

Original Research

Comparative Efficacy of Temephos and Pyriproxyfen on *Aedes aegypti*, *Aedes albopictus*, and *Culex quinquefasciatus* Collected from Different Ecological Zones of Punjab, Pakistan

Muhammad Irfan Akram¹, Waseem Akram², Adeela Anwar Rana³, Rashid Iqbal^{4,5**}, Allah Ditta^{6,7*}, Zahid Mahmood Sarwar⁸, Rashid Ayub⁹, Mohammad Abul Farah¹⁰

¹Department of Entomology, Faculty of Agriculture and Environment, The Islamia University of Bahawalpur, Punjab, Pakistan

²Department of Entomology, Faculty of Agriculture, University of Agriculture Faisalabad, Punjab, Pakistan

³Nawab Sir Sadiq Muhammad Khan Abbasi Hospital, Bahawalpur 63100, Pakistan

⁴Department of Agronomy, The Islamia University of Bahawalpur-63100, Pakistan

⁵Department of Life Sciences, Western Caspian University, Baku, Azerbaijan

⁶Department of Environmental Sciences, Shaheed Benazir Bhutto University Sheringal, Dir (U), Khyber Pakhtunkhwa 18000 Pakistan

⁷School of Biological Sciences, The University of Western Australia, 35 Stirling Highway, Perth, WA 6009, Australia

⁸Department of Entomology, Bahauddin Zakariya University Multan, Pakistan

⁹College of Science, King Saud University, P.O.Box-2454, Riyadh-11451, Saudi Arabia

¹⁰Department of Zoology, College of Science, King Saud University, Riyadh 11451, Saudi Arabia

Received: 7 February 2024

Accepted: 28 June 2024

Abstract

Management of mosquitoes accentuates mostly the use of both larvicides and adulticides. The dengue control program in Punjab, Pakistan, has long been under the influence of temephos (1G and 50EC) for larviciding. The present study was therefore planned to use larvicides that were currently being used in a dengue control program along with insect growth regulators (IGR) against mosquito species (*Culex* and *Aedes*) collected from various ecological regions of Punjab (Pakistan) regarding disease outbreaks to assess their efficiency by performing larval bioassays as suggested by the World Health Organization (WHO) at three different doses, i.e., low dose (D₁), recommended dose (D₂), and high dose (D₃). Results revealed that among the three larvicides used, pyriproxyfen 0.5WDG showed significantly higher control than temephos 1G and temephos 50EC, but in the case of temephos 1G and temephos 50EC, temephos 1G showed slightly better control. A marginal difference was

*e-mail: allah.ditta@sbbu.edu.pk;

**e-mail: rashid.iqbal@iub.edu.pk

observed between the recommended doses (D_2) and the higher doses (D_3), but a substantial difference was observed between the lower doses (D_1) and those of the recommended and higher doses (D_2 and D_3). Pyriproxyfen 0.5WDG at the recommended dose should be applied in mosquito control programs relying heavily on larvicides as it proved to be a promising control agent, followed by temephos 1G and temephos 50EC.

Keywords: *Aedes aegypti*, *Aedes albopictus*, *Culex quinquefasciatus*, temephos, pyriproxyfen, larval bioassay

Introduction

Mosquitoes are being controlled by a large number of chemical insecticides. Since the introduction of organic insecticides in the 1940s, chemical control has been a commonly used method in mosquito control [1] (pp. 197-210). Mosquitocides are diverse and include pesticides from classes like organophosphates (OP), insect growth regulators (IGRs), and pyrethroids [2, 3]. The use of larvicides is the ultimate approach to reducing mosquito breeding; therefore, the majority of mosquito control programs aim at the larval stage in their breeding sites with larvicides because the adulticides may only decrease the adult population for the time being [4, 5].

Organophosphates (OP's) and insect growth regulators (IGRs) are used for larval control.[6] Temephos (an OP) is the most extensively used larvicide worldwide because of its reasonable residual effect, easy handling, low mammalian toxicity, low cost, and safety in application to drinking water [7, 8]. Temephos is among a small number of organophosphates registered and produced commercially for the control of *Aedes* mosquito larvae [9]. Insect growth regulators are relatively safer than non-targeted organisms [10] and have been suggested for mosquito control. For example, pyriproxyfen, diflubenzuron, and methoprene are some important insect growth regulators registered as mosquito larvicides [6]. Pyriproxyfen is safe for non-target organisms like mammals and fish, along with mosquito predators like dragonflies [10]. Pyriproxyfen is a juvenile hormone pesticide to which various larval instars of a variety of mosquito species differ in sensitivity [11]. According to the World Health Organization (WHO), pyriproxyfen is judged a 'reduced-risk pesticide' or 'unlikely to present acute hazard' which means it is almost harmless to animals or birds and is neither genotoxic nor carcinogenic and can be safely added at a concentration of 0.01 ppm to drinking water for mosquito control [7, 12].

Thus, the present study is of substantial status as it focused on the knowledge of common mosquito species from different ecological zones of Punjab (Pakistan) formed because of dengue prevalence along with evaluation of the efficacy of larvicides and IGR currently being used in dengue control programs.

Experimental

Mosquito Collections and Rearing

Collection of mosquitoes was done from the areas that have been exposed to heavy mosquitocides during the past few years to minimize dengue outbreaks, i.e., Lahore (31°32'58.992"N, 74°20'36.996"E), Rawalpindi (33°36'2.52"N, 73°04'4.44"E), and Faisalabad (31°25'33.60"N, 73°05'56.40"E). The populations of *Culex quinquefasciatus*, *Aedes aegypti*, and *Aedes albopictus* collected from respective localities were maintained under laboratory conditions for bioassay. From all the three districts, the *Culex quinquefasciatus* population was found and collected, though, regarding *Aedes* species, *Aedes aegypti* was found and collected from districts of Faisalabad and Lahore, while *Aedes albopictus* was found and collected from district Rawalpindi. The population was reared at 27±2°C with a relative humidity of 65±5% at L/D 12:12 h photoperiod at Dengue Vector Research Laboratory, Department of Entomology, University of Agriculture Faisalabad, Pakistan. Adults were kept in culture cages provided with 10% sugar solution. Larvae were fed on fishmeal, while pupae were collected daily and transferred to the adult cages. The female mosquitoes were fed on the blood of live white rats twice a week [13].

Bioassay Procedure

Larval bioassay was done against the larval population of mosquitoes collected from respective localities to check the efficacy of different insecticides under laboratory conditions. WHO-recommended larvicides for mosquito control were used, i.e., Temephos (1G and 50EC) from organophosphates and pyriproxyfen from IGRs. Insecticidal activities were tested using standard WHO protocols. Recommended dose, high dose, and low dose were used. For Temephos (1G and 50EC), low dose (D_1), recommended dose (D_2), and high dose (D_3) were used at 0.5 ppm, 1 ppm, and 2 ppm, respectively. In the case of pyriproxyfen 0.5 WDG, low dose (D_1), recommended dose (D_2), and high dose (D_3) were used at 0.005 ppm, 0.01 ppm, and 0.02 ppm, respectively. Each dose was tested in three replicates and a control. For bioassay, twenty-five larvae were placed in the glass beaker in each replication, and the total number of larvae tested per concentration was 100.

Data Collection

Mortality due to temephos 1G and temephos 50EC was observed after 24 hours, and the treatments were exposed for 4-5 months at weekly intervals. For pyriproxyfen (IGR), adult emergence inhibition was observed, and treatments were exposed to larvae fortnightly for 4-5 months. The threshold bioefficacy was 80% mortality/emergence inhibition.

Statistical Analysis

Mortality data, where necessary, was corrected by Abbott's formula [14]. Data so collected was analyzed using three factors CRD for drawing inferences.

Results

The results indicated in the early weeks of the study that mortality induced by all the tested larvicides at all doses was quite similar between the species. Over time, there was observed a significant difference among the tested doses in causing mortality.

Response of the chemicals varied, i.e., temephos 1G showed marginally higher residual efficacy than temephos 50EC i.e. for *Culex quinquefasciatus*, it was about 3 weeks at a lower dose (0.5 ppm), 1.25-1.75 months at the recommended dose (1 ppm), and 1.5-2 months at the higher dose (2 ppm); and for *Aedes aegypti/albopictus*, it was about 1-1.5 months at the lower dose (0.5 ppm), 3-3.5 months at the recommended dose (1 ppm), and 3-4 months at the higher dose (2 ppm), as shown in Fig. 1.

Temephos 50EC showed the least residual efficacy, i.e., for *Culex quinquefasciatus*, it was about 3 weeks at a lower dose (0.5 ppm), 1-1.25 months at the recommended dose (1 ppm), and 1-1.5 months at a higher dose (2 ppm); and for *Aedes aegypti/albopictus*, it was about 1-2 months at a lower dose (0.5 ppm),

2.5-3.5 months at the recommended dose (1 ppm), and 3-3.5 months at the higher dose (2 ppm) (Fig. 2).

Pyriproxyfen induces inhibition emergence in adults for longer periods. So, when it is compared with that of temephos 1G and temephos 50EC in terms of controlling the larva, it showed a significant difference and greater residual efficacy, i.e. for *Culex quinquefasciatus*, it was about 1.5-2 months at the lower dose (0.005 ppm), 2-2.5 months at the recommended dose (0.01 ppm), and about 2.5 months at the higher dose (0.02 ppm); and for *Aedes aegypti/albopictus*, it was about 2.5-3 months at the lower dose (0.005 ppm), 3.5-4 months at the recommended dose (0.01 ppm), and 3.5-4.5 months at the higher dose (0.02 ppm), as shown in Fig. 3.

The marginal difference between temephos 1G and temephos 50EC in causing the mortalities of *Culex quinquefasciatus* and *Aedes aegypti/albopictus* in terms of both residual efficacy (i.e., 80% mortality) and percent mortality, but there was observed a significant difference between pyriproxyfen 0.5WDG with that of temephos 1G and temephos 50EC in controlling *Culex quinquefasciatus* and *Aedes aegypti/albopictus* (Table 1).

However, marginal variation was observed between the recommended doses (D_2) and the higher doses (D_3), but a significant difference was observed between the lower doses (D_1) of the recommended and higher doses (D_2 and D_3). Table 2 shows Species \times District interaction, while Table 3 shows Dose \times District interaction. Overall Species \times Dose \times District interaction has been indicated in Table 4. According to Fig. 1, 2, and 3, the number of days at which 80% mortality/emergence inhibition (LT 80) remained at different doses of chemicals in respective districts. *Aedes aegypti/albopictus* showed more susceptibility in terms of percent mortality as compared to *Culex quinquefasciatus*. The population of *Culex quinquefasciatus* and *Aedes aegypti* from Lahore was found less responsive to the chemicals tested as compared to the population from Faisalabad and Rawalpindi.

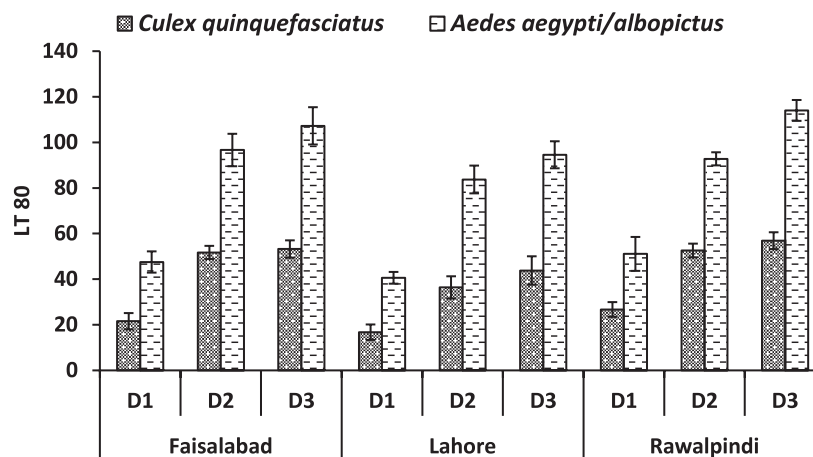


Fig. 1. LT 80 of *Culex quinquefasciatus* and *Aedes aegypti/albopictus* population of Faisalabad, Lahore, and Rawalpindi at different doses ($D_1 = 0.5$ ppm; $D_2 = 1$ ppm; $D_3 = 2$ ppm) of Temephos 1G.

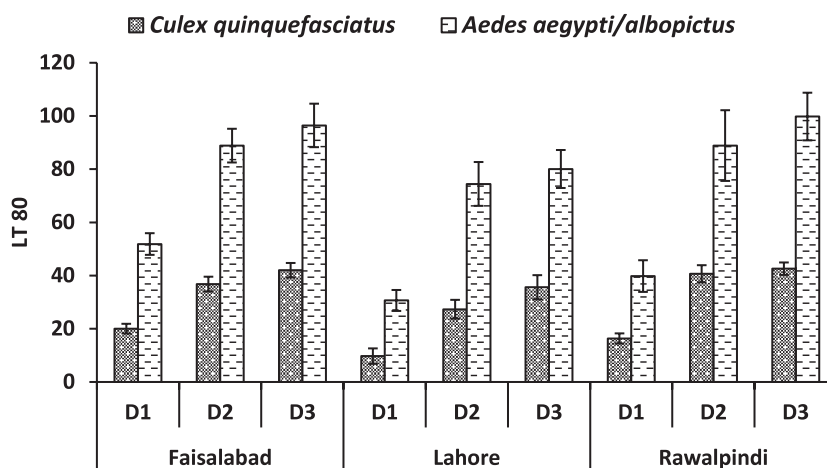


Fig. 2. LT 80 of *Culex quinquefasciatus* and *Aedes aegypti/albopictus* population of Faisalabad, Lahore, and Rawalpindi at different doses ($D_1 = 0.5$ ppm; $D_2 = 1$ ppm; $D_3 = 2$ ppm) of Temephos 50EC.

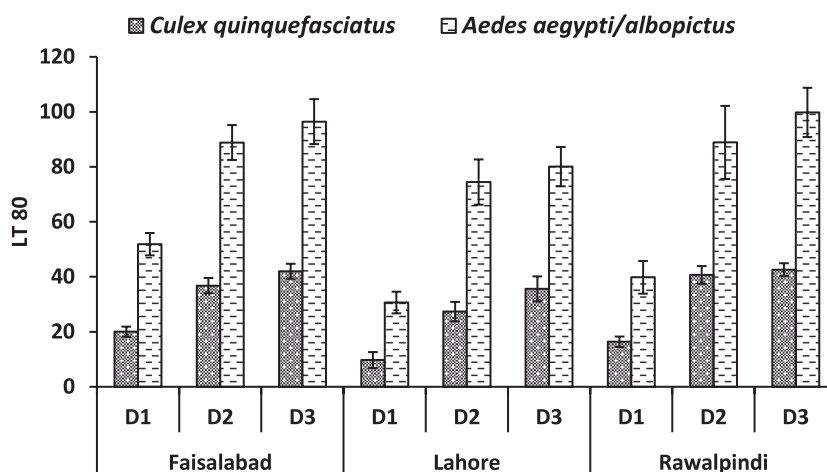


Fig. 3. LT 80 of *Culex quinquefasciatus* and *Aedes aegypti/albopictus* population of Faisalabad, Lahore, and Rawalpindi at different doses ($D_1 = 0.005$ ppm; $D_2 = 0.01$ ppm; $D_3 = 0.02$ ppm) of Pyriproxyfen 0.5WDG.

Table 1. Analysis of variance (mean squares) table for mortality/emergence inhibition at different larvicide/IGR.

Source of variation	Degrees of freedom	Mean squares		
		Temephos 1G	Temephos 50EC	Pyriproxyfen 0.5WDG
Species (Sp.)	1	22639.0 **	24020.2 **	20666.7 **
District	2	852.6 **	926.0 **	130.8 ^{NS}
Dose	2	9793.5 **	7426.7 **	1531.9 *
Species × District	2	1.6 ^{NS}	85.2 ^{NS}	43.0 ^{NS}
Species × Dose	2	996.3 **	960.6 **	233.7 ^{NS}
District × Dose	4	39.9 ^{NS}	48.8 ^{NS}	237.2 ^{NS}
Sp. × District × Dose	4	17.7 ^{NS}	20.5 ^{NS}	40.8 ^{NS}
Error	36	74.3	105	439.2
Total	53			

NS = Non-significant ($p > 0.05$); * = Significant ($p \leq 0.05$); and ** = Highly significant ($p \leq 0.01$)

Discussion

Temephos (granular formulation) at the rate of 1 ppm (1 mg/L) provided 3 months of effective control against *Aedes aegypti* as reported by various scientists [15-18], and against *Aedes aegypti/albopictus* and *Culex quinquefasciatus* as reported by Garza-Robledo et al. [19], which is similar to our results of *Aedes aegypti/albopictus* and close to that of *Culex quinquefasciatus*.

The residual efficacy of temephos 1G (1 mg/L) lasted for 15 weeks (105 days) under laboratory conditions against *Aedes aegypti*, as reported by Chen and Lee [20], which is very close to our results. Temephos (sand granules) at the concentration of 1 mg/L was found highly effective for breeding places of *Aedes aegypti* and *Aedes albopictus* for 3-3.5 months reported earlier by Brown et al. [21], which is very close to our results about *Aedes aegypti* and *Aedes albopictus*. Temephos granular

Table 2. Mean mortality/emergence inhibition±SE for Temephos 1G, Temephos 50EC, and Pyriproxyfen 0.5WDG (Species × District interaction) ^a.

Species	District			Total
	Faisalabad	Lahore	Rawalpindi	
Temephos 1G				
<i>Cx. Quinquefasciatus</i>	42.17±5.43	32.32±4.74	45.40±4.99	39.96±3.01B
<i>Aedes aegypti/albopictus</i>	83.81±9.81	72.97±8.63	85.96±9.62	80.92±5.31A
Total	62.99±7.42A	52.65±6.86B	65.68±7.20A	
Temephos 50EC				
<i>Cx. quinquefasciatus</i>	32.95±3.54	24.22±4.25	33.21±4.41	30.13±2.41B
<i>Aedes aegypti/albopictus</i>	79.05±7.60	61.72±8.50	76.16±10.5	72.31±5.17A
Total	56.00±6.91A	42.97±6.47B	54.68±7.58A	
Pyriproxyfen 0.5WDG				
<i>Cx. quinquefasciatus</i>	89.77±4.77	87.30±7.45	89.82±3.66	88.97±3.08B
<i>Aedes aegypti/albopictus</i>	132.41±8.18	124.13±9.25	127.73±6.31	128.09±4.49A
Total	111.09±6.92A	105.72±7.29A	108.78±5.80A	

^aMeans sharing similar letters in a row or a column are statistically non-significant ($p>0.05$). Small letters represent the comparison among interaction means and capital letters are used for the overall mean.

Table 3. Mean mortality/emergence inhibition±SE for Temephos 1G, Temephos 50EC, and Pyriproxyfen 0.5WDG (Dose × District interaction).

Dose	District			Total
	Faisalabad	Lahore	Rawalpindi	
Temephos 1G				
D ₁	34.56±06.37	28.68±05.66	38.91±06.54	34.05±3.52C
D ₂	74.19±10.62	60.06±11.15	72.69±9.18	68.98±5.82B
D ₃	80.24±12.74	69.19±11.98	85.45±13.05	78.29±7.03A
Temephos 50 EC				
D ₁	35.95±07.39	20.18±05.16	28.09±05.94	28.07±3.72B
D ₂	62.82±12.04	50.89±11.27	64.77±12.39	59.50±6.63A
D ₃	69.23±12.77	57.83±10.64	71.19±13.44	66.08±6.84A
Pyriproxyfen 0.5WDG				
D ₁	104.36±15.81	100.34±19.3	90.68±6.02	98.46±8.15B
D ₂	113.22±10.70	103.74±9.42	114.71±11.3	110.56±5.82AB
D ₃	115.70±10.46	113.08±8.04	120.94±8.78	116.57±5.03A

Table 4. Mean mortality/emergence inhibition \pm SE for Temephos 1G, Temephos 50EC, and Pyriproxyfen 0.5WDG (Species \times Dose \times District interaction) ^a.

Species	Dose	District			Total
		Faisalabad	Lahore	Rawalpindi	
Temephos 1G					
<i>Cx. quinquefasciatus</i>	D ₁	21.58 \pm 3.56	16.75 \pm 3.39	26.72 \pm 3.23	21.68 \pm 2.23D
	D ₂	51.72 \pm 2.90	36.40 \pm 4.88	52.59 \pm 3.01	46.90 \pm 3.22C
	D ₃	53.23 \pm 3.81	43.82 \pm 6.22	56.88 \pm 3.71	51.31 \pm 3.06C
<i>Aedes aegypti/albopictus</i>	D ₁	47.54 \pm 4.66	40.61 \pm 2.58	51.09 \pm 7.44	46.41 \pm 3.06C
	D ₂	96.65 \pm 7.12	83.73 \pm 6.09	92.79 \pm 2.87	91.06 \pm 3.42B
	D ₃	107.25 \pm 8.18	94.56 \pm 5.93	114.02 \pm 4.58	105.28 \pm 4.29A
Temephos 50 EC					
<i>Cx. quinquefasciatus</i>	D ₁	20.05 \pm 1.83	9.72 \pm 2.90	16.36 \pm 1.91	15.38 \pm 1.89C
	D ₂	36.79 \pm 2.78	27.33 \pm 3.54	40.67 \pm 3.23	34.93 \pm 2.55B
	D ₃	42.01 \pm 2.73	35.60 \pm 4.55	42.59 \pm 2.34	40.07 \pm 2.01B
<i>Aedes aegypti/albopictus</i>	D ₁	51.86 \pm 4.07	30.64 \pm 3.94	39.81 \pm 5.95	40.77 \pm 3.88B
	D ₂	88.85 \pm 6.34	74.45 \pm 8.24	88.87 \pm 13.29	84.06 \pm 5.43A
	D ₃	96.44 \pm 8.17	80.07 \pm 7.14	99.79 \pm 8.95	92.10 \pm 5.08A
Pyriproxyfen 0.5WDG					
<i>Cx. quinquefasciatus</i>	D ₁	87.20 \pm 16.0	82.99 \pm 24.6	78.01 \pm 2.97	82.73 \pm 8.61
	D ₂	89.62 \pm 2.55	83.56 \pm 3.51	89.87 \pm 3.02	87.68 \pm 1.84
	D ₃	92.49 \pm 1.86	95.36 \pm 1.46	101.58 \pm 2.05	96.48 \pm 1.62
<i>Aedes aegypti/albopictus</i>	D ₁	121.52 \pm 26.4	117.68 \pm 30.9	103.35 \pm 3.42	114.18 \pm 12.1
	D ₂	136.81 \pm 3.01	123.92 \pm 4.92	139.55 \pm 3.72	133.43 \pm 3.12
	D ₃	138.91 \pm 2.29	130.80 \pm 2.71	140.29 \pm 2.51	136.67 \pm 1.94

^a Means sharing similar letters in a row or a column are statistically non-significant ($P > 0.05$). Small letters represent the comparison among interaction means and capital letters are used for the overall mean.

formulation provided between 4-10 weeks of absolute control of *Aedes* spp. and *Culex* spp., as reported by Pérez et al. [22], is similar to our results. Different concentrations of temephos produced 100% control of *Aedes albopictus* ranging from 7-8 weeks to 5 months as suggested by Morris et al. [23], which is close to our results. The efficacy of temephos was reported to be 7-9 weeks against *Aedes aegypti* and *Aedes albopictus* in a study conducted in Malaysia by the World Health Organization [24], which resembles our findings. The validity period of temephos reported by Liu et al. [25] against *Aedes albopictus* and *Cx. pipiens pallens* was only 10-14 days at 0.4-0.8 mg/L and 21 days at 1.2 mg/L and the effective control time of 0.25 mg/L of 1% temephos was about 28 days to larvae of *Culex pipiens pallens* and *Ae albopictus*, both results in favor of our observations about *Culex* but significantly less than that of *Aedes albopictus*. The granular formulation of 1.0% temephos at rates of 2.0 and 5.0 mg/L in the selected septic tanks provided 22 days of effective larval

control against *Culex pipiens*, as reported by KANG et al. [26], which is also like our results about *Culex* spp.

Temephos provided effective control against *Aedes aegypti* ranging from 130-140 days as reported by Marcombe et al. [27] and 120 days as reported by Boewono and Widyastuti [28], which is higher than our results. Shorter residuality was reported by Gürtler et al. [29] and Grisales et al. [30], in which temephos at the rate of 1 ppm provided approximately 6-9 weeks of effective control against *Aedes aegypti*, which is significantly lower than our findings. Treatment of temephos 50EC (100 ml/ha) in the field trials provided 4-5 weeks of absolute control and an additional 2-3 weeks of partial control against *Culex* spp. as described by Marina et al. [31] that resembles our observations under the laboratory conditions. Temephos 50EC at 0.5 ppm and 1 ppm resulted in 100% control of *Culex* spp. up to 7 days and 18 days of post-treatment reported by Ahmed and Adam [32], which is significantly lower than our results.

The results of larval bioassays conducted by Karunaratne et al. [33] in the laboratory against *Aedes aegypti* and *Aedes albopictus* at 1 ppm showed that temephos produced 100% mortality for ten months, significantly higher results than our findings. Temephos, when used in controlled release capsules, showed high efficacy against the *Culex pipiens* larvae, resulting in 100% mortality up to 120 and 92 days in stagnant and running water, respectively, reported by Badawy et al. [34], which is also significantly higher than our results because of the capsule formulation.

Temephos (5% granules) applied at 1 mg/L in simulated field studies showed effectiveness for 14 weeks against *Aedes albopictus* and 10 weeks against *Cx. quinquefasciatus*, respectively, as reported by WHO [35], which resembles our results as temephos showed more efficacy against *Aedes* spp. as compared to *Cx. quinquefasciatus*. The larval population of *Aedes aegypti* is still susceptible to temephos reported by Thongwat and Bunchu [36] and endorses our results. The less responsive behavior and low mortality of *Cx. quinquefasciatus* against temephos in our findings is due to the possible development of resistance as described by Thomas et al. [37], as they found a significant difference in the larval mortality of *Cx. quinquefasciatus* in urban zones (ranging between 2.8 and 56.5%) and rural zones (ranging from 45.0 to 71.0%).

Pyriproxyfen was found highly effective at a dose of 0.01 mg/L (recommended dose) and 0.02 mg/L (high dose) against *Aedes aegypti* for 16 weeks under a laboratory trial and simulated field trial [38]. Also, 0.1 mg/L granular sand formulations of pyriproxyfen remained active for over 4 months (>16 weeks), as reported by Seccacini et al. [39]. Both results are in favor of our findings in terms of the residual efficacy of pyriproxyfen. Pyriproxyfen resulted in total inhibition of adult emergence of *Aedes aegypti* for 90 days at a 0.05 ppm dose, which is concurrent with our results, and 45 days at a 0.01 ppm dose is dissimilar to our findings as reported by Resende and Gama [40]. The results of Nayar et al. [41] are parallel to our findings in which they used a granular formulation of pyriproxyfen 0.5G at 0.02-0.05 ppm against *Aedes aegypti* and *Cx. quinquefasciatus* and found effectiveness for the entire 6-week test period in both laboratory conditions and outdoors.

Emergence of *Cx. quinquefasciatus* adults was inhibited for 4-11 weeks when treated with 0.1 mg/L of pyriproxyfen under field conditions reported by Chavasse et al. [42], which is close to our results in terms of residual efficacy of pyriproxyfen. A single treatment of pyriproxyfen provided up to 2 months of control against *Culex quinquefasciatus*, as reported by Mulligan and Schaefer [43], which is close to our results in terms of efficacy. About 60-70% inhibition of emergence was seen by Ansari et al. [44] for 3 months in disused

wells against *C. quinquefasciatus* when pyriproxyfen 0.5% GR formulation was applied at the rate of 0.1 mg/L, which followed our results in which 60-70% emergence inhibition (EI) was seen up to 3 months. The results of various studies [45-47] follow our results in which the larvicidal efficacy of pyriproxyfen (IGR) was found to be greater than that of temephos (organophosphates). Ammar et al. [47] reported that even the lower doses of IGRs gave effective control against *Aedes albopictus* compared to organophosphate.

Complete inhibition of adult emergence of *Culex pipiens pallens* and *Cx. tritaeniorhynchus* was observed for 3 weeks only under field conditions when pyriproxyfen 0.5% granules were applied at different dosages of 0.01, 0.05, and 0.1 ppm, as reported by Kamimura and Arakawa [48], also when pyriproxyfen (Sumilarv 0.5G) applied at concentrations of 1.0 and 5.0 mg/L showed complete inhibition of adult emergence of *Aedes aegypti* for 22-28 and 36-42 days, respectively [49]. The granular formulation of 0.5% pyriproxyfen at 0.05-0.1 mg/L inhibited the emergence of *Aedes togoi* in brackish water in rock pools from 5 to 40 days, as reported by Lee [50]. Field trials of pyriproxyfen conducted by Okazawa et al. [51] against immature stages of *Anopheles punctulatus* at 4 different dosages resulted in inhibition of adult emergence for 2 months at a dosage of 0.1 ppm, for one month at 0.05 ppm and 0.01 ppm, and for 20 days at 0.02 ppm. Inhibition of adult emergence of *Aedes aegypti* and *Aedes albopictus* was observed up to 40, 57, and 56 days when treated with 0.001 ppm, 0.1 ppm of pyriproxyfen, and 1 ppm temephos 1% granules, respectively [52]. Studies by WHO [53] reported complete EI against *Aedes aegypti* for 6 weeks in plastic tubs placed outdoors. All these results are significantly lower than our results because of the field conditions.

The residual activity of pyriproxyfen reported by Marcombe et al. [27] in permanent breeding containers against *Aedes aegypti* was up to 28 weeks. The EI of pyriproxyfen GR decreased to < 80% after 160 and 260 days for 0.02 mg/L and 0.05 mg/L, respectively. Mortality of *Aedes aegypti* was reported to be greater than 80% for 5 months by Sihuinchu et al. [54] in field trials in water tanks treated with pyriproxyfen. Complete inhibition of adult emergence of *Aedes aegypti* was observed by Andrighetti et al. [55] for 160 days in shaded areas and 46 days at sunlight exposure when treated with pyriproxyfen at 0.05 ppm. Residual activity of pyriproxyfen was reported at 43 weeks (indoor) and 26 weeks (outdoor) against *Aedes aegypti* [44]. All these results are significantly higher than our findings. Results of Romeo et al. [56] indicate that pyriproxyfen (SUMILARV® 0.5G) is somewhat less effective on *Aedes albopictus* as compared to *Cx. pipiens*, which contrasts with our results, revealed that *Aedes aegypti/albopictus* are more susceptible to pyriproxyfen as compared to *Culex quinquefasciatus*.

Conclusions

As a conclusion of our studies, a marginal difference was observed between temephos 1G and temephos 50EC in causing the mortalities of *Culex quinquefasciatus*, *Aedes aegypti*, and *Aedes albopictus* in terms of both residual efficacy (80% mortality) and percent mortality but there was observed a significant difference of results between pyriproxyfen 0.5WDG compared to that of temephos 1G and temephos 50EC. In the early weeks of the study, mortality induced by all the tested larvicides at all doses was quite similar between the species, but over time, there was a significant difference among the tested doses in causing mortality, i.e., a marginal difference was observed between the recommended doses (D_2) and the higher doses (D_3), but a significant difference was observed between the lower doses (D_1) with that of the recommended and higher doses (D_2 and D_3).

Acknowledgments

The authors would like to extend their sincere appreciation to the Researchers Supporting Project number (RSPD2024R694), King Saud University, Riyadh, Saudi Arabia.

Conflict of Interest

The authors declare no conflict of interest.

References

- CHATTERJEE S., BAG S., BISWAL D., PARIA D.S., BANDYOPADHYAY R., SARKAR B., MANDAL A., DANGAR T.K. Neem-based products as potential eco-friendly mosquito control agents over conventional eco-toxic chemical pesticides-A review. *Acta Tropica*, 106858, **2023**.
- MONTENEGRO-QUIÑONEZ C.A., LOUIS V.R., HORSTICK O., VELAYUDHAN R., DAMBACH P., RUNGE-RANZINGER S. Interventions against *Aedes*/dengue at the household level: a systematic review and meta-analysis. *EBioMedicine*, 93, **2023**.
- WORLD HEALTH ORGANIZATION - Global Insecticide Use for Vector-Borne Disease Control: A 10-Year Assessment [2000-2009], 5th ed. 2011. Available online: <https://apps.who.int/iris/handle/10665/44670> (accessed on 22 October 2023).
- IBRAHIM A.M., THABET M.A., ALI A.M. Physiological and developmental dysfunctions in the dengue vector *Culex pipiens* (Diptera: Culicidae) immature stages following treatment with zinc oxide nanoparticles. *Pesticide Biochemistry and Physiology*, **192**, 105395, **2023**.
- HILLARY V.E., CEASAR S.A., IGNACIMUTHU S. Efficacy of plant products in controlling disease vector mosquitoes, a review. *Entomologia Experimentalis et Applicata*, **172**, 195, **2024**.
- WORLD HEALTH ORGANIZATION - Pesticides and their application: for the control of vectors and pests of public health importance, 6th ed. 2006. Available online: <https://www.who.int/publications/i/item/who-cds-ntd-whopes-gcdpp-2006.1> (accessed on 22 October 2023).
- WORLD HEALTH ORGANIZATION - Dengue guidelines, for diagnosis, treatment, prevention, and control. 2009. Available online: <https://www.who.int/publications/i/item/9789241547871> (accessed on 22 October 2023).
- HONG H., EOM T.H., TRINH T.T.T., TUAN B.D., PARK H., YEO S.J. Identification of breeding habitats and KDR mutations in *Anopheles* spp. in South Korea. *Malaria Journal*, **22** (1), 381, **2023**.
- SAAVEDRA-RODRIGUEZ K., CAMPBELL C.L., LOZANO S., PENILLA-NAVARRO P., LOPEZ-SOLIS A., SOLIS-SANTOYO F., RODRIGUEZ A.D., PERERA R., BLACK IV W.C. Permethrin resistance in *Aedes aegypti*: Genomic variants that confer knockdown resistance, recovery, and death. *PLoS Genetics*, **17** (6), e1009606, **2021**.
- HAFEZ A.M., ABBAS N. Insecticide resistance to insect growth regulators, avermectins, spinosyns and diamides in *Culex quinquefasciatus* in Saudi Arabia. *Parasites & Vectors*, **14**, 1, **2021**.
- LUPENZA E.T., KIHONDA J., LIMWAGU A.J., NGOWO H.S., SUMAYE R.D., LWETOIJERA D.W. Using pastoralist community knowledge to locate and treat dry-season mosquito breeding habitats with pyriproxyfen to control *Anopheles gambiae* SL and *Anopheles funestus* SL in rural Tanzania. *Parasitology Research*, **120**, 1193, **2021**.
- WORLD HEALTH ORGANIZATION - Pyriproxyfen in Drinking Water: Use for Vector Control in Drinking-Water Sources and Containers. 2008. Available online: <https://cdn.who.int/media/docs/default-source/wash-documents/wash-chemicals/pyriproxyfen-background.pdf> (accessed on 22 October 2023).
- SHAALAN E.A., CANYON D.V., BOWDEN B., YOUNES M.W., ABDEL-WAHAB H., MANSOUR A.H. Efficacy of botanical extracts from *Callitris glaucophylla* against *Aedes aegypti* and *Culex annulirostris* mosquitoes. *Tropical Biomedicine*, **23**, 180, **2006**.
- ABBOTT W.S. A method of computing the effectiveness of an insecticide. *Journal of Economic Entomology*, **18**, 265, **1925**.
- MARINA C.F., BOND J.G., MUÑOZ J., VALLE J., QUIROZ-MARTÍNEZ H., TORRES-MONZÓN J.A., WILLIAMS T. Comparison of novaluron, pyriproxyfen, spinosad, and temephos as larvicides against *Aedes aegypti* in Chiapas, Mexico. *Salud Pública De México*, **62** (4), 424, **2020**.
- SIVABALAKRISHNAN K., THANIHACHELVAN M., THARSAN A., ESWARAMOHAN T., RAVIRAJAN P., HEMPHILL A., RAMASAMY R., SURENDRAN S.N. Resistance to the larvicide temephos and altered egg and larval surfaces characterize salinity-tolerant *Aedes aegypti*. *Scientific Reports*, **13** (1), 8160, **2023**.
- MULDERIJ-JANSEN V., PUNDIR P., GRILLET M.E., LAKIANG T., GERSTENBLUTH I., DUTS A., TAMI A., BAILEY A. Effectiveness of *Aedes*-borne infectious disease control in Latin America and the Caribbean region: A scoping review. *Plos One*, **17** (11), e0277038, **2022**.
- PIRES S., ALVES J., DIA I., GOMEZ L.F. Susceptibility of mosquito vectors of the city of Praia, Cabo Verde, to Temephos and *Bacillus thuringiensis* var israelensis. *PLoS One*, **15** (6), e0234242, **2020**.

19. TANTAWY A.H., FARAG S.M., ABDEL-HALEEM D.R., MOHAMED H.I. Facile synthesis, larvicidal activity, biological effects, and molecular docking of sulfonamide-incorporating quaternary ammonium iodides as acetylcholinesterase inhibitors against *Culex pipiens* L. *Bioorganic Chemistry*, **128**, 106098, **2022**.
20. KHALEYLA F., ROHMAH E., MULYATNO K. Susceptibility of *Aedes aegypti* larvae in East Java towards commercial Temephos at different doses. *Jurnal Biologi Udayana*, **25** (2), 165, **2021**.
21. BROWN R., HING C.T., FORNACE K., FERGUSON H.M. Evaluation of resting traps to examine the behavior and ecology of mosquito vectors in an area of rapidly changing land use in Sabah, Malaysian Borneo. *Parasites & Vectors*, **11**, 1, **2018**.
22. MICHAELAKIS A., PAPACHRISTOS D.P., RUMBOS C.I., BENELLI G., ATHANASSIOU C.G. Larvicidal activity of spinosad and its impact on oviposition preferences of the West Nile vector *Culex pipiens* biotype molestus - A comparison with a chitin synthesis inhibitor. *Parasitology International*, **74**, 101917, **2020**.
23. MORRIS C.D., DAME D.A., ROBINSON J.W. Control of *Aedes albopictus* in waste tire piles with reduced rates of temephos-treated granules. *Journal of American Mosquito Control Association*, **12**, 472, **1996**.
24. WORLD HEALTH ORGANIZATION - Report of the seventh WHOPEs working group meeting WHO/HQ, Geneva 2-4 December 2003. 2004. <https://www.who.int/publications/i/item/who-cds-whopes-2004.8> (accessed on 22 October 2023).
25. STOOPS C.A., QUALLS W.A., NGUYEN T.V.T., RICHARDS S.L. A review of studies evaluating insecticide barrier treatments for mosquito control from 1944 to 2018. *Environmental Health Insights*, **13**, 1178630219859004, **2019**.
26. KANG J.O., JEONG D.K., LEE D.K., KANG H., KIARIE-MAKARA M. Field efficacy and non-target effects of temephos granules against *Culex pipiens* (Diptera: Culicidae) and microorganisms in septic tanks, Republic of Korea. *Entomology Research*, **41**, 60, **2011**.
27. FRANCIS S., CRAWFORD J., MCKENZIE S., CAMPBELL T., WRIGHT D., HAMILTON T., HUNTLEY-JONES S., SPENCE S., BELEMVIRE A., ALAVI K., GUTIERREZ C.T. Comparative toxicity of larvicides and growth inhibitors on *Aedes aegypti* from select areas in Jamaica. *Royal Society Open Science*, **7** (3), 192041, **2020**.
28. ARYAPREMA V.S., XUE R.D. Effectiveness and public acceptance of a slow release formulation of *Bacillus thuringiensis israelensis* (Mosquito Dunks®) in controlling the population density of dengue vectors in Sri Lanka. *International Journal of Pest Management*, **67** (2), 108, **2021**.
29. RUBIO A., CARDO M.V., MELGAREJO-COLMENARES K., VIANI M.J., VEZZANI D. Control of container mosquitoes with triflumuron in key urban land uses and effects on non-target dipterans. *Journal of Pest Science*, **1**, **2023**.
30. GUEDES R.N.C., BEINS K., NAVARRO COSTA D., COELHO G.E., BEZERRA H.S.D.S. Patterns of insecticide resistance in *Aedes aegypti*: meta-analyses of surveys in Latin America and the Caribbean. *Pest Management Science*, **76** (6), 2144, **2020**.
31. OGUNAH J.A., LALAH J.O., SCHRAMM K.W. Malaria vector control strategies. What is appropriate for sustainable global eradication? *Sustainable Chemistry and Pharmacy*, **18**, 100339, **2020**.
32. AHMED I.M., ADAM B. Field Evaluation of Temephos and Agnique® MMF Against Immature Stages of *Anopheles arabiensis* Patton (Diptera: Culicidae) The Vector of Malaria in Khartoum, Sudan. Thesis (Masters), Universiti Sains Malaysia, Penang, Malaysia, 15 July 2007. Available online: <http://eprints.usm.my/9038/> (accessed on 22 October 2023).
33. DALPADADO R., GUNATHILAKA N., AMARASINGHE D., UDAYANAGA L. A challenge for a unique dengue vector control program: assessment of the spatial variation of insecticide resistance status amongst *Aedes aegypti* and *Aedes albopictus* populations in Gampaha District, Sri Lanka. *BioMed Research International*, **2021**, 6619175, **2021**.
34. DE OLIVEIRA J.G., PILZ-JÚNIOR H.L., DE LEMOS A.B., DA COSTA F.A.D.S., FERNANDES M., GONÇALVES D.Z., VARIZA P.F., DE MORAES F.M., MORISSO F.D.P., MAGNAGO R.F., ZEPON K.M. Polymer-based nanostructures loaded with piperine as a platform to improve the larvicidal activity against *Aedes aegypti*. *Acta Tropica*, **230**, 106395, **2022**.
35. WORLD HEALTH ORGANIZATION - Guidelines for laboratory and field testing of mosquito larvicides. 2005. Available online: <https://apps.who.int/iris/handle/10665/69101> (accessed on 22 October 2023).
36. SAEUNG M., NGOEN-KLAN R., THANISPONG K., MUENWORN V., BANGS M.J., CHAREONVIRIYAPHAP T. Susceptibility of *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae) to temephos in Thailand and surrounding countries. *Journal of Medical Entomology*, **57** (4), 1207, **2020**.
37. RAI P., BHARATI M., SAHA D. Insecticide resistance to Temephos and synthetic Pyrethroids in *Culex quinquefasciatus* say from sub-Himalayan West Bengal, India. *International Journal of Tropical Insect Science*, **40**, 809, **2020**.
38. GUALBERTO D.A. Laboratory and field evaluation of Sumilarv 0.5 G, a commercial Pyriproxyfen on the larval strains of *Aedes aegypti* and *Aedes albopictus*. *Arthropods*, **11** (4), 164, **2022**.
39. HARBURGUER L.V., MENDOZA J., GONZALEZ P.V. A Review of the Use of Pyriproxyfen for Controlling *Aedes aegypti* in Argentina. *Current Tropical Medicine Reports*, **9** (4), 261, **2022**.
40. MOURA L., DE NADAI B.L., CORBI J.J. What does not kill it does not always make it stronger: high temperatures in pyriproxyfen treatments produce *Aedes aegypti* adults with reduced longevity and smaller females. *Journal of Asia-Pacific Entomology*, **23** (2), 529, **2020**.
41. SU T., SU H. Evaluation on the activity and efficacy of Omniprene 20cs against *Aedes*, *Anopheles*, and *Culex* mosquitoes in outdoor microcosms. *Journal of the Florida Mosquito Control Association*, **70** (1), 7, **2023**.
42. BELL M.J. The Impact of Latrine Construction on Densities and Pathogen Infection Rates of Synanthropic Flies and *Culex quinquefasciatus* Mosquitoes in Odisha, India (Doctoral dissertation, London School of Hygiene & Tropical Medicine), **2020**.
43. DEVILLERS J. Fate and ecotoxicological effects of pyriproxyfen in aquatic ecosystems. *Environmental Science and Pollution Research*, **27** (14), 16052, **2020**.
44. ANSARI M.A., SHARMA V.P., MITTAL P.K., RAZDAN R.K. Evaluation of juvenile hormone analogue JHM/S-31183 against immature stages of mosquitoes in

- natural habitats. *Indian Journal of Malariology*, **28**, 39, **1991**.
45. HERATH J.M.K., DE SILVA W.P.P., WEERARATNE T.C., KARUNARATNE S.P. Efficacy of the insect growth regulator novaluron in the control of dengue vector mosquitoes *Aedes aegypti* and *Ae. albopictus*. *Scientific Reports*, **14** (1), 1988, **2024**.
 46. JUDE J., GUNATHILAKA N., UDAYANGA L., FERNANDO D., PREMARATHNE P., WICKREMASINGHE R., ABEYEWICKREME W. Comparative analysis of the larvicidal activity of temephos (EC₅₀) and novaluron (EC₁₀) to control *Anopheles stephensi* in Sri Lanka. *Parasitology International*, **92**, 102686, **2023**.
 47. FANSIRI T., PONGSIRI A., KHONGTAK P., NITATSUKPRASERT C., CHITTHAM W., JAICHAPOR B., PATHAWONG N., KIJCHALAO U., TIANGTRONG S., SINGKHAIMUK P., PONLAWAT A. The impact of insect growth regulators on adult emergence inhibition and the fitness of *Aedes aegypti* field populations in Thailand. *Acta Tropica*, **236**, 106695, **2022**.
 48. FOXI C., DELRIO G., LUCIANO P., MANNU R., RUIU L. Comparative laboratory and field study of biorational insecticides for *Culicoides* biting midge management in larval developmental sites. *Acta Tropica*, **198**, 105097, **2019**.
 49. ALKENANI N.A. Semi-field assessment of Spinosad in combination with Altosid briquet and Dudim DT tablets against *Aedes aegypti* mosquito larvae reared in pond water. *Saudi Journal of Biological Sciences*, **26** (7), 1737, **2019**.
 50. HUSSAIN A., AKRAM W., TAYYIB M., USMAN M. Response of long-lasting pyriproxyfen larvicide (SumiLarv® 2MR) against the immature stage of *Aedes* mosquito: A vector of dengue in Pakistan. *Pakistan Journal of Agricultural Sciences*, **58** (5), 1563, **2021**.
 51. FOXI C., DELRIO G., LUCIANO P., MANNU R., RUIU L. Comparative laboratory and field study of biorational insecticides for *Culicoides* biting midge management in larval developmental sites. *Acta Tropica*, **198**, 105097, **2019**.
 52. NAKAZAWA M.M., ARAÚJO A.P., MELO-SANTOS M.A.V., OLIVEIRA C.M.F., SILVA-FILHA M.H.N.L. Efficacy and persistence of *Bacillus thuringiensis* svar. israelensis (Bti) and pyriproxyfen-based products in artificial breeding sites colonized with susceptible or Bti-exposed *Aedes aegypti* larvae. *Biological Control*, **151**, 104400, **2020**.
 53. WORLD HEALTH ORGANIZATION. Report of the fourth WHOPEP working group meeting: WHO/HQ, Geneva, 4-5 December 2000: a review of IR3535; KBR3023; (RS)-Methoprene 20% EC, Pyriproxyfen 0.5% GR; and Lambda-Cyhalothrin 2.5% CS. **2001**. Available online: <https://apps.who.int/iris/handle/10665/66683> (accessed 22 October 2023).
 54. HUSTEDT J.C., BOYCE R., BRADLEY J., HII J., ALEXANDER N. Use of pyriproxyfen in control of *Aedes* mosquitoes: A systematic review. *PLoS Neglected Tropical Diseases*, **14** (6), e0008205, **2020**.
 55. CAMPOS K.B., MARTINS A.J., RODOVALHO C.D.M., BELLINATO D.F., DIAS L.D.S., MACORIS M.D.L.D.G., ANDRIGHETTI M.T.M., LIMA J.B.P., OBARA M.T. Assessment of the susceptibility status of *Aedes aegypti* (Diptera: Culicidae) populations to pyriproxyfen and malathion in a nationwide monitoring of insecticide resistance performed in Brazil from 2017 to 2018. *Parasites & Vectors*, **13**, 1, **2020**.
 56. DRAGO A., SIMONATO G., VETTORE S., MARTINI S., MARCER F., DI REGALBONO A.F., CASSINI R. Efficacy of Aquatain® against *Culex pipiens* complex and *Aedes albopictus* in catch basins in Italy. *Journal of the American Mosquito Control Association*, **36** (1), 51, **2020**.