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Colonization and Temephos (Abate) insecticide susceptibility status of *Aedes aegypti* mosquitoes from selected townships in Yangon Region

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ABSTRACT

Aedes mosquitoes are primary vectors for dengue fever, dengue hemorrhagic fever, yellow fever, chikungunya, and Zika virus. Temephos, a widely used larvicide in Myanmar, has been employed for dengue vector control for over a decade. Thus, it is important to assess the larvicide susceptibility status of *Aedes* mosquitoes, particularly *Aedes aegypti*, which is dominant in Yangon Region. Establishing dengue vector colonies in an insectary is crucial for larvicide susceptibility experiments and also for vector competence study. *Aedes aegypti* larval collection activities were conducted in five townships of Yangon Region (Insein, Shwe Pyi Thar, Dagon, North Dagon, and South Dagon) from September 2023 to August 2024 to establish a laboratory colony. The temephos larvicide susceptibility status of laboratory-reared F1 generations of third and fourth instar *Aedes* larvae was tested using laboratory doses of 0.1 g/L and field doses of 1 g/L temephos. Egg-laying ability, hatching ability, and production of offspring were also recorded. Results revealed that 74%, 84%, 97.5%, 97.5%, and 100% mortality was observed within one hour of exposure to the laboratory dose from Dagon, Insein, North Dagon, Shwe Pyi Thar, and South Dagon townships, respectively. However, all F1 generation *Aedes aegypti* larvae exhibited 100% mortality within 24 hours when exposed to both laboratory and field doses of temephos. Meanwhile, the notable high mean for egg-laying was observed in the F_2 generation (76.77 ± 23.14 eggs, range: 40-105) and the F_3 generation (74.95 ± 23.14 eggs, range: 60-101). The F_2 generation also exhibited the highest egg hatching ability (73.29 ± 6.3%). In all generations, the emergence of female pupae was higher than that of male pupae. Field dose of temephos remains effective against *Aedes aegypti* larvae within 1 and 24 hours, although some tolerance to laboratory doses was observed within one hour. Based on these results, it can be concluded that the test larvae from all areas remain susceptible to temephos. The high egg-laying capacity of F_2 and F_3 generations, coupled with the higher emergence of females, poses a potential risk for dengue transmission. Therefore, temephos can still be used as a larvicide, particularly for controlling *Aedes aegypti* larvae.

Keywords: *Aedes aegypti*; Adults; Colony; Dengue; Eggs; Generation; Knockdown; Larvae; Mortality; Susceptibility; Status; Temephos.

1. Introduction

Laboratory rearing of *Aedes aegypti* (L.) has been a common procedure among entomologists and other scientists for about 50 years. Many improvements in equipment and techniques have been developed [1-3], and production by these methods has proved adequate for studies using as many as several thousand specimens. Experiments on dispersal and control by the release of sexually sterile males [4,5] require the sustained production of millions of specimens.

Aedes mosquitoes such as *Ae. aegypti* and *Ae. albopictus* are vector of Dengue Fever and Dengue Hemorrhage Fever, Yellower fever and Zika virus, these viral pathogens are transmitted to humans by the bite of these mosquito species and are abundantly found in tropical regions in Southeast Asia, Asia, and the Pacific [6]. The breeding places of *Ae. aegypti* are almost entirely confined to artificial collections of water, small artificial collections of water; water receptacles of all kinds artificial containers or things resembling these; any receptacle holding fresh water [7] (Barraud, 1934). Such breeding places may be in the dwelling itself, for example the earthenware pots (chatties) or calabashes almost universal in native huts for storing water; metal drams, Bago jars, glassed earthen pots, flower vases, neglected cups or jugs or other household collections of water in better-class houses, or another type of breeding place about the house arises from defects or neglected features of the building itself, for example uncovered cisterns [8-10], roof gutters [11] (Jones, 1925), cracks in the masonry, traps of drains, or flush tanks or pans of water-closets when temporarily out of use.

Aedes aegypti habit is more diurnal than nocturnal and is widely recognized in the tropics as the 'day biting mosquito'. Nevertheless, it shows a marked preference for shade and dark corners and in general, avoids bright light and open spaces. It will feed, however, readily in moderate or bright daylight at any time of the day.

An estimate, 50 million people are at risk in dengue endemic countries [12]. In South East Asia and Western Pacific Regions, which bear nearly 75% of the current global disease burden due to dengue [13]. Globally, 3.5 billion people are at risk of contracting dengue fever and 1.3 billion live in dengue-endemic areas in 10 countries, except in DPR Korea [14]. In 2023, and as of 8 June, 2162214 cases and 974 dengue deaths have been reported globally [15]. Temephos (Abet) is a very effective larvicidal agent and used in all tropical country for controlling *Aedes* larvae in man-made and artificial water storage containers [14].

Dengue Fever and DHF are increasingly becoming serious public health problems in Myanmar, especially among the 5-10, and 11-15-years old age groups and now noted 15 years above, a vast majority of the cases occur in 5-8 years old age group [16,17]. In Myanmar, the highest numbers of DHF cases were reported from Irrawaddy, Kachin, Magway, Mandalay, Mon, Rakhine, Sagging, Tanintharyi and Yangon regions [18].

Clinically recognizable DHF was first noticed in 1969 in Yangon Children's Hospital, followed by the first epidemic in Yangon in the year 1970 [18]. High number of dengue hemorrhagic fever (DHF) transmissions occurs during raining season from May to October. Water storage practices in Yangon city provide year-round breeding opportunities for the vector. In States and Regions, mostly *Aedes* breeding sites become established in raining season when the locals store rain water for the domestic usages. Although colonies of *Aedes* species have been established in the laboratory in the past, no reported in the literature and thus no satisfactory method of rearing this species has been yet described. This species is considered a potential threat to transmit DF and DHF in young children and now infect all age of population.

To assess the potential risk of dengue in Myanmar, it is necessary to conduct vector competence studies to establish the vector colony. In this study, several experiments were conducted to describe the successful rearing conditions of the colony and the key biological attributes of the laboratory-reared *Aedes* mosquitoes. Consequently, as a first step, the aim of the present study is to establish a laboratory colony of *Aedes* mosquitoes from Dagon, Insein, Shwe Pyi Thar, Dagon Myo Thit North and Dagon Myo Thit South townships and provide a detail rearing protocol for further laboratory study of dengue vector research on vector competence as egg laying ability, hatchability and susceptibility status for better understanding of behavior of *Aedes* mosquitoes.

Recently, it has been reported that *Ae. aegypti* has developed resistance to one of the four classes of insecticides most commonly used for its control (pyrethroids, organochlorines, carbamates, and organophosphates) [19], 26 countries have detected resistance to all four classes [20]. An Indonesian study revealed that all field strains were resistant to temephos at a dose of 0.012 mg/l, with mortality rates at 24 hours of 22% to 60% [21]. Temephos, a widely used larvicide in Myanmar, has been employed for dengue vector control for over a decade. Resistance in *Ae. aegypti* populations were relatively higher due to endophilic behavior [22]. Therefore, the present study is needed to establish a laboratory colony of *Aedes* mosquitoes and to assess the temephos insecticide susceptibility status of *Aedes* mosquitoes to control dengue disease.

1.1. Study Objectives

(1) To establish mosquito colonies from the selected townships in the insectary of Medical Entomology Research Division, Department of Medical Research.

(2) To assess the temephos larvicidal insecticide susceptibility status of laboratory reared *Aedes aegypti* mosquito larvae from the selected townships.

2. Materials and Methods

2.1. Study design

Laboratory and field base experimental study was performed.

2.2. Study period

Study period was determined from September 2023 to August 2024.

2.3. Study area

Study was conducted in Insein, Shwe Pyi thar, Dagon, North Dagon, and South Dagon Township, Yangon Region.

2.4. Study population

Aedes adults, larvae, Temephos (Abate), containers and discarded water storage containers.

2.5. Mosquito larvae collection

Aedes larvae were collected from discarded water storage containers from Shwe Pyi Thar, Insein, Dagon, Dagon Myo Thit North and Dagon Myo Thit South Townships and colony was maintained in DMR Entomology laboratory for laboratory test.

2.6. Species identification of mosquitoes

Larvae and adult mosquitoes emerged from larval survey were identified by morphological methods according to the key of Rampa and Prachong [23].

2.7. Laboratory tests

In the laboratory, Egg-laying ability, hatching ability, and production of offspring were also recorded till F_{10} generations. And Temephos larvicide susceptibility status of *Aedes* larvae were tested in laboratory.

2.8. Temephos

Temephos is an organophosphate larvicide used to treat water infested with disease carrying insects including mosquitoes, midges, and black fly larvae. Temephos affects the central nervous system through inhibition of cholinesterase.

2.9. Procedure of susceptibility test

F_1 generation of 3^{rd} & 4^{th} instar *Aedes* larvae 50 each were put into laboratory dose 0.1g/Lit and field dose 1g/Lit of Temephos concentrations from each Township. Mortality was checked and recorded at 1hour and after 24hours according to WHO [24]. 5 replicates test was done with F_1 generation of *Aedes* larvae from each Township.

2.10. Data analysis

Excel sheet was used. Larvicidal susceptibility status of mosquito larvae were calculated in percent. Egg-laying ability, hatching ability, and production of offspring were also calculated in percent and analyzed by using standard method [25].

3. Results

Figure 1 shows that mean number of egg laying ability of wild and F_1 to F_{10} generation of *Aedes aegypti* mosquito from different townships in the laboratory was found that the high mean number of egg-laying was observed in the F_2 generation (76.77 ± 23.14 eggs, range: 40-105) and the F_3 generation (74.95 ± 23.14 eggs, range: 60-101) and followed by F_4 generation range between 50 to 90 eggs.

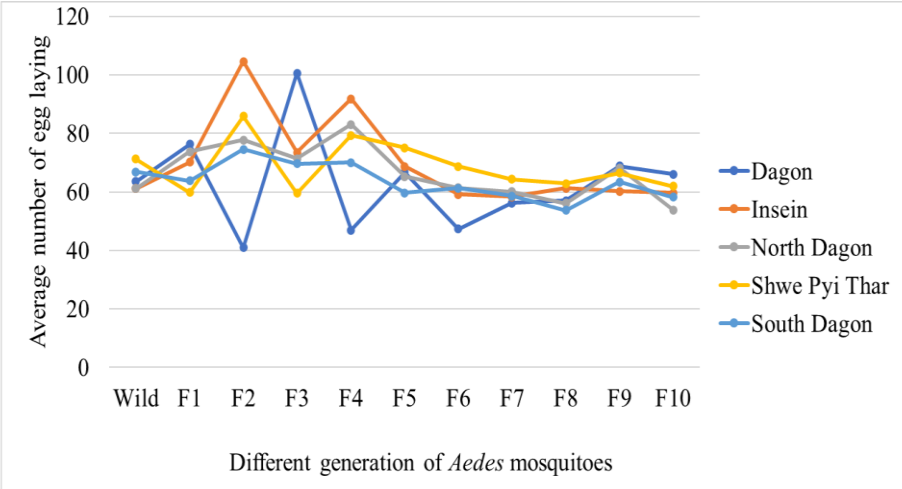

Figure 1. Mine number of eggs laying ability of wild caught and F_1 to F_{10} generation of *Aedes aegypti* mosquito from different Township in the laboratory

The F_2 generation also exhibited the highest egg laying, hatching ability and emerging pupae (76.77±3.3%, 73.29± 6.3% and 67.54±2.6%). Followed by F_3 generation (74.95±2.1%, 66.33±3.5%, and 61.15±2.7% and lowest was observed F_7 generation (54.21±3.1%, 46.75±3.6 % and 39.40±2.8%).

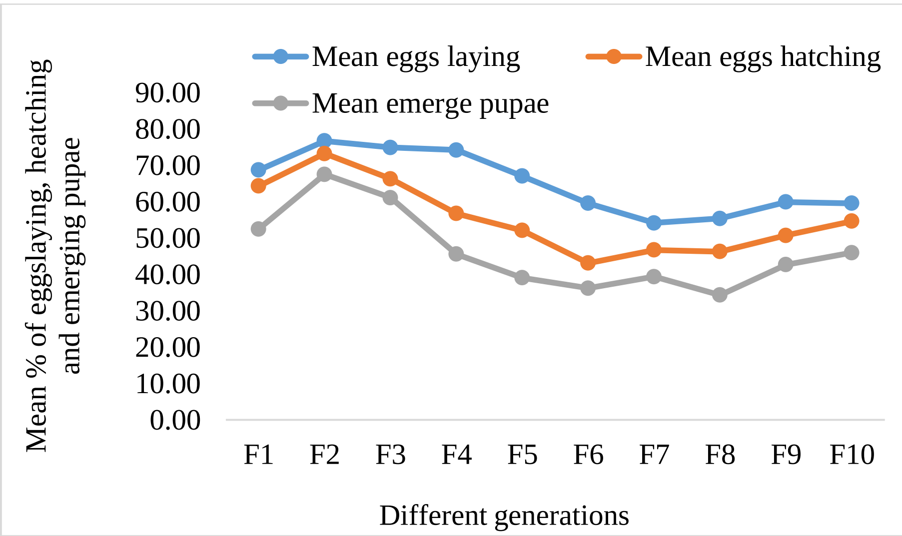

Figure 2. Mean % of eggs laying, hatching and emerging pupae by different generations in laboratory condition

Figure 3 Shows that emerging of male and female *Aedes* mosquitoes by different generation and found that female mosquito was higher emerging rate than male emerging rate in all generation of laboratory raring *Aedes* mosquitoes.

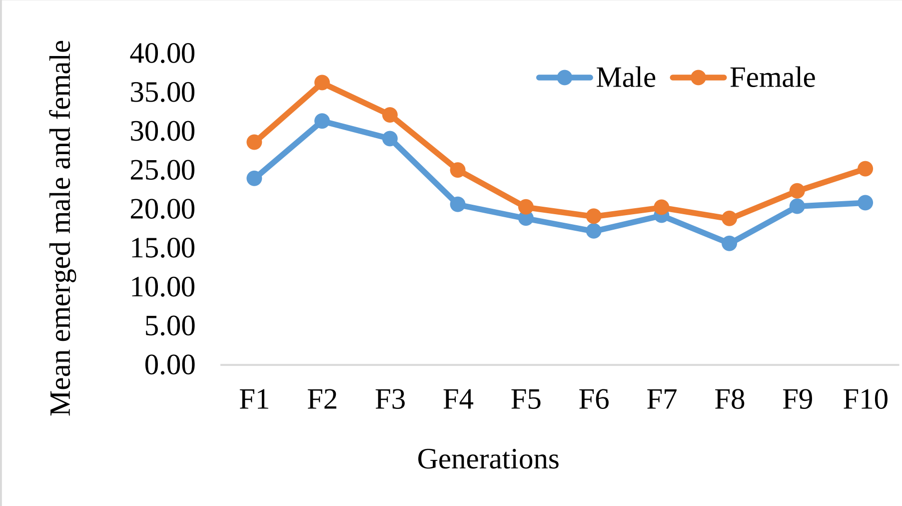

Figure 3. Emerging of male and female *Aedes* mosquitoes by different generation in laboratory condition

Figure 4 shows that susceptibility status of F_1 generation of 3rd & 4th instar *Aedes* larvae in laboratory dose of Temephos (Abate) 0.1g/ Lit and found that 100% mortality was found within 24hours in 0.1%Temephos solution in laboratory. Knock down effect of *Aedes* larvae was began after 20 minutes of exposure period, knock down effect was found 15% at 25min from Shwe Pyi Thar, 20% at 30min from South Dagon, 24% at 33 min from Insein and 5% from North Dagon and14% knock down at 40min from Dagon Townships and 100 knockdown was observed after 60min from South Dagon and Shwe Pyi Thar Township. Some *Aedes* larvae from Dagon, Insein and North Dagon Townships were found alive till 60minutes of exposure period in 0.1g/lit Temephos solution. All the larvae from different Townships were found 100% die after 24 hours of exposure periods.

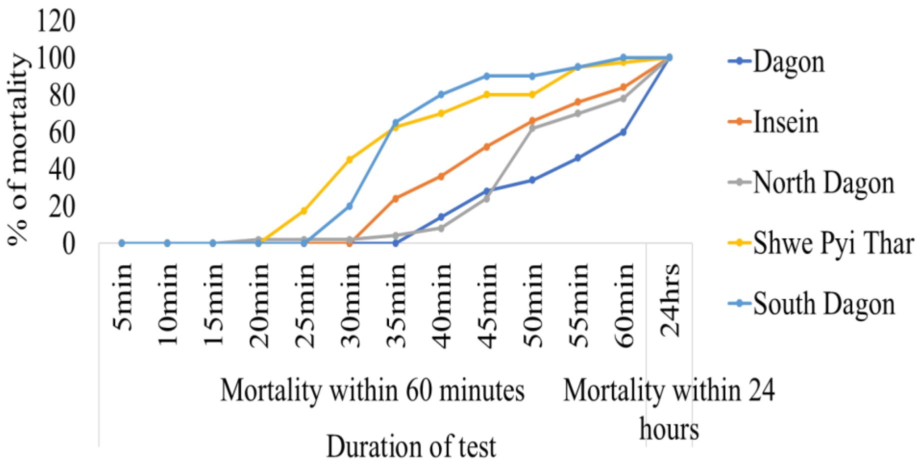

Figure 4. Susceptibility status of F_1 generation of 3rd & 4th instar *Aedes* larvae in laboratory dose of Temephos (Abate) 0.1g/ Lit.

Figure 5 shows that mortality rate of F_1 generation of 3rd and 4th instar *Aedes* larvae in field dose 1g/Lit of Temephos (Abate) and found that all the F1 generation of *Aedes* larvae from different Townships were 100% died at field dose 1g/lit of Temephos within 24 hours of exposure period. Knock down effect was started at 10min from Dagon Myo

Thit South, at 20min from Shwe Pyi Thar, at 25min from Dagon, Dagon Myo Thit North and Insein Townships. *Aedes* larvae from all townships were found 100% knockdown within 60 min in field dose concentration.

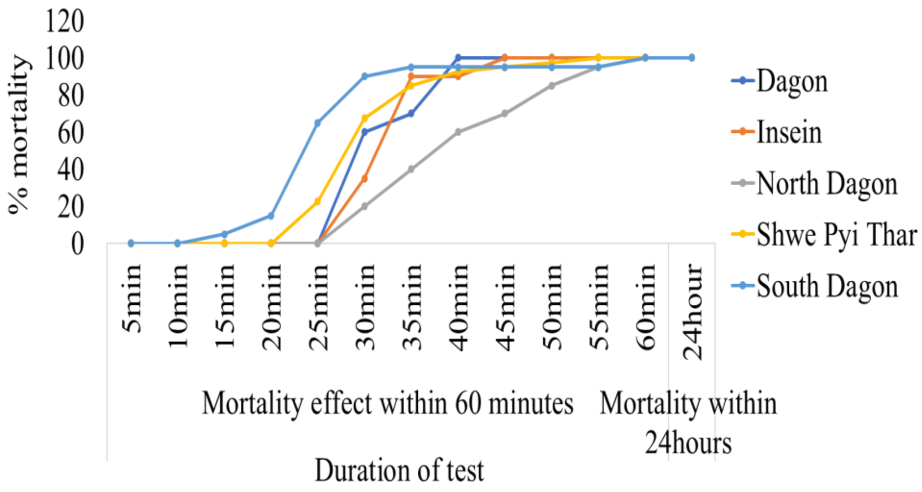

Figure 5. Mortality of F_1 generation of 3rd and 4th instar Aedes larvae in field dose of Temephos (Abate) 1g/Lit.

Figure 6 shows that comparison of mean mortality rate of F_1 generation of 3rd and 4th instar *Aedes* larvae against laboratory dose (0.1g/Lit) and field dose (1g/Lit) of Temephos (Abate) and found that 100% mortality was found within 24 hours in both laboratory and field dose of Temephos concentration. Mean knockdown was started at 15 min in Field dose and at 20 min in Laboratory dose, knockdown effect was gradually increased till 60min. In field dose all F_1 generation of larvae were found 100% knockdown within 60min and 100% mortality within 24 hour. And in laboratory dose, 90.3 of the larvae were knockdown within 60min and 100% mortality was observed within 24hour. 9.7% knockdown resistance was found within 60 min in laboratory dose (0.1g/Lit) of Temephos concentration in laboratory condition.

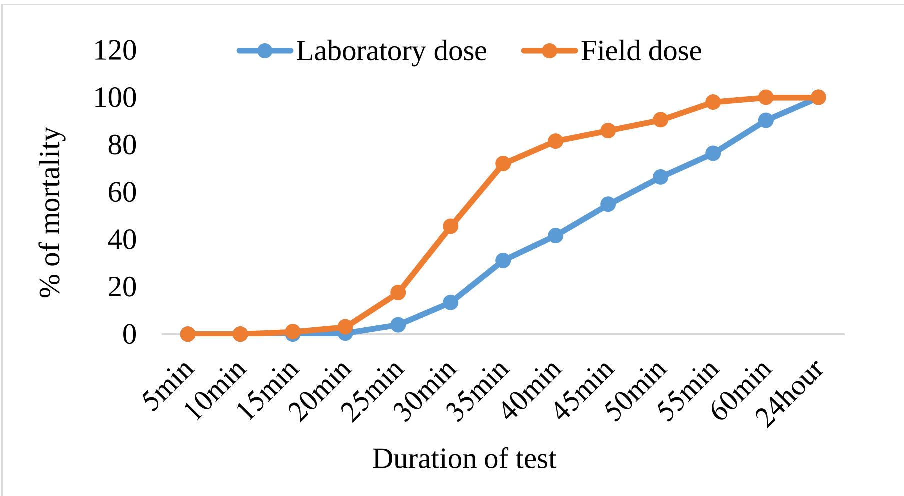

Figure 6. Comparison of mean mortality rate of F_1 generation of 3rd and 4th instar *Aedes* larvae against laboratory dose (0.1g/Lit) and field dose (1g/Lit) of Temephos (Abate)

4. Discussion

Aedes aegypti, and *Ae. albopictus* are potential vectors of Dengue and Dengue Heamorrhage fever caused by fibrin viruses mostly harmful to human being especially in children age between 6 to 12 years. Although the diseases caused all age [16,17]. Human activities and poorly maintained sanitation in the surrounding area can trigger the breeding of mosquitoes [26]. High- density of water storage containers due to lack of water supply and have not that made cleaning it may offer the best breeding conditions for mosquitoes as regions with improper drainage and piping systems show high potential in becoming *Aedes* habitats [27]. The abundance of these species is influenced by the preference and inherent behaviors in oviposition of the female mosquitoes, as well as other biotic and abiotic factors [28,29]. Environmental factors such as relative humidity, wind and temperature influence the occurrence and density of these species. Anthropogenic changes in the environment will also influence the abundance and distribution of these species [30]. There are a lot of potential breeding sites in high-rise buildings and it is difficult to control by just focusing on a singular control method. The increase in the number of breeding mosquitoes within the region is the main cause of dengue outbreaks [31]. The disease is transmission by adult *Aedes* mosquitoes not transmitted in larval stage. In global and in Myanmar Temephos is used to control *Aedes l*arvae in water storage containers to reduce transmission of diseases via to larvae control. Temephos is widely used after Surname in Myanmar. It is a very effective control tool for larval control. Although, it was used about 2 decades in Myanmar. In others researchers in different countries revealed that Temephos resistance was observed in larval stage. Therefore, the present study focusing laboratory dose 0.1g/lit dose and field dose 1g/lit dose of Temephos were tested against laboratory reared Dagon, Insein, Dagon Myo Thit north, Dagon Myo Thit south and Shwe Pyi Thar strains *Aedes* F1 generation of 3rd and 4th instar larvae in laboratory condition. Results revealed that 74%, 84%, 97.5%, 97.5%, and 100% mortality was observed within one hour of exposure to the laboratory dose from Dagon, Insein, North Dagon, Shwe Pyi Thar, and South Dagon townships, respectively. However, all F1 generation of *Aedes aegypti* larvae exhibited 100% mortality within 24 hours when exposed to both laboratory and field doses of Temephos. And also, F1 generation of larvae from selected townships showed 100% knockdown was observed within 60 min exposure in Field dose 1g/Lit. same results of laboratory and field dose were observed from Moun Ywar Township in Magway Region and Hintada Townships in Ayeyarwady Region in Myanmar, Thailand and India [32]. Although Paeporn and his associated revealed that insecticide susceptibility of *Aedes aegypti* in Tsunami-affected Areas in Thailand and found that permethrin was used in many places for more than five years for routine control of adults during outbreak of this disease. This was followed by deltamethrin, cyfluthrin and fenitrothion use in some selected areas. The results from all studied areas indicated 100% susceptibility to fenitrothion. Permethrin resistance could have resulted from cross resistance with DDT. Moreover, the use of household insecticides, especially aerosol where the active ingredient is mainly permethrin, might have contributed to the resistance of the species. Deltamethrin, the insecticide that followed permethrin, induced resistance in some studied areas [33,34]. Another study using Temephos in Thailand also revealed that *Ae. aegypti* mosquitoes of one collection site each in Bangkok, Nakhon Sawan (northcentral), and Nakhon Ratchasrima (northeast) were resistant to temephos, with mortality ranging from 50.5 to 71.4%. Moreover, there was a trend of resistance to temephos among *Ae. aegypti* populations of all studied districts of Nakorn Ratchasima and most areas of Nakhon Sawan, of which those in one area were susceptible [35]. Meanwhile, the notable high mean for egg-laying was observed in the F_2 generation (76.77 ± 23.14 eggs, range: 40-105) and the F_3 generation (74.95 ± 23.14 eggs, range: 60-101). The F_2 generation also exhibited the highest egg hatching ability (73.29 ± 6.3%). Jitpakdi and associated revealed that the hatching factor using hay-fermented water was necessary and/or at least the most important factor in the stimulation of egg hatching. The heating rate of *Ae. lineatopennis* in natural water and various concentrations of hay-fermented water provided no statistically significant differences – i.e. 0.25% (65%); 0.5% (61%); 1% (64%); 2% (66%) and 4% (59%) ($\chi 2= 1.46$, $p > 0.05$) [36].

Many species of *Aedes* mosquitoes, as *Ae. aegypti, Ae. albopictus* and *Ae. togoi* – are easily reared in the laboratory and also successfully mated in a 30 x 30 x 30 cm sized cage or smaller [37]. Observation of the free mating ability of *Ae. lineatopennis* in a 30 x 30 x 30 cm cage revealed that *Ae. lineatopennis* was strongly eurygamous. After emergence from the pupa stage, the male seeks to mate at the first opportunity, which is usually the first evening at dusk in night-mated mosquitoes, and during the day time in day-mated mosquitoes. In general, the males form dancing swarms at the time of mating and the females may enter these swarms in small numbers. Each female is promptly grabbed by a male, which locates her through the antennae, and the couple can be seen to fall in tandem out of the swarms. In some *Anopheles* species, the swarms of mosquitoes are 15-20 feet above the ground, which is a long distance for the couple to fall [38,39], whereas many species of *Aedes* and *Mansonia* mate without the males forming swarms [40]. When mosquitoes breed in the laboratory, mating of the swarm forming species is markedly variable according to the space limitation. Thus, some species of mosquitoes that mate without males forming swarms free-mate easily in small cages such as one of 30 x 30 x 30 cm, whereas others do not [36]. Many *Aedes* species–as *Ae. triseriatus, Ae. hendersoni and Ae. harinasutai* – that fail to mate in a small cage can be successfully colonized by artificial mating [41,42]. Although, in the present study *Ae. aegypti* can mate easily in 30x30x30 cm mosquito rearing cage in the laboratory.

5. Conclusion

In all generations, the emergence of females was higher than that of males. Field dose of temephos remains effective against *Aedes aegypti* larvae within 1 and 24 hours, although some tolerance to laboratory doses was observed within one hour. Based on these results, it can be concluded that the test larvae from all areas remain susceptible to Temephos. The high egg-laying capacity of F_2 and F_3 generations, coupled with the higher emergence of females, poses a potential risk for dengue transmission. Therefore, Temephos can still be used as a larvicide, particularly for controlling *Aedes aegypti* larvae. Other diseases such as malaria, filariasis, and Japanese encephalitic vector mosquitoes, should be colonized for further studies. Insecticide susceptibility status of *Aedes* and other vector mosquitoes should be tested annually. Larval habitats of dengue vectors and other vectors should be searched in indoor and outdoor water storage containers of the houses and all water sources of the environment. Transmission of dengue cases should be measured in the studied areas. Temephos efficacy on mosquito larvae should be measured in different dengue endemic areas.

Declarations

Source of Funding

This study received no external funding.

Competing Interests Statement

All the contributing authors declare no conflicts of interest.

Consent for publication

The authors declare that they consented to the publication of this study.

Authors' contributions

All the authors took part in literature review, analysis, and manuscript writing equally.

Informed Consent

Not applicable.

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