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**Original article**

# Comparison between WHO standard method and CDC alternative one for detecting resistance levels of filariasis vector, *Culex pipiens* to some larvicides in Egypt

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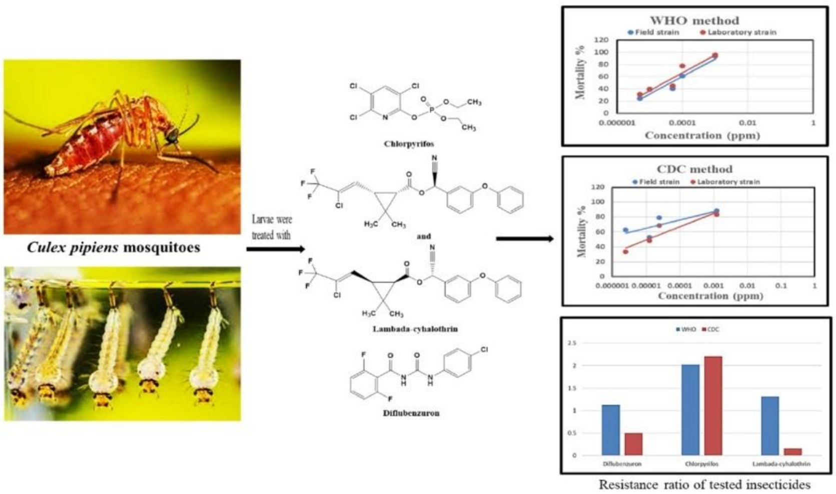
**ABSTRACT**

*Culex pipiens* mosquito is a well-known vector of many vector-borne diseases such as filariasis, Rift Valley Fever and West Nile Fever. This study aimed to compare the resistance by World Health Organization (WHO) and Center for Disease Control and Prevention (CDC) methods. *Culex pipiens* larvae were collected from Qalubiya governorate and colonized with the laboratory population. Larvae were treated with different concentrations of three insecticides, lambda-cyhalthrin; diflubenzuron and chlorpyrifos. The mortality percent, as well as the parameters of regression analysis including $LC_{50}$ and $LC_{90}$, were calculated separately for both WHO and CDC methods. The results showed that in the case of laboratory population $LC_{50}$ values were 0.000043, 0.000089 and 0.00535 ppm for lambda-cyhalthrin, diflubenzuron and chlorpyrifos, respectively for WHO method. While $LC_{50}$ in CDC method were 0.0000094, 0.0002 and 0.005 ppm of the tested insecticides, respectively. From the previous results, we concluded that lambada-cyhalothrin was the most effective insecticide followed by diflubenzuron and chlorpyrifos. Also, based on the lethal concentrations ($LC_{50}$ and $LC_{90}$) there was difference between two methods against the laboratory population in all insecticide treatments. On the other hand, there was no difference between the two methods against the field populations except the lambda-cyhalthrin treatment. According to calculated resistance ratio (RR), all tested insecticides recorded low RR in both two assays however, results showed that chlorpyrifos displayed the highest RR value in both WHO and CDC assays. The slopes obtained for both assays were almost like each other indicating the promising of the two assays.

## Graphical abstract

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## 1. Introduction

*Culex pipiens* mosquito is the primary vector for many vector-borne diseases in Egypt that affecting human beings, particularly Rift Valley Fever, West Nile Fever and filariasis [1, 2]. Some of these diseases are fatal unless treated, while others lead to lifelong disabilities and impairment [3]**.** Therefore, we need to control or manage mosquito vectors for preventing mosquitos-borne disease, especially in the absence of the effective vaccines. Chemical control is an important component of the management programs by using insecticide [4], but insecticides sometimes fail to control mosquitoes. The main reason for the failure of management mosquito vectors is the development of insecticide resistance. Many mosquito species had already developed resistance to synthetic chemicals because of their overuse [5, 6].

The early detection of insecticide resistance aids in rational pesticide selection for use against disease vectors [**7**]. Insecticide-based vector control faces the issue of resistance to organophosphates and pyrethroids, including the *Culex pipiens* complex. Now, each of the four chemical groups of insecticides available on the market, organophosphates, organochlorines, carbamates, and pyrethroids, has been the subject of resistance, whether they are larvicides or adulticides [8].

Chlorpyriphos, lambada-thyalothrin, and diflobenzuron resistance in Egypt have been recorded on *Culex pipiens* [9-11]**.** For the detection of resistance of Chlorpyriphos, lambada-thyalothrin, and diflobenzuron insecticides those belong to organochlorines, pyrethroids. In this study two techniques were used. Although the WHO technique has been widely used for detecting resistance of insecticide. In this technique the mortality is recorded after 24 hours of mosquito's exposure to insecticides [12]. On the other hand, the alternative technique developed by CDC depends on counting mortality during 1 to 2 hours of mosquito's exposure to insecticides, and in this case, the larvae will be tested in a less time-consuming. CDC method always, is used in detection of insecticide resistance of the adult populations [13, 14]. Therefore, in this study, CDC method was used in determination of insecticide resistance of the larval populations of *Culex pipiens* and compared to WHO standard one.

## 2. Materials and Methods

### 2.1 Rearing technique

*Culex pipiens* mosquitoes were collected in larval stage from Qalubiya governorate and colonized in the laboratory conditions at the insectary of the Research Institute of Medical Entomology, Dokki, Giza, Egypt. Mosquito larvae were reared in metal trays containing distilled water and fish food at standard conditions (28°C, 12 h: 12 h light/dark period, 75% relative humidity). When larvae reached the pupal stage, the pupae transferred to cups. These cups were put inside net cages until adults emerged and were supplied with cotton pieces soaked in a 10% sucrose solution. Females were fed on blood of pigeon for egg development [15].

### 2.2 Insecticides Used

Three insecticides of three different groups were used as illustrated in figure 1

**A. Chlorpyrifos (40% EC)**

**Chemical group:** Organophosphate
**Trade name:** (Reldanon Extra) was purchased from Kanza Group 6th of October city, Giza, Egypt.
**Empirical formula:** $C_9H_{11}Cl_3NO_3PS$
**Mode of action:** non-systemic insecticide with contact, stomach, a vapour action, cholinesterase inhibitor [16].

**B. Lambada-cyhalothrin (3.5% EC)**

**Chemical group**: Pyrithroid
**Trade name:** Karasay was purchased from Kafr El Zayat Co. Gharbiya governorate, Egypt.
**Empirical formula:** $C_{23}H_{19}ClF_3NO_3$
**Mode of action:** non-systemic insecticide with contact and stomach action, and adult repellent properties [16].

**C. Diflubenzuron (50% WP)**

**Chemical group:** Benzoylurea.
**Trade name:** Diflucin was purchased from Egypt Chem International, Alexandria, Egypt.
**Empirical formula**: $C_{14}H_9ClF_2N_2O_2$
**Mode of action**: Diflubenzuron acts by inhibition of chitin synthesis and so interferes with formation of the insect cuticle [16].

### 2.3 Bioassays

Two insecticide resistance bioassay methods were used to determine and compare the susceptibility in *Culex pipiens* larvae when treated with chlorpyrifos, lambda-cyhalothrin, and diflubenzuron insecticides.

#### 2.3.1 WHO Bioassay

Twenty-five late 3rd or early 4th larval instars of *Culex pipiens* from both laboratory and field population mosquitoes were transferred to glass beaker for treatment with chlorpyrifos and lambda-cyhalothrin, while the 2nd larval instar was used for diflubenzuron one. Five concentrations of chlorpyrifos (0.001, 0.005, 0.05, 0.02, &0.1 ppm), while concentrations used in diflubenzuron were 0.000001, 0.00001, 0.0005, 0.001, & 0.005 ppm, and lambda-cyhalothrin insecticides concentrations were 0.000001, 0.000005, 0.00001, 0.00005, 0.0001, & 0.001 ppm. In the experiments 1 ml of insecticides were added to 224 ml of distilled water in the beaker then waiting for 15-30 minutes, finally 25 ml of water containing larvae were added to large beaker (500ml) and maintained under standard conditions (25 ± 2 ° C and humidity of 70-80%). The mortality was recorded after 24 h for chlorpyrifos, lambda-cyhalothrin, while in case of diflubenzuron the mortality was recorded after 72h. All treatments were tested with three replicates [17]. The $LC_{50}$ (Lethal concentration 50% of population and $LC_{90}$ (Lethal Concentration 90% of population) values were calculated, and slope and heterogeneity analysis were also determined according to WHO [18].

#### 2.3.2 CDC bioassay

According to CDC [19], twenty-five mosquito larvae from both laboratory and field populations were transferred to a small beaker containing 20 ml of distilled water, then added to a large glass beaker that contained 79 ml distilled water and 1ml insecticide (the total volume will be 100 ml). As in WHO, late 3rd or early 4th larval instars of *Culex pipiens* mosquitoes were used for chlorpyrifos and lambda-cyhalothrin while the 2nd larval instar was used for diflubenzuron. Five concentrations (0.0025, 0.0125, 0.05, 0.125 & 0.25 ppm) and (0.0000025, 0.000025, 0.000125, 0.0025 & 0.0125 ppm) were used for chlorpyrifos and diflubenzuron, respectively, while four concentrations (0.0000025, 0.0000125, 0.000025 & 0.00125 ppm) were used in lambda-cyhalothrin treatment. Larvae were maintained under standard conditions (25 ± 2 ° C and humidity of 70-80%).

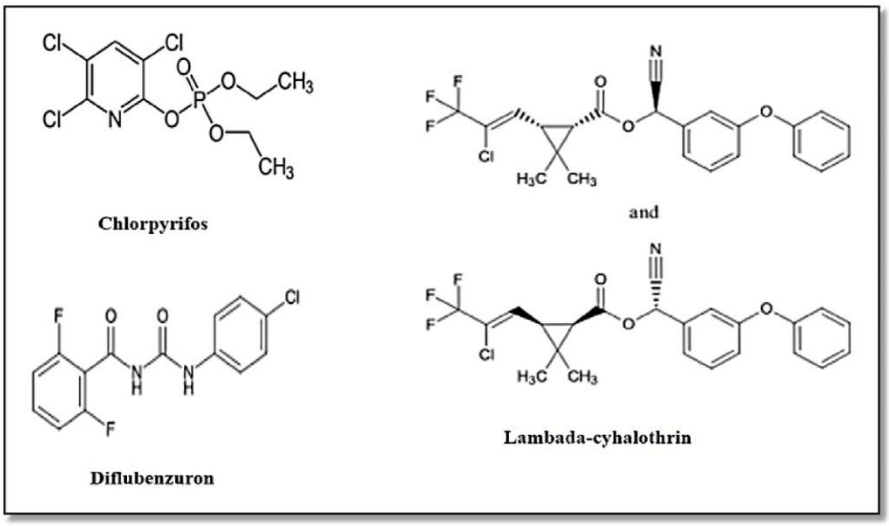


**Figure 1. Chemical structure of the tested insecticides.**

A timer was started and the mortality was recorded every 15 minutes during 2 h. for both chlorpyrifos and lambda-cyhalothrin, while in case of diflubenzuron the larvae were exposed to insecticides for 2 h and then carefully removed from contaminated water to clean water and the mortality was recorded after 72h. All treatments were tested with three replicates. The $LC_{50}$ and $LC_{90}$ values were calculated, and slope and heterogeneity analysis were also determined.

### 2.4 Resistance ratio (RR) calculation

Resistance levels of *Culex pipiens* larval field population in the two WHO and CDC method were calculated as follows:

Resistance ratio RR = $LC_{50}$ of the field population ⁄ $LC_{50}$ of Laboratory population [20].

### 2.5 Statistical analyses

Data of both WHO and CDC assays were analyzed by using SAS software [21] to calculate the median lethal concentration, $LC_{50}$ and $LC_{90}$ and chi-square ($\chi 2$). The mortality of each bioassay was corrected by that of the control using Abbott's formula [22].

## 3. Results

### 1. Toxicity of tested insecticides against laboratory and field population of Culex pipiens

Late 3rd or early 4th larval instars of both laboratory and field populations of *Culex pipiens* were treated with serial concentrations of the three insecticide, lambada-cyhalothrin, chlorpyrifos, using WHO and CDC methods. As shown in figures (2 and 3), the mortality percentage was concentration dependent, i.e., mortality increased as the concentration of the tested compound increased. Data presented in tables (1 and 2) showed that $LC_{50}$ values of chlorpyrifos and lambada-cyhalothrin were 0.00535, 0.0111, 0.000043, & 0.000013 ppm for laboratory and field populations, respectively in case of WHO method. While in CDC method $LC_{50}$ values of chlorpyrifos and lambada-cyhalothrin were 0.005, 0.0113, 0.0000049, & 0.0000015 ppm for laboratory and field populations, respectively. As well as slope, Chi ($\chi 2$) and correlation coefficient (R) values were presented in tables (1 and 2).

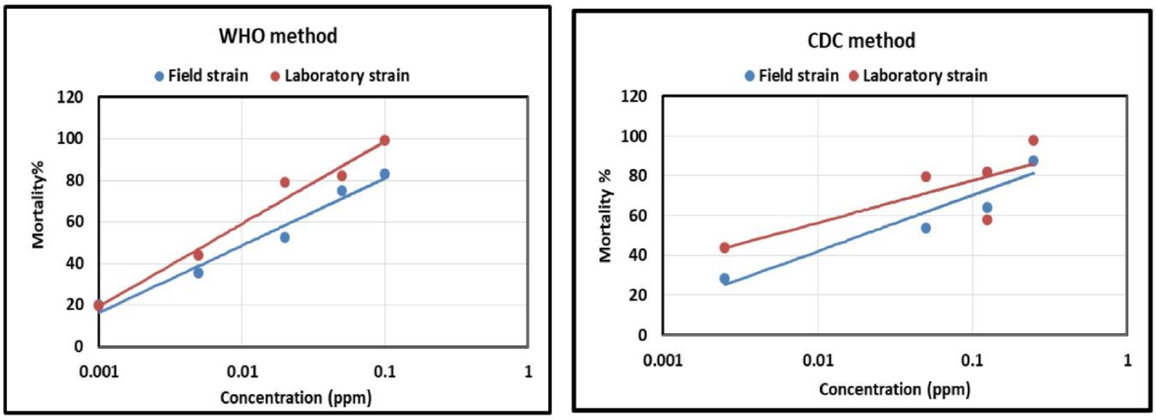


**Figure 2. Effect of chlorpyrifos on laboratory and field population *Cuex pipiens* using WHO and CDC methods**

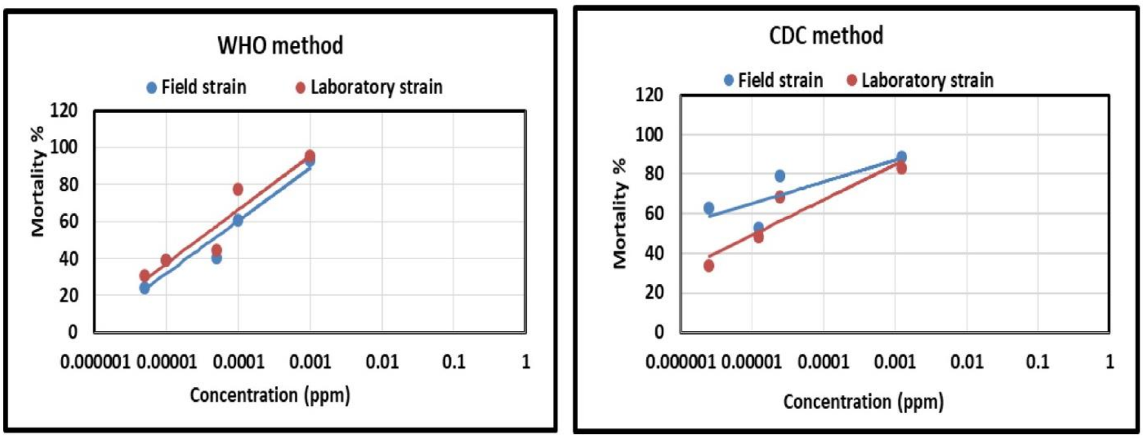


**Figure 3. Effect of lambada-cyhalothrin on laboratory and field population *Cuex pipiens* using WHO and CDC methods.**

The toxicity of diflubenzuron against 2nd instar larvae of *Culex pipens* expressed as mortality percentages at different concentrations was presented in figure 4. These results obviously showed that larval mortality after 72 h was positively correlated with different concentrations. $LC_{50}$ of diflubenzuron were 0.000089 & 0.0001 ppm of laboratory and field populations, respectively in case of WHO method. While in CDC method, $LC_{50}$ values were 0.0002 & 0.0001 ppm of laboratory and field populations, respectively. The slope value for field and laboratory populations was (0.4834±0.0467 & 0.5543±0.0671) and (0.4307±0.0466 & 0.4673±0.0426) in WHO and CDC methods, respectively. Also, Chi ($\chi 2$) and correlation coefficient(R) values were recorded in tables (1 and 2).

Data presented in (Table 1 & 2) demonstrated the comparison between treatment with different concentration of chlorpyrifos, lambada-cyhalothrin and diflubenzuron on laboratory and field populations *Culex pipiens* larvae using WHO and CDC methods. Depending on $LC_{50}$ and values lambada-cyhalothrin was the most effective insecticide, it had the lowest $LC_{50}$ and $LC_{90}$. On the other hand, chlorpyrifos insecticide was the lowest effective insecticide, with the highest value of $LC_{50}$ and $LC_{90}$.

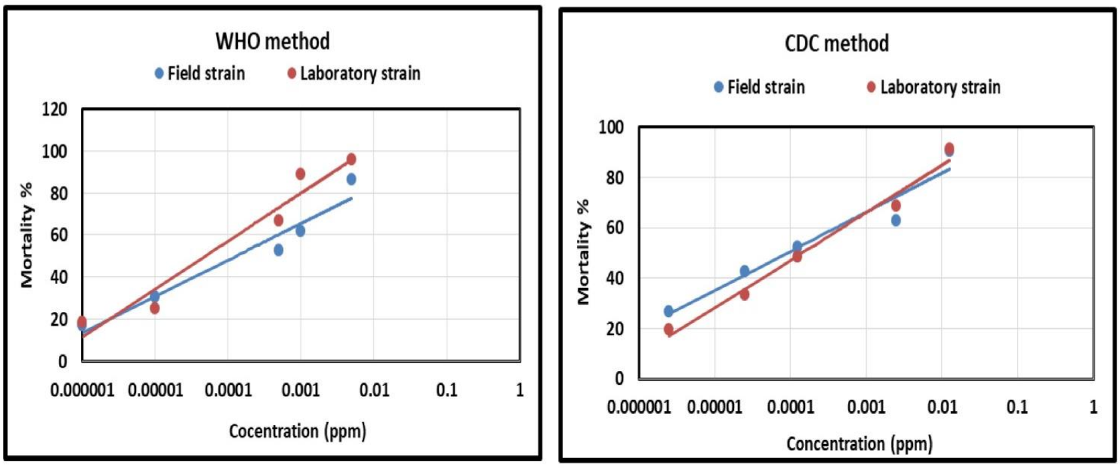


**Figure 4. Effect of diflubenzuron on laboratory and field strain *Cuex pipiens* using WHO and CDC methods.**

**2. Resistance ratio (RR) of *Culex pipiens* to the tested insecticides in WHO and CDC methods**

The results in figure 5 showed that the resistance ratio was low in all tested insecticides that ranged from 0.16 to 2.2 for both WHO and CDC. However, the two methods were compared; we demonstrated that RR was lower in CDC than WHO for both lambda-cyhalothrin and diflubenzuron. In contrast, RR was higher in CDC than WHO for chlorpyrifos. Also, results showed that chlorpyrifos recorded the highest RR value in both WHO and CDC.

## 4. Discussion

In the vector control field, the WHO insecticide susceptibility test is the most common and standard assay for assessing resistance status. In order to search for a simple, rapid and more reliable method in the assessment of insecticide resistance, CDC developed as alternative assays [23], in this study we compared the WHO tests with the CDC larval bioassay in the Egyptian *Culex pipiens* populations where insecticide resistance has been widely reported to most insecticide groups [24].

The susceptibility of *Culex pipiens* populations to three tested insecticides, were examined and the result of the investigation indicated that, lambada-cyhalothrin was the most effective insecticide followed by diflubenzuron and chlorpyrifos. The susceptibility was tested using two assays; the standard WHO and CDC one. The results indicated that there is difference between two methods in laboratory populations in all insecticides. The present results are in contrast to the studies of Aïzoun *et al*. [25] who found that the WHO and CDC bioassays gave similar results when treated adults of *Anopheles gambiae* mosquitoes with deltamethrin and bendiocarb in southern Benin.

**Table 1. Toxicity data of diflubenzuron, chlorpyrifos, and lambda-cyhalothrin insecticides against laboratory larval population of *Culex pipiens* showing comparison between WHO and CDC methods**

| Tested insecticide | WHO method | | | | | CDC method | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | $LC_{50}$ | $LC_{90}$ | Slope ±SE | Chi ($\chi 2$) | R | $LC_{50}$ | $LC_{90}$ | Slope ±SE | Chi ($\chi 2$) | R |
| **Chlorpyrifos** | 0.00535 | 0.0535 | 1.2814±0.09 | 3.35 (0.3395) | 0.994 | 0.005 | 0.1878 | 0.8230±0.09 | 9.48 (0.024) | 0.923 |
| **Lambda-cyhalothrin** | 0.000043 | 0.000018 | 0.7381±0.05 | 14.8 (0.0113) | 0.956 | 0.0000094 | 0.0003 | 0.8242±0.11 | 2.53 (0.28) | 0.980 |
| **Diflubenzuron** | 0.000089 | 0.0182 | 0.5543±0.06 | 4.1 (0.2489) | 0.973 | 0.0002 | 0.0989 | 0.4673±0.04 | 0.28 (0.96) | 0.999 |

**Table 2. Toxicity data of diflubenzuron, chlorpyrifos, and lambda-cyhalothrin insecticides against field larval population of *Culex pipiens* showing comparison between WHO and CDC methods**

| Tested insecticide | WHO method | | | | | CDC method | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | $LC_{50}$ | $LC_{90}$ | Slope ±SE | Chi ($\chi 2$) | R | $LC_{50}$ | $LC_{90}$ | Slope ±SE | Chi ($\chi 2$) | R |
| **Chlorpyrifos** | 0.0111 | 0.3484 | 0.8716±0.09 | 2.87 (0.238) | 0.24 | 0.0113 | 0.5767 | 0.7608±0.08 | 18.96 (0.0003) | 0.91 |
| **Lambda-cyhalothrin** | 0.000013 | 0.0019 | 0.7859±0.06 | 15.23 (0.004) | 0.96 | 0.0000015 | 0.00004 | 0.53±0.11 | 16.93 (0.0002) | 0.79 |
| **Diflubenzuron** | 0.0001 | 0.0668 | 0.4834±0.04 | 8.90 (0.031) | 0.96 | 0.0001 | 0.0796 | 0.4307±0.04 | 10.77 (0.013) | 0.94 |

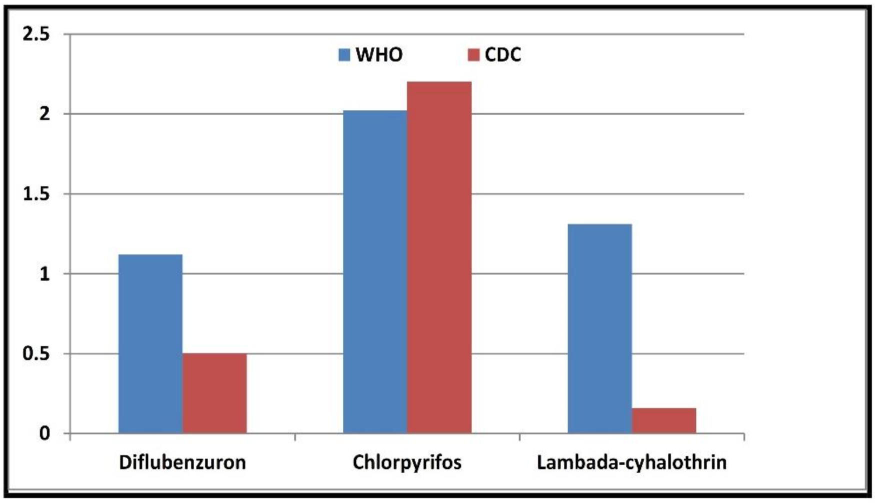


**Figure 5. Resistance ratio (RR) of *Culex pipiens* to the tested insecticides in WHO and CDC method**

Also, the present results showed that laboratory populations were more susceptible to all tested insecticides than the field ones. These results are similar to the study of Shoukat *et al*. [26] who demonstrated that the susceptibility was higher in the larvae of laboratory populations when compared to the field one. The present results indicated that lambda-cyhalothrin which was not recommended for mosquito larvae control was more effective in killing *Culex pipiens* larvae than the two other insecticides recommended for control mosquito larvae [27]. Hence, from the obtained results, we suggest that the lambda-cyhalothrin (pyrethroid insecticide) could be recommended for the control of *Culex pipiens* mosquito larvae after more studies about its persistence in field conditions.

A reverse result obtained by Rahman and Mahfuzur [28], who found that fenitrothion, organophosphrous (OP) exhibited comparatively higher toxicity about 3 and 8 times more toxic than deltamthrin (pyrethroid). They stated that, the fenitrothion and Deltamethrin could be suggested for *Culex pipiens* mosquito larvae management. Also, Ali and Andrui-de xue [29] found that all observations of tested insecticides except for malathion, were highly effective against *Ae.albopictus* larvae and were considered economically effective, while the IGRs showed exceptional activity.

Mazzarri and Georghiou [20], classified the resistant ratio into three levels: low (RR<5), moderate (5 <RR<10), high (RR>10). In the present results, the RR values ranged from 0.16 to 2.2 for both WHO and CDC assays in all treatments. These ratios were low according to previous classification. Although this RR was lower in CDC assay than WHO one for both lambda-cyhalothrin and diflubenzuron. Additionally, results showed that chlorpyrifos recorded the highest RR value in both WHO and CDC assays.

Even the CDC method is suitable for evaluating formulated insecticides in the field within a short time and a minimalnumber of mosquitos, while WHO one not recommended to formulated concentrations. Also, there are complementarities between both assays and some specificity was illustrated for each of the two methods used [30]. The present findings correspond well with Gaaboub *et al* [30] who found that Chlorpyrifos-methyl and diflubenzuron play an important role in the toxic effect of the larval and adult stages of *Culex pipiens* as well as the biochemical components, which were found positively reflected on their role in the control of the two stages. Chlorpyrifos-methyl insecticide was the best in toxicity, while diflubenzuron was the best in effect on the biochemical components. This finding is in contrast to the study of Gamal [31] who indicated that the *Culex pipiens* mosquito population from Egypt can increase resistance to malathion and lambda-cyhalothrin, were that insecticides are continuously or rotationally used to control this species.

The obtained results indicate that the CDC assay is viable alternative to the WHO one for detecting insecticide resistance in mosquito larval populations. However, it is important to note that the CDC assay is more suitable for active ingredients as well as formulated concentrations.

## 5. Conclusion

We can conclude that there was difference between WHO and CDC methods two methods against the laboratory population in all insecticide (lambda-cyhalthrin, diflubenzuron and chlorpyrifos) treatments. While there was no difference between the two methods against the field populations except the lambda-cyhalthrin treatment. The results described herein showed that lambada-cyhalothrin was the most effective insecticide followed by diflubenzuron and chlorpyrifos. Besides, both lambda-cyhalthrin, diflubenzuron recorded low RR in the two assays however, chlorpyrifos displayed the highest RR value in both WHO and CDC assays. Our findings in this research, indicated that the CDC assay is viable alternative to the WHO one for detecting insecticide resistance in mosquito larval populations. However, it is importance to note that the CDC assay is more suitable for active ingredient as well as formulated concentrations.

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Not applicable.

**Conflicts**

The author has no conflicts of interest that are concerned with this article.

**Ethics approval and consent to participate**

Not applicable.

**Consent for publication**

Not applicable.

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