiled rice straw after cooling. The initial colony was left for 1 wk to allow the *Paramecium* sp. to increase in number. The presence of *Paramecium* sp. in the culture was determined using a stereomicroscope. Two hundred ml of paramecium suspension was added daily and 0.3 g of fish food was added every 3 d to each copepod culture container. The copepod reproductive cycle was quite short, usually taking 1 wk to develop from egg to adult. Using this rearing technique, copepods could increase 100-fold within a 2-wk period.

**Laboratory predation**

In order to confirm predation efficiency and to find the optimum ratio of copepods to mosquito larvae that would be released into the water storage jars for simulated field experiment, the local Thai strain, *M. thermocyclopoides*, was tested against *Ae. aegypti* 1st instars in the laboratory. The predation experiment was conducted in 1-L beakers containing 25 copepods and 4 larval densities of 25, 50, 75, and 100 *Ae. aegypti* larvae. Survivorship of mosquito larvae was assessed 24 h later. Four replicates were used and numbers of live larvae in containers with copepods were compared with those of larvae in control containers (no copepods).

**Mosquito strains**

*Aedes aegypti* mosquitoes used in Bti bioassays were obtained from a susceptible colony maintained at the Department of Medical Sciences, National Institute of Health, Thailand. This colony was established from eggs collected in Bangkok and maintained since 1989 without exposure to any insecticides or Bti products.

To determine the efficacy of the combined copepods and liquid Bti in a simulated field experiment, an *Ae. aegypti* colony was established from wild field-caught mosquitoes. Twenty ovitraps were placed at Hau Sam Rong Subdistrict, Plaeng Yao District, Chachoengsao Province, for 2 wk. This location was selected because the field trial using the combined treatments of copepods and Bti against *Ae. aegypti* was planned to be conducted there. Collected eggs were used to establish the new mosquito colony in the laboratory. The colony was kept at 25 ± 2°C and 75 ± 10% RH with a light-dark cycle of 12:12 h. Larvae were fed with fish food powder and adults were fed with 10% sugar solution and blood-fed on hamsters. Larvae from this colony were used in the following simulated field experiment.

**Simulated field experiment**

The outdoor experiment was carried out for 16 wk from October 2001-January 2002 in a greenhouse of the Faculty of Science, Mahidol University, Bangkok. The average ambient temperature was 32 ± 4°C and the humidity was 65 ± 10% RH. Sixteen 200 L earthen jars filled with 100 L of dechlorinated tap water were divided into 4 treatments: copepods only, liquid Bti only, both copepods and Bti, and no copepods or Bti (control). Each control agent (copepods, Bti, or both) was introduced once only into the jars on the first day of the experiment. Fifty gravid *M. thermocyclopoides* females were released into each jar of the copepod treatment. For the Bti treatment, 0.53 ml of the liquid Bti product (=10X LC90) was introduced into each jar. For the combined treatment, 50 gravid female copepods and 0.53 ml of liquid Bti were placed into each jar.

In order to simulate natural productivity of *Ae. aegypti* (Southwood et al. 1972), 50 1st instar larvae were
added into each jar daily until termination of the 16-wk experiment. A fine mesh net was used to cover the mouth of each jar in order to prevent contamination from outside mosquitoes or other organisms (Figure 1). The net cover had a 20 x 20 cm opening in the side covered by a net door through which all activities were conducted.

Larvae and pupae of mosquitoes and all stages of copepods (nauplii and adults) were sampled each week by using a round sweep net of 25-cm perimeter. The net was swept gently in the water 4 times around the perimeter of the jar from the surface layer of water to the bottom and the immature mosquitoes and copepods that were caught were transferred into a tray with water. This regimen of sampling was repeated 4 times for each jar after which the larvae, pupae, and copepods were counted. To simulate natural conditions, pupae (in low numbers and not reported) and adult mosquitoes found in the jars were eliminated during weekly sampling while the mosquito larvae and copepod samples were transferred from trays back into the jars after counting.

Data analysis

Numbers of mosquito larvae were compared among treatments by using one-way analysis of variance (ANOVA) followed by Duncan’s Multiple Range Test. (SPSS Inc. Chicago, IL). To homogenize the data, the numbers were log10 (X+1) transformed prior to statistical comparisons. Copepod numbers were also compared between the copepods only and the copepods with Bti treatment by using the t-test analysis.

The efficacy of different treatments was assessed by the calculation on percent reduction using the formula,

\[ \% R_a = \left( \frac{C_a - T_a}{C_a} \right) \times 100 \]

where \( R_a \) is the reduction in larval number at week a, \( C_a \) is the average number of larvae per control jar at week a and \( T_a \) is the average number of larvae per treated jar at week a (Tietze et al. 1994).

RESULTS

Potency of Thai Bti product

The lethal concentrations at the 50th and 90th percentiles of the liquid Bti product were 0.2375 mg/l and 0.5268 mg/l, while those of the standard Bti IPS 82 were 0.0084 mg/l and 0.0162 mg/l respectively. From the bioassays data, the International Toxic Units (based on LC50) showing potency of the liquid Bti product was obtained from:

\[ \text{Potency of Bti product} = \frac{15000 \times 0.0084}{0.2375} = 531 \text{ ITU/mg} \]

The local Bti product was not concentrated or formulated and therefore possessed low level of activity as compared to other Bti products. It nevertheless had considerable larvicidal activity and was suitable for use in the simulated field experiment.

Copepod predation trial

In the laboratory test, *M. thermocyclopoides* were shown to be an effective predator upon 1st instar larvae of *Ae. aegypti*. These copepods killed 100% of larvae stocked at larval densities of 25 (1:1 copepods:larvae), 50 (1:2), and 75 (1:3); however, efficacy was reduced slightly, giving a mortality of 98% at the larval density of 100 (1:4) during the 24-h test period. Mortality rates of larvae in the control were 0% for all replicates during the same period.

Simulated field experiment

Copepod only treatment. *M. thermocyclopoides* survived and established quickly in water storage jars under the simulated field conditions in the greenhouse after only one inoculation. They reduced mosquito larvae in the jars by maintaining the larval numbers < 5 per sample for 7 wk (Figure 2) as compared to the more than 20 larvae per sample for the same period in the control. After this period, the larval density increased in copepods but was still in the low range (not more than 12 larvae per sample) as compared with those of the control where the larval numbers per sample were > 20 throughout the experiment (Figure 2). Comparing the larval numbers per sample in the copepods alone treatment with that of the joint action of copepods and Bti, numbers in the former treatment were higher than the joint treatment for 14 wk (except the 13th wk) and
Figure 2. Mean number of *Aedes aegypti* larvae per sample each week in treatments of copepods, copepods and Bti combined, Bti, and control.

Figure 3. Percent reduction of *Ae. aegypti* larvae for each week after treatments with combined copepods and Bti, Bti alone, and copepods alone.
then were lower for the remaining 2 wk of experiment. When the larval numbers under the control of copepods alone were compared with those under the control of Bti alone, it was noted that larval numbers per sample under the control of copepods were slightly higher than those under the control of Bti for 5 wk. After that, the two populations were essentially similar for the next 6 wk, and then they were quite different until the termination of the experiment, when the numbers of larvae were markedly higher in the Bti treatment. The mean numbers of larvae sampled were the lowest in the joint treatment followed by copepods alone and Bti alone treatments. All three treatments were significantly different from the control and there was no difference between copepod and Bti treatment ($F = 23.083$, $df = 3/60$, $P<0.01$) as shown in Table 1.

**Joint action of Bti and copepods**

When liquid Bti was used together with copepods, overall, it produced better results. The larval numbers per sample in this treatment were zero for the first 8 wk, after which mosquito larvae were noted in low numbers (1-2) in the samples. The larval populations were still lower than those of other treatments for 12 wk. The efficacy of the combined treatment declined after this period. The larval numbers per sample increased sharply from 2 to 14 from the 13th wk and were 6 to 14 until the termination of the experiment. Overall, the joint treatment induced highest level of suppression in mosquito larvae (See Table 1).

**Bti only treatment**

The liquid Bti product reduced the larval numbers in the jars to zero per sample for 4 wk. After that, the sampled larval numbers increased moderately until the 12th wk and then increased sharply after this period to a level almost the same as that of the control in the remaining 4 wk (Figure 2). It was noted that the sampled larval numbers in the Bti alone treatment increased concomitantly as those of the joint treatment of copepods and Bti, but the joint treatment exhibited lower larval numbers than Bti. Overall, the Bti and copepod treatments showed similar suppression of mosquito larvae (Table 1).

The percent reduction in larval numbers sampled in the different treatments was compared on a weekly basis (Figure 3). Copepods alone produced a high level of reduction of 79 to 97% during the first 7 wk. The percent reduction ranged from 52% to 77% during the remaining 9 wk of the experiment. Bti alone, in contrast, gave reduction of 100% for 4 wk. Level of reduction was still high (87-96%) during wk 5 to 7. Thereafter the level of control dropped to below 80% during the remaining period (See Figure 3). Percent reduction in larval
numbers sampled from the combined copepods and Bti treatment was 100% for 8 wk and was still high by wk 12, yielding 91-96% of reduction. However, the percent reduction dropped to 46 to 76% during wk 13 to 16 of the experiment. The results indicated that the joint action of copepods and Bti gave long-lasting control of *Ae. aegypti* larvae in water storage jars when compared with the treatments that used only one type of strategy.

During the course of the experiment we noted an upward trend in copepod populations in the joint treatment of copepods plus Bti. Copepod populations in the joint treatment were significantly higher (Figure 4) for the first 8 wk of the experiment than in the copepod alone treatment ($t = -4.97, df = 14, P<0.01$). The mean number of copepods per sample during this period in the copepod alone treatment was $10.25 \pm 0.77$ per weekly sample as compared to $16.63 \pm 0.98$ per sample in the joint treatment. The density of copepods, however, for the whole 16-wk period was not significantly different in these two treatments ($t = -1.51, df = 30, P>0.1$). The mean number of copepods without Bti for the entire period was $9.47 \pm 0.60$, while the mean number of those in the joint treatment was $12.11 \pm 1.26$ per sample. There seems to be a direct relationship between Bti activity and copepods at least during the early period of the experiment when Bti larvicidal activity was at its maximum. As the Bti activity declined, so did the density of copepods.

In the copepod-Bti treatment, high density of copepods during the initial 10-12 wk positively correlated with reduction of *Ae. aegypti* larvae (See Figure 4). As the copepod density declined to $<10$ per sample, the *Ae. aegypti* larvae showed increased survivorship. This trend was also noted to a certain extent in the copepod alone treatment (Figure 4). When the copepod density remained at 10 per sample, mosquito larval populations were suppressed adequately. As the copepod density decreased to 7 per sample, mosquito larval survivorship increased.

**DISCUSSION**

Predacious copepods, *Mesocyclops* spp., have been known for their predation upon mosquito larvae. However, no investigations on the predatory ability of the copepod *M. thermocyclopoides* have been carried out in Thailand. In this study, a local Thai strain of this copepod was chosen for study to obtain baseline data to see the potential of this predator for possible use in a control program of dengue vectors in Thai villages. In our laboratory test carried out in 1-L beakers by introducing *Ae. aegypti* 1st instar larvae (25, 50, 75, 100) but keeping the number of *M. thermocyclopoides* constant at 25, this copepod showed excellent predatory efficiency by producing mortality of 98-100% of larvae in 24 h at the predation-prey ratios ranging from 1:1 to 1:4. Similar laboratory evaluation of the potential of this copepod was reported by Mittal et al. (1997) in India in a test varying the numbers of *M. thermocyclopoides* (from 2 to 20) while the number of *Ae. aegypti* larvae was kept constant at 25. The results of this study indicated that the copepod:larvae ratio required to produce over 90% predation in 24 h should be 1:2. In another area, a laboratory study on six species of Costa Rican copepods was carried out by Schaper (1999) using only 1 copepod per 10 *Ae. aegypti* larvae in 50 ml of water, a volume much smaller than in our study. *M. thermocyclopoides* proved to have a higher predatory action than the other 5 species. The result of our simulated field experiment carried out in 200-L jars placed in a greenhouse showed that this copepod (*M. thermocyclopoides*) could not completely eliminate all daily inoculated larvae in the 16 wk of the experiment. Results of cage simulated experiments on the efficacy of some species of copepods against *Ae. aegypti* larvae conducted by Jennings et al. (1995), Kay et al. (1992) and Schaper (1999) were different from our results. *M. guangxiensis* and *M. aspericornis* eliminated all mosquito larvae produced by 25 pairs of *Ae. aegypti* in 3-L tins placed in screen cages that were inoculated by 50 gravid female copepods 6 wk after the start of the experiment (Jennings et al. 1995). Fifty copepods of each *M. longisetus* and *M. aspericornis* killed all *Ae. aegypti* larvae in 5-L earthenware pots placed in cages within 3 wk (Kay et al. 1992). In the test carried out by Schaper (1999) using predation-prey ratio 1:1 (*M. thermocyclopoides : Ae. aegypti = 20:20*) in one inoculation into 2 L of water in plastic containers, no larvae were found 4 wk later. The difference between our results and those of the other studies is due to the large size of the container and the large volume of water (100 L) which we used in our experiment. In such a large volume of water, the frequency of encounter between predator and prey is greatly reduced. In small-sized containers with a small volume of water, the chance of encounter is high. To compensate for the level of reduced encounter, it will be desirable to stock with larger number of predators or augment the predator by additional timely inoculations. A single inoculation of *M. thermocyclopoides* into 200-L jars filled with 100 L of water was enough for the copepods to persist in our study and produced results similar to those of Tietze et al. (1994) where copepods were introduced once into tires inundated with 4 L of water. In the Costa Rican study (Schaper 1999), *M. thermocyclopoides* survived from 2 to 5 mo in bromeliad leaf axils and 3 to 6 mo in used car tires under the field
trial conditions. In our simulated field study this copepod survived for a minimum of 4 mo. A population of copepods still prevailed in the jars at the end of the experiment.

The highest level of control of Ae. aegypti larvae in our study (91-100% for 12 wk) was the use of combined treatment of copepods and Bti. The treatment combination produced results similar to those of Tietze et al. (1994) where M. longisetus was combined with Bti, Bacillus sphaericus, or methoprene controlled field populations of mosquito larvae developing in waste tires for 20 wk. The combined agents provided better mosquito reduction than either copepods or the other control agents alone. The percent reduction of Ae. aegypti larvae in our study was essentially the same as that of Tietze et al. (1994). The reduction of larvae in our study was maintained at 100% for 4 wk with Bti alone and 8 wk with copepods plus Bti, while reduction due to copepods alone was somewhat below 100%. Reapplication of each control agent was not carried out so the percent reduction in our study declined in the later part of the experiment.

The local Thai liquid Bti product was chosen for evaluation in this study for its cost effectiveness and the need to develop local production. Although potency of the Bti was low, its effectiveness was adequate in the simulated field experiment. Waste tires treated with Bti (Teknar HP-D) had cyclic mosquito abundance and Bti was found to be effective for up to 2 to 3 wk after each application (Tietze et al. 1994). Batra et al. (2000) evaluated the efficacy of VectoBac in both tablet and granular formulations and Bacticide powder against Ae. aegypti larvae in desert coolers and tires. A 100% reduction of late larval instars of this mosquito was obtained for a period of 2 and 3 wk respectively. Similarly Dominic et al. (2000) evaluated the efficacy of VectoBac aqueous suspension in stream pools for the control Anopheles larvae and more than 80% reduction of immatures was observed for 2-8 d.

In our study, we noted a dependent interaction between copepod numbers and Bti. Bti was compatible with M. thermocyclopoides with no ill effects on the predator. The ten times LC<sub>90</sub> was with no deleterious effects on copepods. Similarly, Bti at 10X the maximum label rate was compatible with M. longisetus (Tietze et al. 1994). One interesting relationship between copepods and Bti that we noted was the prevalence of high numbers of copepods in the joint treatment as compared with the copepod alone treatment, as long as Bti was present at larvicidal concentrations. Such a relationship between copepod abundance and Bti was also reported by Tietze et al. (1994), where high numbers of copepods prevailed under repeated Bti applications. We postulate that this phenomenon was due to the higher consumption of larvae by the copepods in the joint treatment. Larvae ingesting Bti Cry toxins become sluggish and were more prone to predation by the predator than healthy larvae. As the larvicidal efficacy of Bti declined in our study 5 to 6 wk post-treatment, copepod populations began to decline.

In order to improve the efficacy and potency period of one time inoculation of copepods and one treatment of Bti, no water was drawn out or added into the jars. The study indicated that under outdoor conditions, special food was not essential for the nauplii stage of copepods and M. thermocyclopoides should survive in field releases without providing food sources. The reapplication of these two control agents needs further study for the long-term control of mosquito vectors in actual field situations.

In this study, the use of copepod M. thermocyclopoides in conjunction with Bti for mosquito control in jars gave the best results when compared with the application of copepods or Bti alone treatments. The approach of combined copepods and Bti may, therefore, also prove useful for mosquito control in other types of breeding containers both under simulated field and actual field situations.

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