



## Evaluation of some organophosphorous and carbamate pesticides against *Culex pipiens* transmitting Rift Valley fever: toxicological and enzymatic studies

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**Abstract:** The present study aimed to Study the toxic effects of some phosphorous and carbate compounds against the end of the third and the beginning of the fourth life of larval stage of *Culex pipiens* this study, carried out with pesticides, is still used to this day, raising the alarm about its use and its impact on workers in the field of control. [Sundws Sadiq Munshi Naser A. Alkenani and Khatter A. Najat **Evaluation of some organophosphorous and carbamate pesticides against *Culex pipiens* transmitting Rift Valley fever: toxicological and enzymatic studies.** *Life Sci J* 2020;17(5):37-46]. ISSN: 1097-8135 (Print) / ISSN: 2372-613X (Online). <http://www.lifesciencesite.com>. 3. doi:[10.7537/marslsj170520.03](https://doi.org/10.7537/marslsj170520.03).

**Keywords:** Organophosphorous, Carbamate, pesticides, *Culex pipiens*

### 1. Introduction

Mosquitoes, as we mentioned earlier, are insects of medicinal importance, as they are able to transmit many diseases to humans or animals, in addition to that, mosquitoes are found in all tropical and temperate regions and even to the northern side of the Arctic, (Schorb. 2013). It also has the ability to breed in different environments such as ponds, swamps, and in rock and tree holes, etc. (Redua, 2008). What also helped to increase the density of mosquitoes are those various human activities that have caused an environmental imbalance, such as the reclamation of vast agricultural lands and the use of traditional irrigation methods or the establishment of irrigation channels, the construction of dams, as well as the urban development and the subsequent existence of sanitation areas and the accumulation of industrial containers such as tires Glass, empty cabinets, etc. (Patz *et al.*, 2000; Norris 2004; Rattanarithikul *et al.* 2005a).

All these and other factors have led many studies and scientific research to study mosquitoes and other medicinal insects and find the best ways to control them. Also, these human efforts included the creation of specialized organizations such as the World Health Organization (WHO) and the Food and Agriculture Organization of the World (FAO) which some of its tasks are to provide information about these insects in order to reduce and reduce their risk (Al-Ahmad, 2016).

Despite many research and control operations that have taken place over decades, all over the world, the presence of mosquitoes remains a major global health problem, especially in those developing

countries (Becker *et al.*, 2010). In the Kingdom of Saudi Arabia, mosquitoes transmit 44% of the diseases prevalent in the world, especially dengue fever, malaria, Rift Valley fever and elephantiasis, and in spite of the efforts made to make coastal cities in the Kingdom of Saudi Arabia, including Yanbu Governorate, among the most important investment attraction centers At the global level, however, those in charge of developing these cities face many environmental problems related to the spread of disease-carrying insects, and therefore more field studies are required to determine the geographical distribution of mosquitoes of medicinal importance, their seasonal activity and their dynamic fluctuation. In order to control his clans and societies in the environment to the extent that he does not cause any harm to humans and the environme

Although there are many types of chemical insecticides that are still highly effective against disease carriers, the continued use of these pesticides has led to the emergence of the resistance characteristic of mosquitoes in addition to the occurrence of environmental pollution and thus the urgent need for the diversity of pesticide groups and improving Methods of application and making them more effective against mosquitoes and safer for the environment (WHO, 1990)

Thompson and Meisch (1977) mentioned that the manufactured pyrethroid pesticides played a crucial role in combating the third and fourth age of larvae of *Cx Pipiens* mosquitoes. quinquefasciatus as well as *Aedes vexans* meigen.

Mulla, *et al* 1980 ) Referring to The pyrethroid pesticides are highly effective against six species of

mosquitoes and their effectiveness was at the fourth age of different mosquito larvae, where the impact rate was from 1 - 50 ppb at LC90, and I found it to be effective at the mosquito excretion rate at a rate of 1 - 10ppb at LC90.

Jinfu (1999) confirmed the resistance of mosquito type *Cx Papiens* (L) pallens. In Zhejiang Province of China, Deltamethrin was found to have a rate of resistance in the excretory stage of (5.9-24.73) at LC50 (5.9-24.73) at LC50 and resistance rate at LC95 (95.8-294.5) indicating excessive use of pyrethroid in Control work

The use of chemical insecticides is one of the traditional methods in controlling mosquitoes, and it is characterized by the fact that it gives quick and positive results against mosquitoes as a troublesome pest for human and animal comfort, and it is also an effective way to face emergencies when an outbreak of any disease transmitted by mosquitoes taking into account that the use of control means Chemical against mosquitoes must be done with great care based on sufficient knowledge of the life and behavior of mosquitoes and the epidemiology of the disease, in addition to the availability of expertise and full knowledge of using chemical pesticides and their various groups and field application methods and the extent of toxicity of these pesticides to People, animals, and natural enemies of mosquitoes and their pollution of the environment and methods of field application. In general, the use of chemical pesticides in mosquito control programs in all countries is usually directed towards controlling both adult insects and immature phases (2005, WHO).

The scientist Kalyanasundaran. *et al.*, (2003) tested the efficacy of a new compound and is a toxic phosphorous organism Reldan (Chlorpyrifos-methyl) and compared its efficacy with Dursbban (Chlorpyrifos-ethyl) against different larvae of vector mosquitoes under laboratory conditions and against *Culex quinquefasciatus* in three Larval stages. The sensitivity of the various vector mosquito larvae to Reldan and Dursbban was determined using a standard procedure and LC50 values in mg / l were collected by Probit analysis. The decrease in the mosquito density ratio was calculated by the Mullas formula. The duration of the presentation showed a decrease of more than 80% in the percentage collected and estimated in the graph by a decrease in the ratio planning of the larval density against the days after treatment. The efficacy of Dursbban and Reldan against *Anophles fluviatilis* larvae under LC50 low values were from  $5.90 \times 10^{-7}$  and  $1.07 \times 10^{-9}$  mg / l respectively compared to other mosquito species. Dursbban and Reldan were equally effective against *Aedes aegypti*, *An.stphensi* and *An.culicifacies*. Reldan's effectiveness was nine times more than

Dursbban against *Cx. quinquefasciatus* in LC50 sequences  $1.17 \times 10^{-4}$  and  $1.34 \times 10^{-3}$  mg / l. Field evaluation showed these pesticides against *Cx. quinquefasciatus* that when Reldan and Dursbban are applied at a rate of 500 g (ai) / ha, the abnormal phase density decreases in more than 80% of the mean duration of 4,0, 4,5 days, 4,6, and 3.7 days in sewage and cement ponds, respectively. In drains at 500 g (ai) / ha Reldan was effective for 3,6 days, while Dursbban was ineffective. Efficacy was only 4.0 days at 1000 g (ai) / ha. There was no significant increase in efficacy survival for Dursbban and Reldan as application rates increased.

Micheal, *et al.* (2007) explained. In a study conducted in Doha and Al Rayyan, the effect of Chlorpyrifos, propetamphos (organophorous), cyphenothrin, cyfluthrin (Synthetic) and etofenprox on *Cx pipiens* larvae was demonstrated. Where it was found that the Rayyan area is more effective for the mentioned pesticides in the Doha region, due to the rationalization in the use of pesticides

## 2. Material and methods

In the present study a field strain of *Cx Papiens* mosquitoes was used. were collected in the form of larvae of different ages from Taif Governorate, which is located in the southeast of the Makkah Al-Mukarramah region, at the top of the Sarawat Mountains, at an altitude of between 1700 - 2500 meters above the sea level and located between two longitudes (40,1341,10) and two latitudes ( 52,20,4022). This province was chosen for its high population density and high *Cx.Papiens* growth intensity. The larvae were transported with their reproductive water in small plastic boxes with a tight cover to the Research Unit of Dengue Fever & Vector Control, Department of Life Sciences - Faculty of Science - King Abdulaziz University in Jeddah. It is equipped with a mosquito rearing room at  $27 \pm 1$  ° C,  $70 \pm 5$  % RH, 14-hour lighting periods and 10-hour darkness

### A - Breeding of mosquito larvae

The field mosquito larvae were transferred by a plastic pipette to a rectangular Pottery bowls porcelain dish of 40 cm long, 20 cm wide and 6 cm deep, white in color for easy viewing of mosquito larvae with a quantity of tap water .It was taken into consideration, as far as possible, when transferring field larvae to the breeding dishes, to be homogeneous in their ages and free from impurities related to reproductive water. The larvae were fed on a mixture consisting of equal proportions of powder to ornamental fish scales

(Gold Fish, Flake Food, USA) and dry baking powder

### B- Breeding of mosquito adults

Virgins are hatched in cages for complete insect breeding. Cubic Cages with Equal Metal Frames (30 x

30 x 30 cm). Covering all sides of the cage with a white cloth except for the back side, it is a transparent white muslin cloth to easily see and follow the mosquitoes inside, as the front face is equipped with a cloth sleeve of about 20 cm in length and has an opening of about 15 cm in diameter to allow the insertion of the cups of virgins and diabetic solution

Whole insects of male and female mosquitoes are fed on a 10% sugar solution to maintain the vitality and activity of whole insects, where a small piece of cotton is absorbed into the sugar solution and placed in a small plastic cover from the caps of mineral water bottles and inserted into the breeding cage

### C - Egg production

To obtain the eggs of the first generation, the female mosquitoes of the previous strain were fed after 4-5 days after hatching the A blood meal from a living pigeon. The bird is equipped with removing feathers from the chest area with the legs and wings tied with a rope of linen. Then the bird is placed for an hour on the top surface of the breeding cage so that the bird's chest comes into contact with the cloth so that the mosquitoes can feed and obtain a blood meal. A small plastic cup with water in the breeding cage is placed inside the breeding cage. Oviposition on water-filled filter papers begins about four days after feeding to the blood. Eggs containing filter papers are collected and placed in larval rearing dishes containing Tap water. Eggs are hatched after 1-2 days to larvae that complete the life

### Level of sensitivity of mosquito larvae to the tested compounds

#### Determination of the susceptibility level of mosquito larvae to the tested compounds

#### Compounds used in the biological evaluation

##### Organic phosphorous compounds

1- Actikil 500

Active ingredient: Premimos methyl 50% Pirimephos methyl and registered in the Kingdom of Saudi Arabia No) .891-29-100(

2- Safrotin 20 MC

Active material: Propetamphos MC 20% Propetamphos 20 MC Form registered in the Kingdom of Saudi Arabia with number ( 1048-12-154) Carbate compound

3-Metho 900

Active material: Methomyl 90% form (3-13) and registered in the Kingdom of Saudi Arabia No(2778-10204-248)

### Prepare the standard solution

For the pesticide in the liquid form (Actikil 500 and Safrotin 20 MC), 1 ml of both compounds to be evaluated was taken and each compound was placed in 100 ml water in a standard 100 ml beaker separately and moved to homogeneity, while for the pesticide in the form of fine powder (Metho 900) 1 g of powder

was weighed to be evaluated and placed on 100 ml of water in a 100 ml standard beaker and stirred until the powder completely dissolved

In this study, the effects of these traditional phosphorescent organic pesticides Actikil 500, Safrotin 20 MC and Carbamate Metho 900 were evaluated on the larval nervous system of the genus *Culex pipiens* in different concentrations are 0.1, 0.08, 0.05, 0.03 and 0.01 respectively for Actikil 500 and Safrotin 20 MC. The carbicide Metho 900 is 0.1, 0.2, 0.3, 0.4, 0.5. According to the method used by the World Health Organization (WHO 2005)

### Larvae experiments

To conduct larval sensitivity test experiments against selected pesticides through lethal concentrations Lc50,90 treated larvae in the case of pesticides with a rapid effect of organophosphorous and carbamate pesticides and their role in inhibiting the cholinesterase enzyme.

The experiments were carried out in white plastic plates with a diameter of 11 cm, depth 4 cm, and a capacity of 250 ml. They were filled with 100 ml of water from the tap for all concentrations and all sensitivity tests were done using five replicates / concentration so that in each dish there were 20 larvae from the end of life The third and the beginning of the fourth age, known as repeaters, in addition to the control group. They were provided with food during the test. After 24 hours of the test, Dead Larvae are calculated. They are the ones that cannot move when touched with the dissection needle in the siphon or head area. Likewise, the account of the dying Larvae that are not dying Aa rise to the water surface or dive into the water when the water vibration can be used as an equation Abbott (Abbott, 1925) to modify the proportion of death in the case of the death rate of more than 5% and less than 20% of the control group. The dead phases of the treated larvae were examined to identify distortions that appear in their apparent form and become anatomical microscope with a digital camera and connected to a computer

### Statistical analysis

To analyze the laboratory toxicity of compounds used in biochemical evaluation tests of pesticides against *Cx.pipiens* mosquito larvae. Ldp-line program was used to extract other statistical constants such as Lc50 and Lc90 and Slope value as well as Chi square according to Finny method (1952)

### 3. Results and Discussion

Evaluation of the effectiveness of some organophosphorous pesticides against *Cx. pipiens* larvae

In this study, laboratory toxicity was evaluated for two types of organophosphorous pesticides, Actikil

500 and Safrotin 20 MC, and the etiological effect of these two pesticides was estimated after 24 hours after the treatment of the 3rd instar larvae of *Cx. pipiens* mosquitoes as an effector of evaluation by calculating the values of concentrations. Killer of 50% and 90% which is what is known as LC50, LC90 of larvae where these compounds are usually toxic and fast effect and have no cumulative effect

By analyzing the toxicological results of these compounds, it was found that there was a direct proportion between the tested concentrations and the mortality percentages of the treated larvae with these concentrations. The results also showed that the effective concentrations of the Actikil 500 were (0.001, 0.003, 0.005, 0.008, 0.01) ppm ppm. Straight. Death rates for treated larvae ranged from 10-97% from the lowest concentration to the highest concentration in Table (1).

Whereas, the effective concentrations of Safrotin phosphorous were 20 MC (0.01, 0.03, 0.05, 0.08, 0.1) ppm ppm, respectively. The percentages of larval death corresponding to these concentrations ranged from 30% to 0.01 concentration and 98% to the highest concentration 0.1 ppm Table (2)

On the other hand, the laboratory toxicological lines of the tested compounds and the statistical constants derived from them showed the difference of the lethal concentrations values for the half of the number of tested larvae 50% and also for 90%, which is known as LC50, LC90 and treatment with the Actikil 500 pesticide (0.0028, 0.007 ppm). Respectively. The confidence intervals ranged from the minimum to the upper limit corresponding to these concentrations from (0.0025, 0.0031 ppm and 0.0062, 0.0081) ppm at a 95% confidence level. Table (3)

Table (1) Relationship between effective concentrations and percentages of *Cx. pipiens* mosquito death 24 hours after treatment with Actikil 500 phosphorous

Con.	Con. * 1000	Log (Con. * 1000)	Treated	Observed response %	Linear response %	Linear probit
0.001	1	0	100	10	7.91994	3.5892
0.003	3	0.4771	100	50	54.4051	5.1107
0.005	5	0.699	100	77.5	79.3369	5.8183
0.008	8	0.9031	100	95	92.9053	6.4691
0.01	10	1	100	97	96.2106	6.7781

\*Five repeats per concentration, 20 larvae per duplicate

Table (2) Relation between Effective Concentrations and Percentage of *Cx. pipiens* Mosquito Larvae Death 24 hours after treatment with Safrotin 20 MC phosphorous

Con.	Con. * 100	Log (Con. * 100)	Treated	Observed response %	Linear response %	Linear probit
0.01	1	0	100	23	18.88	4.1176
0.03	3	0.4771	100	49	55.2604	5.1323
0.05	5	0.699	100	69	72.714	5.6042
0.08	8	0.9031	100	84	85.0432	6.0383
0.1	10	1	100	95	89.3246	6.2444

Five repeats per concentration, 20 larvae per duplicate

Table (3) Lc50 and Lc 90 Values, Slope Values for Actikil 500 Toxicity Line and Maximum and Minimum Values for Each

Slope	Chi	LC <sub>50</sub> (ppm)	LC <sub>90</sub> (ppm)
3.1889	+/- 0.2383	0.0028	0.007
Lower limit ppm		0.0025	0.0062
Upper limit ppm		0.0031	0.0081

While the obtained results recorded different values for the 50% lethal concentrations, 90% of the *Cx. pipiens* larvae treated with Safrotin 20 MC reached about 0.0425 ppm / gm and 0.1089 ppm / gm and the confidence intervals of the minimum and the

highest up to corresponding to these concentrations of ( 0.0382, 0.0468, 0.0952, 0.01292 ppm / gm Table (4)

The results of the statistical analysis were the high sensitivity of the individuals tested against the phosphorous pesticide 500 Actikil compared to the phosphorous pesticide Safrotin 20 MC The

effectiveness of the pesticide 500 Actikil was more effective than the pesticide Safrotin 20 MC by about 9.286 times and this was confirmed by the values of the relative resistance indicators (RR) Resistance Ratio and also the tendency values Toxic lines Slope Table (5) and Figure (1) and Figure(2).

These indicate that the increase in the toxicity line's tendency towards the y-axis (vertical) indicates evidence of the effectiveness of the compound and the sensitivity of the tested strain. The tabular values of the Chi square indicate that the individuals tested with the Actikic pesticide are more homogeneous than the individuals tested with the phosphorous pesticide, where the lower the calculated values of the Kai square From the tabular at 0.05 and degrees of freedom 3 (n -2), where this indicates the homogeneity of the individuals tested, Figure (3), where it shows the tendency of toxicity lines for the relationship between the two pesticides used.

In general it can be said that the different level of sensitivity of the tested larvae to the selected pesticides may be due to the difference in the type of

active substances included in the formation of each pesticide where the active substance included in the pesticide 500Actikil is Pirimiphos Methyl at a concentration of 50% while the active substance in the pesticide Safrotin 20 MC is Propetamphos and concentrated 20%, and this is one of the reasons for the superiority of the pesticide 500Actikil to the pesticide Safrotin 20 MC, as well as the difference in the intensity of the pesticide use and the application times, especially field application.

It is worth noting that these results obtained from this study are consistent with many previous studies and studies in this field. Mahyoub *et al* (2016) attributed the reason for the difference in the sensitivity of *Cx.pipiens* to Diazinon, Cylfuthrin and Propoxur to the different active substances involved. In the composition of each compound, as well as the difference in the tested concentrations, as this study coincided with what was indicated by Al Thabiani, *et al.* (2016) were of significant differences between *Cx.pipiens* larvae death rates and concentration used from the tested pesticides

Table (4) Lc50 and Lc 90 Values, Slope Values of Safrotin 20MC, and Maximum and Minimum Values for Both

Slope	Chi	LC <sub>50</sub> (ppm)	LC <sub>90</sub> (ppm)
2.1268 +/- 0.1883	6.860 Tabulated 7.8	0.26	0.1041
Lower limit ppm		0.22	0.0857
Upper limit ppm		0.03	0.1345

Table (5) shows the relationship between Actikil 500 and Safrotin 20MC as well as RR resistance

Line name	LC50	Lower limit	Upper limit	Index	RR	Slope
Actikil	0.0028	0.0025	0.0031	100	1	3.189
Safrotin	0.026	0.022	0.03	10.769	9.286	2.127

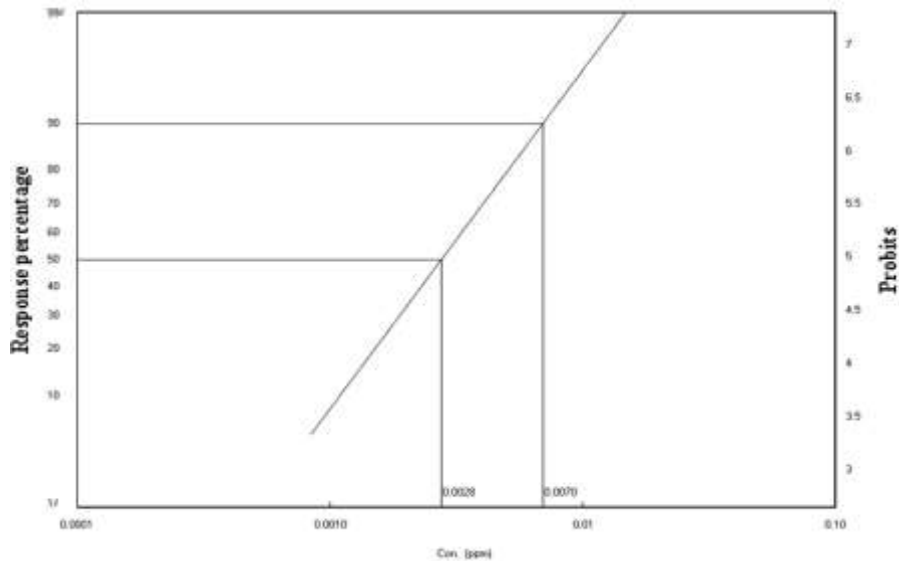
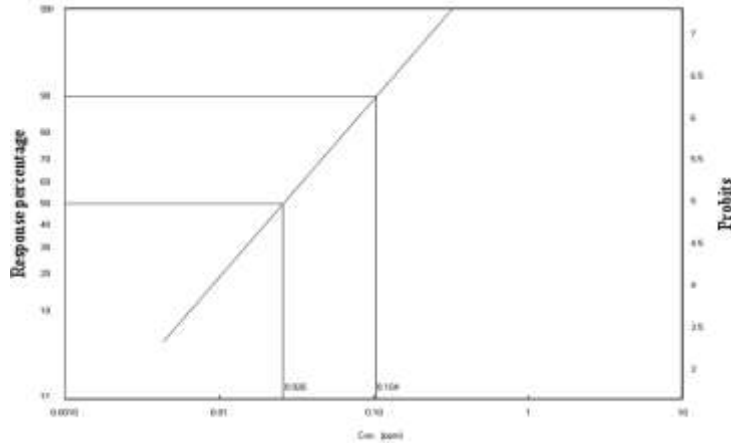
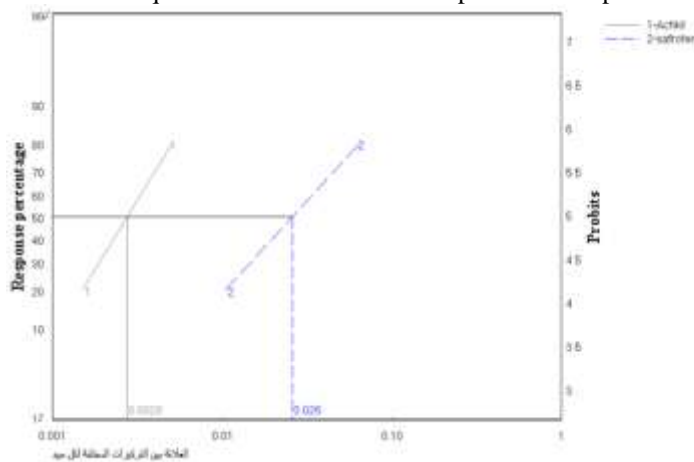


Figure (1) the relationship between Actikil 500 pesticide concentrations and the percentage of *Cx.pipiens* mosquito death. 24 hours after exposure to the pesticide



Figure(2) the relationship between the concentrations of the Safrotin 20 MC and the percentage of *Cx.pipiens* mosquito death. 24 hours after exposure to the pesticide



Figure( 3) Toxicological lines show the relationship between the different concentrations of Actikil 500 and Safrotin 20 MC and the percentage of death of *Cx.pipiens* larvae. And that after 24 hours exposure

The study was also confirmed by Alghamdi, T. (2018) This is in that the concentration of the active substance in the composition of the real pesticide influencing the sensitivity of the tested materials

### Second: To evaluate the efficacy of the methocarbonate 900 methocide against *Cx.pipiens* larvae

#### Evaluation of the Carbamate Metho 900 pesticide against *Cx.pipiens*

Laboratory sensitivity of *Cx.pipiens* mosquito larvae was measured for commercial carbohydrate Metho 900 and the active substance is 90% Methomyl, 24 hours after treatment with this pesticide at concentrations (0.1, 0.2, 0.3, 0.4, 0.5). The results showed that there is a direct correlation between the increase in the concentration of the active substance in the composition of the pesticide and the mortality ratios of larvae Table (6). The laboratory toxicological lines of the tested pesticide and the statistical parameters derived from it showed that the LC50 is

0.3797 as well, LC90 at the concentration of 0.5185 PPM.

The confidence intervals ranged from the lower to the upper limit corresponding to these concentrations, the LC50 was 0.1513 - 0.1944 ppm, and the LC90 was 0.4435 - 0.6417 ppm at the 95% confidence level.

These results indicated that the toxicity line is away from the y-axis and this indicates the lack of effectiveness of the compound (carbamate) as the tabular chi (chi) square appeared at the level of 0.05 and degrees of freedom (n-2) 3 that the tested individuals are homogeneous from among the members of the group tested with the pesticide Metho 900 Table (7). It is noted that the results of the statistical analysis increased the sensitivity of the individuals tested against the carbamate Metho 900 compared to the organic phosphorous Pesticide Safrotin 20 MC and Actikil 500 as well as the slope values of the toxic lines Slope through which it is observed that Metho 900 pesticide is significantly

away from the y-axis indicating the establishment of larvae The high mosquitoes of this pesticide form

Figure( 4)

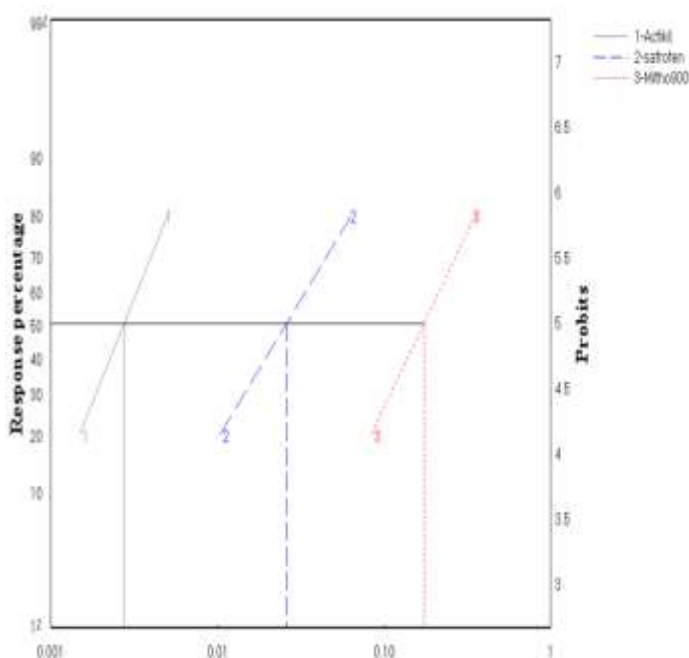
Table (6) the sensitivity level of *Cx pipiens* mosquito larvae. For Metho 900

Con.	Log (Con. * 10)	Treated	Observed response %	Linear response %	Linear probit
0.1	0	100	28.57	25.8923	4.3533
0.2	0.301	100	55.102	56.5681	5.1654
0.3	0.4771	100	68.367	73.9095	5.6405
0.4	0.6021	100	82.653	83.5853	5.9778
0.5	0.699	100	93.878	89.2308	6.2392

\*Five repeats per concentration, 20 larvae per duplicate

Table (7) Lc50 and Lc 90 Values and Slope Values for Metho 900 Pesticide Line and Maximum and Minimum Values for Each

Slope	Chi	LC <sub>50</sub> (ppm)	LC <sub>90</sub> (ppm)
7.698 +/- 0.2616	4.3804	Tabulated 7.8	0.1737
Lower limit ppm		0.1513	0.4435
Upper limit ppm		0.1945	0.6417



Figure(4) Toxicological lines show the relationship between the different concentrations of Actikil 500, Safrotin 20 MC and Metho 900 and the percentage of death of *Cx.pipiens* larvae. And that after 24 hours exposure

The results also indicate that the larvae of *Cx.pipiens* treated with the three pesticides used led to morphological deformation of the larvae, as phases appeared in the tested larvae, such as the intermediate stages between the larvae and the virgins or between the virgins and adults, as well as deformations in some larvae in the chest or abdomen or Neck prolongation, as in some cases, constriction occurred in the body's

rings, and their coloration between the dark and light of the pigmentation larvae. As for some adults, there were deformations in the wings and legs, which prevented them from completing their noha and flying, and some of them adhered to the feet with the peeling ice from the pupal shape . Figure (5),(6)and (7).

These results were in agreement with previous studies, where (Haoues *et al*, 2008) and studies

conducted by (Oakshott, *et al* 2005), as well as with Paul (A, *et al* 2006), which showed significant

differences between death rates in treated larvae and the concentrations used were found.



Figure (5) Albino larvae as well as neck elongation



Figure (6):Moderate phases appear between larvae and virgins as well as albino virgins





Figure (7):Moderate phases appear between virgins and adults, as well as adult insects that have lost the ability to fly due to deformations of the wings or leg adhesion to the skins' skin.

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4/22/2020