



Mosquitoes as pesticide pollution Indicators: A comparative susceptibility analysis of field and laboratory strains of mosquitoes against different conventional insecticides

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ABSTRACT

Thrissur Kole wetlands fall under the category of temporary wetlands that are exceedingly ideal procreation environments for mosquitoes. A broad array of insecticides is used as a competitive weapon in mosquito restriction tactics, including organochlorines, organophosphates, carbamates, and pyrethroids. Indiscriminate use of these chemicals could develop resistance in targeted and nontargeted species present in that environment. The present study is an assessment of the susceptibility status of laboratory and field strain *Aedes albopictus* and *Culex quinquefasciatus* mosquitoes against deltamethrin, lambda-cyhalothrin, and malathion employing the *World Health Organization* protocol. The results show that laboratory strain mosquitoes are more susceptible than the field-collected mosquitoes toward these insecticides. The field strain of *Ae. albopictus* showed 6.36, 11.74, and 18.36 times resistance than the laboratory strain against malathion, deltamethrin, and lambda-cyhalothrin, respectively. *Cx. quinquefasciatus* larvae also repeated this resistance pattern like 4.54 times resistance against malathion and 13.15 times and 12.62 times resistance against deltamethrin and lambda-cyhalothrin, correspondingly. The increased susceptibility of the field strain could also lead to a prospect of treating mosquitoes as an indicator species of pesticide contamination. Finding out the precise dosage of insecticide applications could furthermore help in the vector management program and diminish environmental pollution caused by these chemicals.

1. INTRODUCTION

Wetlands are inimitable waterlogged ecosystems with distinctive abiotic and biotic environmental characteristics. These aquatic systems might support the massive quantity of floral and faunal diversity concerning their origin, topographical position, aquatic organization, and interaction among them [1]. As stated by a public health organization, wetlands are considered favorable procreation grounds for vector mosquitoes transmitting arboviruses and parasites [2,3]. Thrissur Kole wetlands is a shallow-water low-lying strip of the aquatic system acting as an intermediary region between terrestrial and marine ecosystems, which is maintained at

0.5–1 m under sea level. These Ramsar sites are topographically located in the central region of Kerala, which overlays an area of 10,187 ha and spreads across Mukundapuram, Chavakad, and Thrissur Taluks of Thrissur district. Kole lands are inundated around half of the year with monsoon water dispensed by the two major rivers in Thrissur, and in the next half of the year, paddy as well as vegetable cultivation is practiced [4]. Byproduct deposition by agriculture could modify the depth and parameters of the water system, and these favorable changes might provide a reproducing environment for mosquitoes. For example, declining water temperature led to vector species development [5,6].

Many parasites and arboviruses that are causative for diseases like malaria, dengue, chikungunya, zika, filariasis, yellow fever, Japanese encephalitis, etc. have accomplished association with some mosquitoes to disperse their pathogenicity. Mosquito-borne diseases are considered one of the primary reasons for mortality

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and illness around the globe, especially in tropical and subtropical countries. Apart from the disease transmission, mosquitoes are the most annoying human pests with their irritating blood-feeding behavior [7,8]. *Anopheles*, *Aedes*, and *Culex* are the three major genera that comprise disease-spreading vector mosquitoes [9]. *Cx. quinquefasciatus* is one of the most annoying common mosquito species [10]. This mosquito can carry a nematode called *Wuchereria bancrofti* which cause filariasis, West Nile virus, Saint Luis encephalitis virus, Ross river virus, and Japanese encephalitis virus across the Earth [11–15]. *Ae. albopictus* is an indigenous species in Southeast Asia; hence, the name Asian tiger mosquito, which is spread all over the world. It has the potential vectorial capacity to transmit chikungunya, dengue, and some other west Nile viruses [16,17].

Mosquito-borne diseases substantially provide infection load, mortality, poverty, and devitalization to the society in tropical countries where the disease has been dispersed [9]. Over the past few years, unusual disease occurrence and severe annoyance were frequent in infected areas; hence, scientists have found out that mosquito control would be the most appropriate solution for this puzzle [18]. Chemical treatment leftovers as the primary indispensable tactic are used in pest management because of their rapid results in the broad treatment area [19]. Adequate insecticide practice is regarded as an influential defense to achieve better public health manifestations and increase agricultural yield in developing countries [20–22]. From the beginning of pest management programs, insecticide-based mosquito eradication operations could be assessed as the highly efficient approach, but only a limited number of less harmful and highly economical insecticides are used in these practices [23].

Malathion is a chemical compound in the organophosphate insecticide family, which is chemically formed by the esterification of thiophosphoric acid and phosphoric acid. These substances are regarded as neurotoxins due to their detrimental effect on a neurotransmitter called acetylcholine and are mainly used in the pest management program. A group of synthetic insecticides is also used in mosquito control, namely pyrethroids, which contains deltamethrin, lambda-cyhalothrin, permethrin, etc. Pyrethroids act on the nervous system of targeted organisms similar to organophosphate, but instead of the neurotransmitter, pyrethroids can influence the sodium channels of neurons [24,25]. Malathion and pyrethroids are widely used in mosquito management in the public health domain. However, its prolonged and excessive use led to a considerable degree of chemical resistance in the targeted organisms [26]. Screening of insecticide susceptibility and dosage estimation can help in vector control operations and the evaluation of insecticide influence on mosquito behavior [27].

2. METHODOLOGY

2.1. Study Site

Thrissur Kole wetlands is a 10,187 ha area located between 10° 20' and 10° 40' N latitude and between 75° 58' and 76° 11' E longitude in Kerala's central region. Thrissur Kole wetlands extend across Mukundapuram, Chavakad, and Thrissur Taluks of Thrissur district. This area spreads from Velukara in the south to

the northern bank of Chalakudy river and Tholur and Kaiparambu areas of Thrissur Taluk in the north. The following wetland area of Thrissur Taluk is named Ponnani Kole. The significant breeding habitats of mosquitoes in Kole wetlands comprise paddy fields, rocky pools, tree holes, coconut shells, ditches, containers, irrigation canals, and ponds [28].

2.2. Insecticide

Lambda-cyhalothrin, deltamethrin, and malathion (technical grade) were purchased from “New India Surgicals”, Calicut, Kerala, India.

2.3. Mosquito Sampling and Colony Maintenance

Ae. albopictus and *Cx. quinquefasciatus* mosquitoes were collected from different Thrissur Kole wetlands' localities and identified by classical taxonomic methods [29,30]. The collected larvae were reared into adults in laboratory conditions (temperature 26 ± 2°C, larval food was prepared by mixing yeast and dog biscuits). Adult mosquitoes were fed with 5% sucrose and blood meal was provided on the third day of emergence. F1 progeny larvae of field-collected mosquitoes were subjected to larval bioassay. Laboratory colonized insecticide-free *Ae. albopictus* and *Cx. quinquefasciatus* were maintained in the communicable disease research laboratory, St. Joseph's College, Irinjilakuda. These untreated larvae were also used in larval bioassay as laboratory strain.

2.4. Larval Bioassay

The standard *World Health Organization* (WHO) procedure was followed for determining larval susceptibility [31]. Accordingly, the larvae were subjected to different concentrations of insecticides whose stock solutions were prepared using distilled water as the solvent. 1 mg/ml deltamethrin, lambda-cyhalothrin, and malathion stock solution was prepared in water. Test concentrations were prepared by adding 1 ml insecticide-containing solution to 249 ml of water in a 500 ml capacity beaker and stirred vigorously for 30 seconds with a glass stirrer. For the control, 1 ml of distilled water or acetone as required was added to 249 ml of dechlorinated water instead of insecticide. To each of the beakers containing different tests and control, 25 late third or early fourth instar larvae were released with the help of a glass strainer. Six serial test concentrations of insecticides were prepared for larval bioassay. Mortality was recorded after 24 hours. Unmoved and moribund larvae were treated as dead larvae. If 5%–20% mortality was obtained in the control experiment, it was corrected by using Abbott's formula [32]. The LC_{50} and LC_{90} values for insecticides were calculated using Probit analysis by the dosage mortality regression line [33].

3. RESULT

Larvicidal efficacy of malathion, deltamethrin, and lambda-cyhalothrin on field-collected and laboratory-reared *Ae. albopictus* and *Cx. quinquefasciatus* is given in Tables 1 and 2. The results of malathion susceptibility of field-collected and laboratory-colonized *Ae. albopictus* and *Cx. quinquefasciatus* larvae illustrated that the

Table 1: Mosquito larvicidal effect of malathion, deltamethrin, and lambda-cyhalothrin against *Aedes albopictus*.

Insecticide	LC25 (LCL-UCL)	LC50 (LCL-UCL)	LC90 (LCL-UCL)	CHI square value	p value
Field strain					
Malathion	7.4643×10^{-3} ppm (3.4597 $\times 10^{-3}$ –1.0200 $\times 10^{-2}$)	1.2398×10^{-2} ppm (8.6815 $\times 10^{-3}$ –1.7250 $\times 10^{-2}$)	3.2516×10^{-2} ppm (2.1659 $\times 10^{-2}$ –1.0775 $\times 10^{-1}$)	23.362	0.000107202 ^a
Deltamethrin	6.4881×10^{-4} ppm (4.5221 $\times 10^{-4}$ –8.0519 $\times 10^{-4}$)	1.0249×10^{-3} ppm (8.3082 $\times 10^{-4}$ –1.2185 $\times 10^{-3}$)	2.4435×10^{-3} ppm (1.9358 $\times 10^{-3}$ –3.6504 $\times 10^{-3}$)	9.709	0.045634782 ^a
Lambda-Cyhalothrin	5.3587×10^{-4} ppm (3.2222 $\times 10^{-4}$ –7.0675 $\times 10^{-4}$)	9.0259×10^{-4} ppm (6.7797 $\times 10^{-4}$ –1.13.6 $\times 10^{-3}$)	2.4305×10^{-3} ppm (1.8091 $\times 10^{-3}$ –4.2521 $\times 10^{-3}$)	12.671	0.013001896 ^a
Laboratory strain					
Malathion	1.1442×10^{-3} ppm (5.1524 $\times 10^{-4}$ –1.5676 $\times 10^{-3}$)	1.8672×10^{-3} ppm (1.2904 $\times 10^{-3}$ –2.6246 $\times 10^{-3}$)	4.7353×10^{-3} ppm (3.1612 $\times 10^{-3}$ –1.6322 $\times 10^{-2}$)	25.735	0.00003579 ^a
Deltamethrin	4.3115×10^{-5} ppm (1.86603 $\times 10^{-5}$ –6.46454 $\times 10^{-5}$)	8.7237×10^{-5} ppm (5.5912 $\times 10^{-5}$ –1.2074 $\times 10^{-4}$)	3.3284×10^{-4} ppm (2.1763 $\times 10^{-4}$ –8.1799 $\times 10^{-4}$)	14.853	0.005 ^a
Lambda-Cyhalothrin	2.3138×10^{-5} ppm (8.3473 $\times 10^{-6}$ –3.6602 $\times 10^{-5}$)	4.9158×10^{-5} ppm (2.8954 $\times 10^{-5}$ –7.1322 $\times 10^{-5}$)	2.0578×10^{-4} ppm (1.2750 $\times 10^{-4}$ –6.1128 $\times 10^{-4}$)	17.003	0.002 ^a

Table 2: Mosquito larvicidal effect of malathion, deltamethrin, and lambda-cyhalothrin against *Culex quinquefasciatus*.

Insecticide	LC25 (LCL-UCL)	LC50 (LCL-UCL)	LC90 (LCL-UCL)	CHI square value	p value
Field strain					
Malathion	1.5×10^{-2} ppm (8 $\times 10^{-3}$ –2 $\times 0-1$)	2.6×10^{-2} ppm (1.9 $\times 10^{-2}$ –3.3 $\times 10^{-2}$)	7.3×10^{-2} ppm (5.3 $\times 10^{-2}$ –1.38 $\times 10^{-1}$)	14.010	0.007 ^a
Deltamethrin	1.2482×10^{-3} ppm (7.869 $\times 10^{-4}$ –1.5796 $\times 10^{-3}$)	1.9125×10^{-3} ppm (1.4928 $\times 10^{-3}$ –2.3853 $\times 10^{-3}$)	4.3021×10^{-3} ppm (3.2180 $\times 10^{-3}$ –8.1746 $\times 10^{-3}$)	16.778	0.002134925 ^a
Lambda-Cyhalothrin	1.2360×10^{-3} ppm (7.5425 $\times 10^{-4}$ –1.5784 $\times 10^{-3}$)	1.9052×10^{-3} ppm (1.4675 $\times 10^{-3}$ –2.4018 $\times 10^{-3}$)	4.3345×10^{-3} ppm (3.2060 $\times 10^{-3}$ –8.6456 $\times 10^{-3}$)	17.828	0.00133342 ^a
Laboratory strain					
Malathion	2.7717×10^{-3} ppm (1.6451 $\times 10^{-4}$ –5.1244 $\times 10^{-3}$)	5.7159×10^{-3} ppm (1.8352 $\times 10^{-3}$ –1.1258 $\times 10^{-2}$)	2.2612×10^{-2} ppm (1.1416 $\times 10^{-2}$ –7.8949 $\times 10^{-1}$)	44.394	0.000000005 ^a
Deltamethrin	1.0272×10^{-4} ppm (7.45144 $\times 10^{-5}$ –1.228286 $\times 10^{-4}$)	1.4540×10^{-4} ppm (1.2131 $\times 10^{-4}$ –1.7020 $\times 10^{-4}$)	2.8138×10^{-4} ppm (2.277889 $\times 10^{-4}$ –4.253177 $\times 10^{-4}$)	13.518	0.009002003 ^a
Lambda-Cyhalothrin	9.8709×10^{-5} ppm (5.99641 $\times 10^{-5}$ –1.2622 $\times 10^{-4}$)	1.5090×10^{-4} ppm (1.1552 $\times 10^{-4}$ –1.884708 $\times 10^{-4}$)	3.3806×10^{-4} ppm (2.5384 $\times 10^{-4}$ –6.386195 $\times 10^{-4}$)	17.740	0.001 ^a

LCL = lower confidence limit; UCL = upper confidence limit.
 p-value < 0.05 shows a significant difference at 5%.

laboratory strain was more susceptible than field-collected strain. The LC₅₀ value of field-collected *Ae. albopictus* was 1.2398×10^{-2} ppm, which was about 6.64 times greater than the laboratory strain whose LC₅₀ was 1.8672×10^{-3} ppm. LC₅₀ value of field-collected *Cx. quinquefasciatus* 2.6×10^{-2} ppm and LC₅₀ of laboratory strain was 5.7159×10^{-3} ppm.

Deltamethrin susceptibility result displayed that the LC₅₀ value of field-collected *Ae. albopictus* was 1.0249×10^{-3} ppm and laboratory strain *Ae. albopictus* was 8.7237×10^{-5} ppm. LC₅₀ value of field strain *Cx. quinquefasciatus* was 1.9125×10^{-3} ppm, and that of laboratory strain was 1.4540×10^{-4} . Susceptibility results of lambda-cyhalothrin indicated that the laboratory strain was more susceptible than field strain. LC₅₀ of field-collected *Ae. albopictus* 9.0559×10^{-4} ppm and LC₅₀ of laboratory strain was 4.91589×10^{-5} ppm. LC₅₀ of field-collected *Cx. quinquefasciatus* was 1.9052×10^{-3} ppm and LC₅₀ of laboratory strain *Cx. quinquefasciatus* was 1.5090×10^{-4}

Ae. albopictus mosquitoes exhibited more susceptibility than *Cx. quinquefasciatus*, and both field and laboratory strains of all three groups were treated by three different insecticides.

Figures 1–3 show the comparison of susceptibility status of field and laboratory strains of *Ae. albopictus* and *Cx. quinquefasciatus* against three different insecticides. Lambda-cyhalothrin was the most efficient insecticide, and malathion showed the least efficacy when compared to the other two insecticides used. All mosquitoes showed more susceptibility toward lambda-cyhalothrin and less toward malathion. The susceptibility rate can be illustrated in the order of lambda-cyhalothrin > deltamethrin > malathion.

4. DISCUSSION

Organochlorines, organophosphates, carbamates, and pyrethroids are the widespread chemical compounds used in the pest control strategy. Altogether, 12 different insecticides belonging to these four classes were recommended by the WHO for mosquito

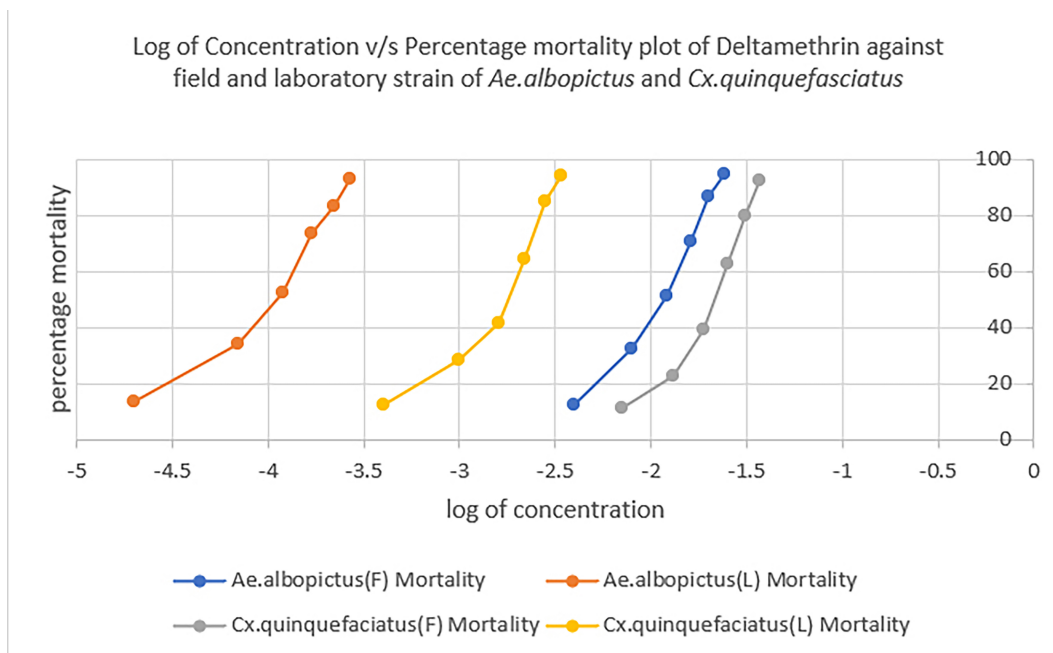


Figure 1: Comparison of susceptibility status of deltamethrin against laboratory and field strains of *Ae. albopictus* and *Cx. quinquefasciatus*.

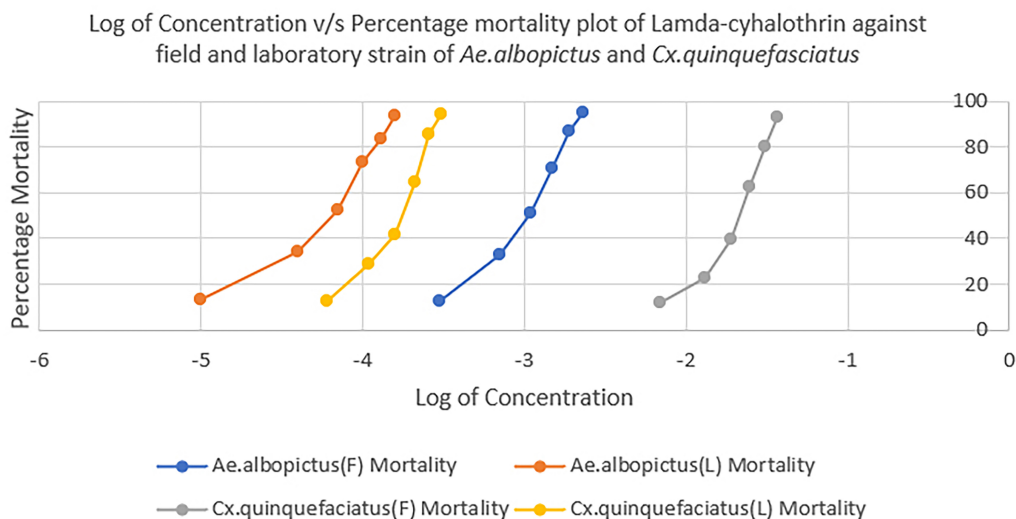


Figure 2: Comparison of susceptibility status of lambda-cyhalothrin against laboratory and field strains of *Ae. albopictus* and *Cx. quinquefasciatus*.

eradication [34,35]. Malathion comes under the organophosphate group of insecticides, is lethal to insects at the same time, and is less harmful to mammals. These second-generation insecticides are globally used for mosquito control only after the prohibition of Dichlorodiphenyltrichloroethane and dieldrin in the USA in the 1970s [24]. The extensive use of malathion arose from some resistance problems in different mosquito species worldwide, and several researchers also conducted many studies. Our present study discloses that laboratory strain *Ae. albopictus* and *Cx. quinquefasciatus* more susceptible than the field strain, which was collected from Thrissur Kole wetlands. Studies on malathion

resistance against mosquitoes were reported in the 1990s itself. Bisset *et al.* [36] and Bracco *et al.* [37] reported on malathion and carbamate resistance in *Cx. quinquefasciatus* in Cuba And Brazil, respectively. In 2010, Selvi *et al.* [38] discussed their result in the study on the susceptibility of *Ae. albopictus* against malathion. They discovered that the laboratory strain mosquitoes were more susceptible than field strain ones because of the recurrent contact with the insecticides used in the field. Organophosphate, carbamate, and pyrethroid resistance were also noted in *Cx. quinquefasciatus* mosquito by some studies conducted in America, Saudi Arabia, and northern Thailand [39–41].

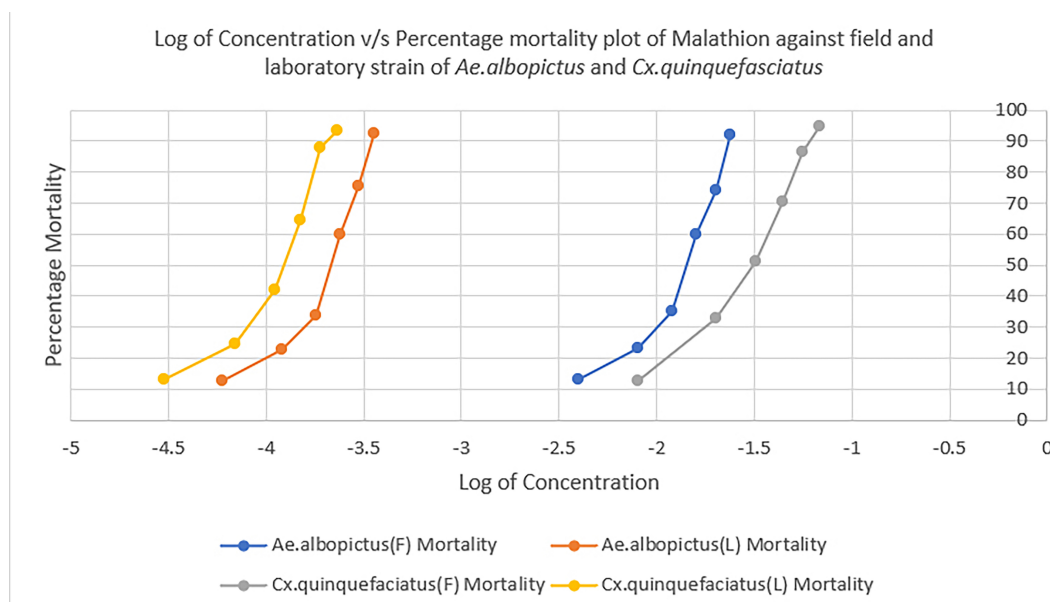


Figure 3: Comparison of susceptibility status of malathion against laboratory and field strains of *Ae. albopictus* and *Cx. quinquefasciatus*.

Kole wetlands is the major rainwater plunked ground of the surrounding area of the Thrissur district. Karuvannur and Kecheri river sediments urban and industrial atrophies with their monsoon flood watercourse to this delta every year [42]. Tessy and Sreekumar [43] reported almost 30 pollutant tolerant algal species from Thrissur Kole wetlands. Our study site is considered one of Kerala's major rice bowls, and the paddy cultivation practices take place during northeast monsoon season to post-monsoon season (September–March). An enormous number of pesticides, weedicides, and fertilizers have been applied by the farmers in these paddy fields to protect their crops and attain maximum yield [44,45]. These chemical combinations bring about some insecticidal tolerance in different mosquito species and other non-targeted organisms. Two individual studies publicized such tolerance in *Ae. albopictus* and *Ae. aegypti*, respectively [46,47].

In the current study, we made a comparative analysis of field-collected and laboratory-colonized *Ae. albopictus* and *Cx. quinquefasciatus* mosquitoes against malathion, deltamethrin, and lambda-cyhalothrin. Laboratory strains of *Ae. albopictus* and *Cx. quinquefasciatus* were more susceptible than the field strains. The outcome unveiled that both *Ae. albopictus* and *Cx. quinquefasciatus* exhibit more susceptibility toward lambda-cyhalothrin and least susceptible toward malathion. The susceptibility rate is in the order of lambda-cyhalothrin > deltamethrin > malathion in these mosquitoes. Hamdan *et al.* [48] discovered some LC_{50} values in *Ae. albopictus* and *Cx. quinquefasciatus* mosquitoes. The result of *Ae. albopictus* susceptibility test showed LC_{50} of malathion is 0.1972 ppm, temephos is 0.0514 ppm, and permethrin is 0.0022 ppm. The LC value in *Cx. quinquefasciatus* against malathion is 0.0163 ppm and permethrin is 0.00001 ppm. The result indicates that *Ae. albopictus* is more susceptible than *Cx. quinquefasciatus* toward all the insecticides tested and could explain the natural larval habitat preference of *Culex* mosquitoes is more polluted larval habitats when compared to *Aedes* species which breeds in freshwater.

Aedes mosquitoes generally adopt freshwater habitats and *Culex* prefers slightly to immensely polluted water for their development. The present study perceived that both species were collected from the same environment, which indicates that these mosquitoes had developed some resistance. The susceptibility assays conducted against different commonly used insecticides in the pest control study area also support these findings. Results from the quantitative susceptibility assays demonstrate the variance between the field and laboratory strains, in which field strains showed more resistance toward all the tested insecticides. The field strain of *Ae. albopictus* showed 6.36, 11.74, and 18.36 times more resistance than the laboratory strain against malathion, deltamethrin, and lambda-cyhalothrin, respectively. *Cx. quinquefasciatus* larvae followed this resistance pattern with 4.54 times resistance against malathion and 13.15 times and 12.62 times resistance against deltamethrin and lambda-cyhalothrin, correspondingly. A study conducted in Thailand observed such resistance array in *Cx. quinquefasciatus* against a group of insecticides, and they suggested that the prevalent interaction of insecticides might have resulted in some resistance in these mosquitoes [49]. The remarkable difference of LC_{50} values between the same species' strains might result from their resistance development to the insecticides, and this trait could be considered a crucial characteristic of a biological indicator of insecticide pollution. There are some early remarks on mosquitoes as possible bioindicators of insecticide pollution [50], and some recent surveys justified *Culex* mosquitoes as lead effluence bioindicators [51]. The utilization of the study area for seasonal paddy cultivation and periodical exposure to the mentioned chemical insecticides for insect pest management makes this study's findings more significant [45].

5. CONCLUSION

Various chemical insecticides have been used for decades in agricultural as well as public health indices to improve crop

productivity and accomplish better mosquito management. However, overdependence and disproportionate use of these chemical insecticides are primarily attributed to the development of resistance in targeted organisms. Mosquitoes collected from the areas contaminated with extensive chemical exposure showed some insecticidal tolerance. Subordinate part of this unsystematic usage is environmental effluence and harmful effects on non-targeted organisms. This study tried to discuss some lethal concentration values of two mosquitoes against three conventional insecticides. These values may help to contribute toward efficiency in mosquito-monitoring tactics with a minimal ecosystem imbalance.

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