

EVALUATION OF TEMEPHOS AS A POTENTIAL LARVICIDE AGAINST IMMATURE STAGES OF *AEDES AEGYPTI* (LINNAEUS) (DIPTERA: CULICIDAE)

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ABSTRACT

Aedes aegypti, a significant public health pest requires consistent insecticidal applications for effective management. Current laboratory study assessed the residual efficacy of two formulations of temephos—i.e., temephos 1G (granular) and temephos 50EC (liquid) against immature stages of *Ae. aegypti* focusing on survival and developmental plasticity. Survivors displayed prolonged developmental periods, with maturation durations of 12 days for temephos 1G, 22 days for temephos 50EC, and 30 days for the combined treatment (temephos 1G + temephos 50EC), compared to less than 6 days in the control group. Additionally, incomplete metamorphosis, including failed pupation and the inability to emerge as adults were observed. The combined treatment led to more pronounced reductions in fecundity, longevity, and egg viability compared to alone formulation. With the following survival durations: combination treatment (54 days), temephos 50EC (44 days), temephos 1G (32 days) and control (22 days), the survivorship curve demonstrated the impact of temephos on the larval population. Treated populations exhibited notable growth and developmental abnormalities. Although *Ae. aegypti* had lower mortality rates, their overall fitness was reduced, indicating a potential long-term decline in population.

Keywords: Temephos, Residual efficacy, Vector control, mosquito management, larval control.

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INTRODUCTION

Novel insecticides that specifically target the immature stages of *Aedes aegypti* (Diptera: Culicidae) in artificial containers located near human dwellings represent a common vector control strategy (Snetselaar *et al.*, 2014). Target-specific insecticides are safe for household animals and fish (Sarwar *et al.*, 2016). In this respect, temephos is an organophosphate pesticide containing a phosphoric-ester group and is widely applied due to its rapid action (Morales *et al.*, 2019; Konan *et al.*, 2021). Temephos remains effective for 30 days against mosquito larvae when applied in abundant aquatic vegetation (Ritchie *et al.*, 2021). WHO recommends optimized temephos dose of 2 g/m² for water sources deeper than 16cm (WHO, 2009). It takes 9-10 days *in vivo* and 12-14 days *in vitro* for complete mortality (Grigoraki *et al.*, 2015). Temephos remained reactive from 15-17 days under field conditions (Gleave *et al.*, 2022) and up to 40 days under laboratory conditions (Wiwatanaratanabutr and Kittayapong, 2006; Arslan *et al.*, 2016).

The potency with respective dissipation of toxic level affects the larval growth and developmental time and poses negative impact on the molting process. This results in substantial increase in larval instars, production of super larvae, abnormal pupation and malformed adults (Alvarez *et al.*, 2018; Hafeez *et al.*, 2022). The substantial increase in molting reduced the agility that resultantly increased the larval weight which further advanced to abnormal pupal formation and adult emergence (Pradani, 2020). In addition to this, the time required to complete larval development gradually increases in succeeding generations (Schechtman and Souza, 2015; Thornton *et al.*, 2020).

Investigation of life parameters in field collected *Ae. aegypti* populations, demonstrated that temephos treated containers resulted in delayed larval growth, exerting negative impact on adult fecundity and fertility (Montella *et al.*, 2007; Diniz *et al.*, 2015). When each gender was compared for survivorship following adult emergence, females survived slightly longer than males (Lima *et al.*, 2003; Belinato *et al.*, 2012). Emerged adults had smaller wings and stunted legs, with reduced behavioral activities (Muturi *et al.*, 2010). Developing

females took smaller blood meals, lost the ability to retain sperm up to their full potential because of chemical intoxication, resulted in smaller batches of eggs laid. The eggs hatching rate dropped and a lesser proportion of females were obtained than males (ratio 1:5) (Rahim *et al.*, 2017; David *et al.*, 2018). Pupation and adult emergence cycles of next-generation populations were disturbed (Sanil and Shetty, 2012; Abbas *et al.*, 2024). Morphology of gonads in the temephos-exposed male population was significantly affected, with rudimentary and atrophied testes, and the size of the vas deferens was adversely affected (Kumar *et al.*, 2009; Martins *et al.*, 2012).

The current study was conducted by using temephos 1G (Granular) and 50EC against *Ae. aegypti* immatures in the laboratory following WHO protocols 2009. It is hypothesized that immature stages of *Ae. aegypti* develop efficiently in the temephos treated containers likely due to the development of resistance, which is associated with enhanced metabolic detoxification mechanisms. The main objective was to study the temephos impact on growth and development of *Ae. aegypti*. The proposed results would provide meaningful findings to curtail *Aedes* mosquito, contributing to reduced engorgement and fecundity.

MATERIALS AND METHODS

Insect Collection and Rearing in Laboratory: *Aedes aegypti* larval populations were collected from motor pool, botanical garden, and hostel warden office of University of Agriculture Faisalabad (31.428532° N; 73.069196° E, 31.429178° N; 73.071088° E and 31.432224° N; 73.065893° E, respectively) with the help of Clark and technologies from the breeding sites. The collected *Aedes* larval populations were shifted to Dengue Vector Research Laboratory (DVRL), University of Agriculture Faisalabad and maintained under standard control conditions (27-30 °C, 70-80% RH) and 14:10 (L:D) hours photoperiod. The larvae were sorted in glass beakers (6 height×2.5-inch width) (Pyrex glass, UK) according to their instars. Larval populations were fed with crushed fish diet (TetraMin, Germany). Larval molting was properly observed. Pupae were shifted into rearing cages (1.5×1.5×1.5 ft) and the newly emerged adults were fed on 10% sugar syrup. An albino rat was used for adult blood feeding (Harrison *et al.*, 2021). Moist cotton and tissue papers were placed for egg laying (Prasadini *et al.*, 2019). These eggs were used for residual bioassay.

Insecticides and Bioassay: Temephos 1G (Temephos[®], CAS#3383-96-8, American Cyanamid Co[®]) (2 g/m²) and temephos 50EC (Abate[®], CAS#3383-96-8, Katyayani Organics[®]) (8ml/L water) were used as larvicide agent of *Ae. aegypti* separately and in combination. All larvicidal

trials were performed in iron trays having water holding capacity of 150 liters and an area of 1m² (20 cm depth) (Kamgang *et al.*, 2011). Iron trays were filled with 100-liter (10cm depth) tap water. A total of 100 *Aedes* eggs were released in each tray. To check the residual efficacy, a standard dose of both formulations was added in each water tray. To check the combined residual effect of both formulations, a recommended dose of both insecticides (combination) was applied. Each treatment was replicated four times and one control group was executed separately. Larvae were observed after regular intervals of 24 hours to collect insecticidal mortality data. Larvae that did not move, float or settled down were considered dead. All alive larvae were kept separately in distilled water for life stage and development studies. This experiment continued when larval mortality reached 50% or larvae progressed to pupal stage (in any treatment).

Growth and Development: Residual efficacy of insecticides against *Aedes* population was recorded after one-time application. The effect of insecticide residues was studied by recording growth and development of different stages, such as larvae to pupae, pupae to adults and then fecundity and egg viability. The abnormality in pupation was registered based on differences in shape, size and weight compared with control treatment samples. The abnormal categories were identified when adults had stunted growth or physical disability (reduced legs, wings, depressed abdomen and half-molted adults). Furthermore, the adults were differentiated based on antenna (male: plumose, female: pilose), proboscis (male: less developed, female: well developed) and genitalia (male: Clasper structure, female: Ovipositor structure). The emerged adults were paired and each pair was released in a separate cage to calculate the fecundity with respect to treatments. The longevity of each adult was estimated up to the death of each individual.

Furthermore, growth indices were computed for larvae, pupae and fitness for *Ae. aegypti* with following formulas (Bourguet *et al.* 2004).

$$\text{Larval growth index} = \frac{\% \text{ Pupation}}{\text{Larval duration} \times \% \text{ Emergence}}$$

$$\text{Pupal growth index} = \frac{\% \text{ Pupation} \times \text{Pupal weight}}{\text{Pupal duration}}$$

$$\text{Fitness index} = \frac{\% \text{ Pupation} \times \text{Pupal weight}}{\text{Larval duration} + \text{Pupal period}}$$

Percentage larval survival was calculated with the help of Abbott (1925). The data was analyzed through ANOVA techniques. Tukey HSD All-Pairwise comparisons test of survival for time and treatment was applied to the collected data. Larval growth and development data means were compared using R software (v4.4.1). The well-known package ggplot2 (Grammar of Graphics) utilizes structural attributes—including data frames, aesthetics, and geometries—to generate parallel plots, bar charts, and smooth line plots.

RESULTS

The survival rate of temephos 1G-treated larvae varied significantly across observation days ($F_{5,18}=93.3$, $P \leq 0.001$) (Figure 1). The response was evident from the 2nd day and survival decreased with respect to succeeding days. The results also indicated that 50% survival was recorded by the 12th day. In contrast, larvae treated with temephos 50EC and temephos 1G+temephos 50EC required observations up to 22nd day ($F_{10,33}=33.7$, $P \leq 0.001$) and 30th day ($F_{3,12}=33.7$, $P \leq 0.001$), respectively. Control-treated-larvae exhibited maximum survival, with pupation recorded by the 6th day ($F_2, 9=1.29$, $P=0.32$). The survivorship curve exhibited the usefulness of temephos in tested larval population wherein maximum life cycle prolongation was recorded in combination, comparing temephos 50EC, temephos 1G and control (54, 44, 32 and 22, respectively) (Figure 1).

Days (Larval duration) to pupation (DTP), pupal duration (PD), male adult duration (MAD) and female adult duration (FAD) (Figure 2). Temephos 1G + temephos 50EC showed statistically significant and

maximum days to pupation ($F_{3,12}=411$, $P \leq 0.001$), pupal duration ($F_{3,12}=153$, $P \leq 0.001$) and adult duration: male adult duration (MAD) ($F_{3,12}=5.02$, $P = 0.017$) and female adult duration (FAD) ($F_{3,12}=3.35$, $P \leq 0.055$) followed by temephos 50EC and temephos 1G.

The control group *Ae. aegypti* population exhibited maximum (100%) healthy pupation ($F_{3,12}=33.7$, $P \leq 0.001$) and adult emergence (96.42%) ($F_{3,12}=13$, $P \leq 0.001$) comparing with temephos 1G (79.58% and 80.417%), temephos 50EC (66.25% and 58.33%) and minimum in temephos 50EC + temephos 1G (43.33% and 25%). While the pupal weight ($F_{3,12}=69.1$, $P \leq 0.001$) was recorded maximum for temephos 50EC + temephos 1G (2.46 mg) followed by temephos 50EC (2.33 mg) and temephos 1G (2.18 mg). The minimum pupal weight was evidenced in control-group (Table 1).

The fecundity ($F_{3,12}=111$, $P \leq 0.001$) and egg viability ($F_{3,12}=1281$, $P \leq 0.001$) exhibited highest in controlled population (109.50 and 97.25, respectively) followed by temephos 1G (41 and 14.52, respectively), temephos 50EC (30.750 and 13.71, respectively) and temephos 1G+temephos 50EC (3.25 and 0, respectively) (Figure 3).

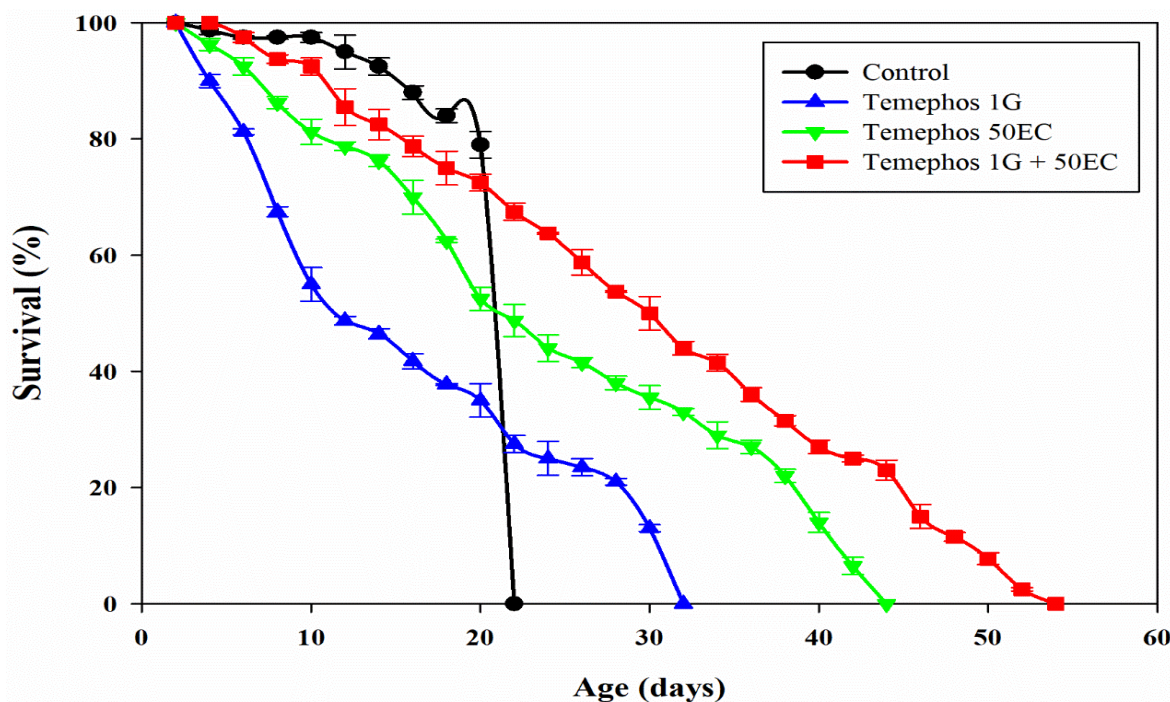


Fig 1. Survival potential of *Ae. aegypti* larvae exposed to temephos 1G ($F=103$, $P \leq 0.001$, Tukey HSD Value=13.56), temephos 50EC ($F=307$, $P \leq 0.001$, Tukey HSD Value=7.72), temephos 1G+50EC ($F=389$, $P \leq 0.001$, Tukey HSD Value=8.67) and control ($F=25.1$, $P \leq 0.001$, Tukey HSD Value=7.05) under laboratory conditions. The x-axis represents the larval age (days), while the y-axis illustrates the survival percentage of larvae after treatment.

The larval, pupal and fitness indices are explained in Table 2. Maximum values of indices were recorded in control-treated population followed by

residual treatment with temephos 1G and temephos 50EC, while the minimum in temephos 1G+temephos 50EC.

Table 1. Effect of temephos 1G, temephos 50EC and temephos 1G+50EC (\pm standard error) on *Ae. aegypti* pupation and adult emergence.

Treatment	Pupal Weight	Pupation		Adult Emergence	
		Healthy	Deformed	Healthy	Deformed
Temephos 1G	2.18 \pm 0.07 ^b	79.58 \pm 0.46 ^b	20.41 \pm 0.46 ^b	80.41 \pm 0.94 ^{ab}	19.58 \pm 0.94 ^{bc}
Temephos 50 EC	2.33 \pm 0.08 ^{ab}	66.25 \pm 0.92 ^b	33.7 \pm 0.92 ^b	58.33 \pm 0.77 ^{bc}	41.66 \pm 0.77 ^{ab}
Temephos 1G+ Temephos 50 EC	2.46 \pm 0.08 ^a	43.33 \pm 0.71 ^c	56.66 \pm 0.71 ^a	25 \pm 1.34 ^c	75 \pm 1.34 ^a
Control	1.54 \pm 0.07 ^c	100 \pm 0 ^a	0 \pm 0	96.42 \pm 0.66 ^a	3.57 \pm 0.66 ^c
Tukey HSD Value	0.21	17.18	17.18	36.03	36.03

*Table showing same letters along the columns are statistically similar with each other

Table 2. Growth and fitness indices for temephos 1G, temephos 50EC and temephos 1G+50EC treated (\pm standard error) *Ae. aegypti* larval population

Parameters	Control	Temephos 1G	Temephos 50EC	Temephos 1G + Temephos 50EC	Tukey HSD Value
Larval growth index	14.66 a	4.47 b	2.20 b	1.63 b	6.33
Pupal growth index	41.54 a	19.08 b	10.96 c	3.82 c	8.52
Fitness index	16.94 a	7.74 b	4.35 c	3.38 c	3.78

*Table showing same letters along the rows are statistically similar with each other

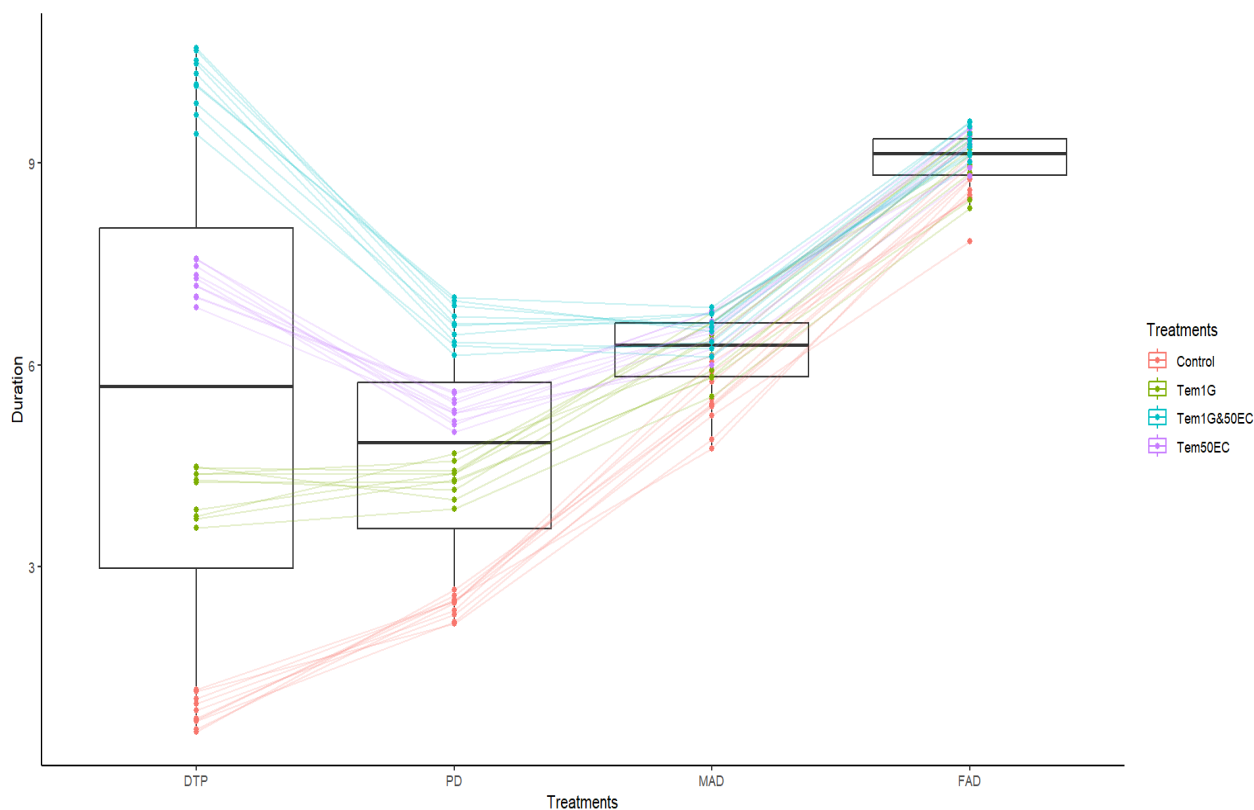


Fig 2. Parallel plot depicting DTP (days to pupation: Tukey HSD = 0.82), PD (pupal duration: Tukey HSD = 0.61), MAD (male adult duration: Tukey HSD = 0.89) and FAD (female adult duration: Tukey HSD = 0.92) across control, Tem1G (temephos 1G), Tem50EC (temephos 50EC) and Tem1Gn50EC (temephos 1G and 50EC). The box (lower and upper) margins explain data distribution while box having thick central lines define medium values. The error-bars showed combination of lower and higher whiskers.

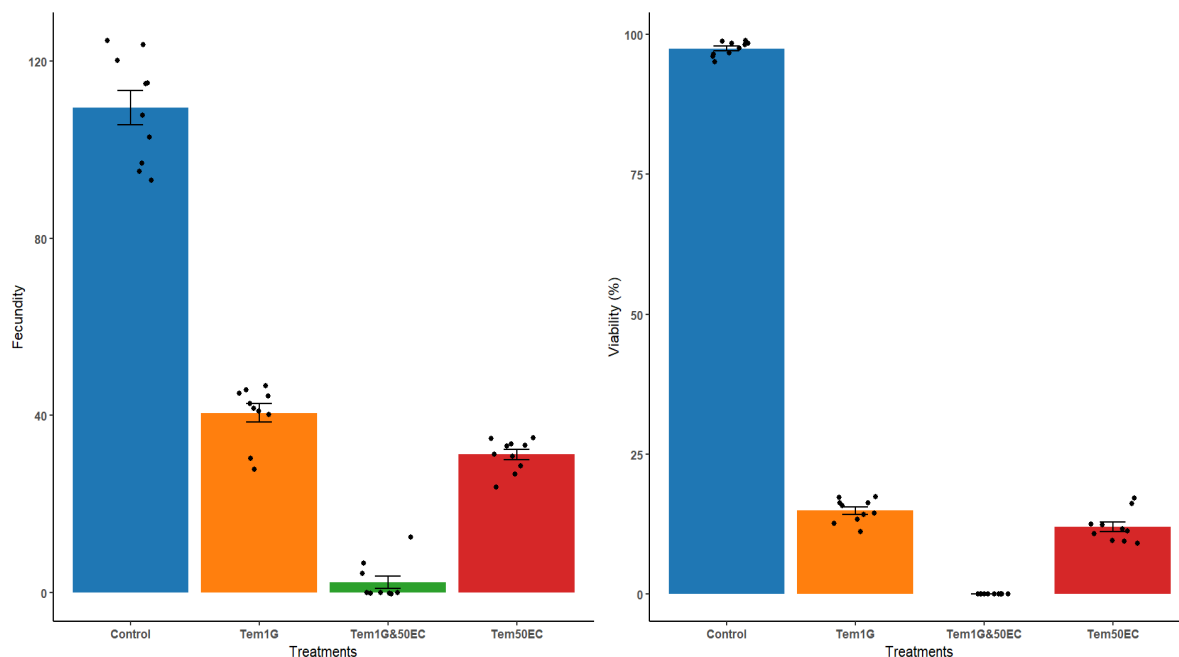


Fig 3. Bar plot depicting fecundity and egg viability across control, Tem1G (temephos 1G), Tem50EC (temephos 50EC) and Tem1Gn50EC (temephos 1G and 50EC). The dotted-points explain data distribution.

DISCUSSION

Insecticide application on *Ae. aegypti* is important for disease transmission like Malaria, Dengue along with continuous study on insecticide resistance monitoring. Insecticides help in targeted mosquito management determine the most effective chemicals, doses, and delivery methods (Chanda *et al.*, 2017). Insecticide studies also drive the development of next generation chemical compounds and integrated vector management (IVM) (Dusfour *et al.*, 2019).

The pre-exposed *Ae. aegypti* larvae to temephos under the current study revealed low to moderate range of resistance. Many morphological anomalies at both the immature and adult stages were revealed in the current study, which also explained the effects of temephos residues on the test population and the surviving larvae when submitted to biological investigation (growth and development). The populations gathered from Argentina (Linás *et al.*, 2010), Bolivia (Biber *et al.*, 2006), and Columbia (Grisales *et al.*, 2013) demonstrated that, *Ae. aegypti* was more likely to be adopted in containers treated with temephos.

The findings of the present study are directly interrelated with Kosoltanapiwat *et al.*, (2020), while working in Thailand, reported 2 to 2.5-fold resistance ratios from Ratchaburi while 2.74-fold from Tambon Thanat, Damnoen Saduak, after 05 generations of *Ae. aegypti*. Our study also highlighted that, *Ae. aegypti* showed great plasticity against temephos-commercial-products. The residual efficacy (up to 50% mortality) has

been reported after 12 and 22 days for temephos 1G and temephos 50EC respectively, and this duration of containment sharply increased, i.e., 30 days when treated in-combination. This treatment period posed adverse effect on immature growth, development and reproductive behavior of test insects such as additional instar formation/super-larvae establishment (Poupardin *et al.*, 2014). Furthermore, repeated applications of temephos against *Ae. aegypti* reduced fitness (Schechtman and Souza, 2015; Vontas *et al.*, 2012) and more than 10-fold resistance ratio observed in Brazil-collected population (Montella *et al.*, 2007).

The influence of temephos concentrations 0.01, 0.06, 0.1 and 0.14 ppm towards the length of larva size (mm) was recorded as 3.3, 3.2, 3.3 and 3.5 respectively. A significant delay in larval development together with a reduction of both adult longevity and fecundity had occurred when resistance to temephos was higher than 40 (Braga *et al.* 2004). However, field populations with lower temephos resistance exhibited decreased blood meal size, lesser number of eggs and inseminated females, when compared to Rockefeller mosquitoes (Diniz *et al.*, 2017). The disrupted endocrinological balance as well as damaged muscles by temephos could be the result of morphological abnormality in mosquitoes (Fallatah and Khater, 2010).

The larval life (days) completed in 15.17 when population treated with temephos 1G followed by temephos 50EC and combination: temephos 1G+temephos 50EC (29.17 and 35, respectively), comparing with control (6.82), while the same trend

reflected in pupae (temephos 1G, temephos 50EC, temephos 1G+temephos 50EC and control (2.32) and adults (4.21, 5.32, 6.53, respectively) stages. Larval developmental time in Brazil for Gelugor-strain was longest (median = 10 days) than USM-strain (median = 7 days). Significant decrease in males ($\chi^2= 33.3$) and females ($\chi^2= 44.9$) of the Gelugor-strain than USM-strains (Rahim *et al.*, 2017).

Resistance has been observed in different *Ae. aegypti* populations (20, 40, 60 and 80% resistance) but these exposed populations exhibited variation in growth and development at immature phase (Diniz *et al.*, 2014). All four *Aedes* strains with high resistance ($RR > 10.0$) were subjected to temephos and found effective 09–13 days, while moderately resistant and susceptible populations displayed effectiveness up to 14–18 days. Temephos also produced a dose-response effect over two Brazilian populations BVT and APG (Belinato *et al.*, 2012). The APG population depicted higher level of temephos resistance ($RR_{50}= 15.7$ and heterogeneity than BVT ($RR_{50}=4.1$)).

According to our observation, larvae that remained submerged in treated water for longer period became sluggish and motionless, further induced disturbance in whipping larval behavior. These characteristic disturbances were recorded most in combination followed by temephos 50EC and temephos 1G-treated populations. Bioassays indicated that temephos induced inactivity and swimming ability that disrupt the normal process of morphogenesis (Gimenez *et al.*, 2020).

The treated *Ae. aegypti* larval population had lesser number of adult formation due to residual effects of temephos. Many surviving pupae hatched into deformed adults. Maximum number of healthy adults was recorded in-case of temephos 1G followed by temephos 50EC and temephos 1G+temephos 50EC. The total life span of female adults from immature to adult stage in temephos 1G, temephos 50EC and combination was recorded 31.25, 46.07 and 43.49 days respectively. The total life span of male adults from immature to adult stage in temephos 1G, temephos 50EC and combination was recorded 28.51, 43.22 and 40.55 days respectively. Overall, female adults had a longer life span than the male adults. Previous research by Thornton *et al.* (2020) demonstrated that temephos-treated population of *Ae. aegypti* had greater longevity, with a mean male and female survival of 29 days. After application of temephos, time needed to complete larval development was progressively increased over generations. At the end of the selection process, 11% larvae transformed into pupae. The observed sex ratio was in accordance with the 1:1. In all cases, females lived longer than male adults (Martins *et al.*, 2012).

In general, female mass decreased with increase in temephos dose (Muturi and Alto, 2011). Morphology

of gonads of male adults in the temephos-exposed population was severely affected and was represented by atrophied and rudimentary testes, and the size of the vas deferens was very much reduced (Sanil and Shetty, 2012).

The current study concludes that temephos treated *Aedes* populations have a struggled sexual performance. Sometimes, gonads of male adults were not able of producing active sperm and abundant sperm count while female had lost the ability to retain eggs in their ootheca (Rahim *et al.*, 2017). The larval population that was treated with combination had the lowest fecundity rates followed by temephos 50EC and temephos 1G. The eggs produced by the population treated with combination had 1.31-fold lesser viable eggs than the control strain. The emerged larvae were seen to be deformed and were dead within 6 hours. This supports 104 and 81 eggs recorded from temephos exposed BVT-populations and APG-populations, reflecting 21% decrease in fecundity. The obtained males-frequency of insemination to females drastically affected (46% males of BVT-population and 68% males of APG-population did not inseminate) (Belinato *et al.*, 2012).

Conclusion: This study accentuates importance of temephos in controlling *Ae. aegypti* populations by maintaining residual insecticidal efficacy, leading to prolonged growth periods and incomplete metamorphosis in survivors under laboratory conditions. The combined treatment of temephos 1G and temephos 50EC had the highest impact on developmental abnormalities, reducing fecundity and longevity significantly compared to individual formulations. This underscores the need for integrated vector management strategies that consider resistance and developmental effects when using insecticides to sustain mosquito control.

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Declarations

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Consent for publication: Not applicable.

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Data availability: Supplementary data will be provided on demand.

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