Effect of Insecticide Dichlorvos on Ovaries of Mosquito Fish *Gambusia affinis* (Baird and Girard)

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Received 18, September, 2010
Accepted 3, January, 2011

Abstract:

The toxicity of insecticide dichlorvos (90%) was tested in ovaries of mosquito fish using three sublethal concentrations (0.3, 0.6, 0.8 µg/L) and studied their effects on the means of body and ovary weights, gonadosomatic index, ovum size and ova number.

Results showed that there was a significant ($P<0.05$) decrease in body and ovary weights, ovum size and ova count. However, there was no significant ($P<0.05$) difference in gonadosomatic index in treated groups with the three concentrations of dichlorvos compared with the control group.

This study was added evidence on the ovular toxicity of dichlorvos concerning its effect on fish production as well as reduction in the means of productive offspring and their survival.

Key words: Dichlorvos, mosquito fish, ovary, ovum size, ova number.

Introduction:

Pesticides are a heterogeneous group of substances used for preventing, destroying or repelling the pests. However, their massive and indiscriminate use in crop protection, food preservation, and pests' control has led to acute or chronic poisoning incidents in humans, domestic animals and wild life, and resulted in widespread ecological adverse effects [1]. In addition, pesticides and their residues are one of the important pollutants that reached the aquatic environment and caused acute and chronic toxicity to fish [2].

One of the most abundant organophosphorous insecticides is dimethyl-2,2-dichlorovinyl phosphate (DDVP). It is used in the pests' control that infected domestic animals, stored grains and in treatment parasite-infected fish. It is also used in the control of domestic pests such as flies, fleas, gnats, mosquito and moth [3]. This insecticide produces toxicity by inhibition the acetylcholinesterase (AchE) enzyme activity in the nervous system and acts as neurotoxin by blocking synaptic transmission in cholinergic neurons [2, 4, 5].

As the lack of studies concerning the sub lethal doses of dichlorovos on ovary of mosquito fish (*Gambusia affinis*) in spite of its highly toxicity, there is a necessity to perform a laboratory study for the toxicity effect of this pesticide on some aspects of mosquito fish ovaries. This study aimed to define the ovular toxicity of dichlorvos on ovary weight, gonadosomatic index as well as ova number and size in mosquito fish. The reason beyond mosquito fish selection in this study is that this fish is wide tolerance to temperature and salinity as well as easy to reproduce in the laboratory [6].

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Materials and Methods:

Fish collection and breeding

Fish were collected from Al-Jaderiyah Canals/ University of Baghdad. Three aquaria were prepared with dimensions 30 X 30 X 60 cm^3, filled with ten liters of dechlorinated water and supplied with artificial airing using air pumping. Fish were bred for 50 days in which continuous births were undergone and the new offsprings were used. Fish were fed with commercial dried food twice daily with average 1 gram per aquarium [6].

Concerning the laboratory conditions, the pH of water was 7.75 ± 0.45, dissolved oxygen was 7.95 ± 0.92 mg/l and the temperature average was about 21 ± 1.53 °C.

Toxicity tests

Dichlorvos, dimethyl-2,2-dichlorovinyl phosphate (DDVP) (90%) from Al-Tariq Company (previously)/ Republic of Iraq was used. The insecticide prepared as stock solution at concentration 1 mg/L and it prepared by dissolving 0.1 mg of dichlorvos (90%) in 100 ml of sunflower oil [3, 7]. Other concentrations were prepared by diluting the concentrated solution, calculated according to the active material and expressed as part per billion (ppb) or µg/L [6]. To determine the lethal concentration for 50% of fish (LC50), 54 fish with weight 0.33 gm were placed in glass beakers. A series of concentrations (control, 1, 3, 4, 6 and 8 µg/L) with three replicates for each concentration and the control were selected. The mortality of fish in each concentration was calculated according to the Abbott' formula:

$$\text{Mortality} = \frac{\text{Treatment mortality - Control mortality}}{\text{100 - Control mortality}} \times 100$$

It appeared that the value of LC50 was 2 µg/L after 48 hour (Figure 1).

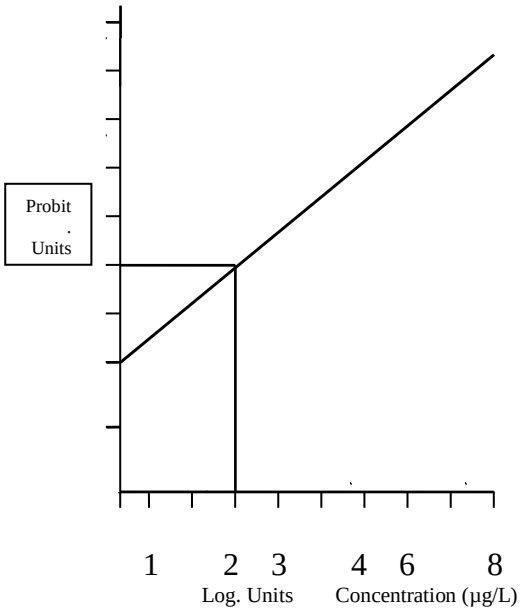

Fig. (1): Toxicity line to determine LC50 of dichlorvos in mosquito fish by probability- logarithmic method.

Then, 36 fish with weight 0.31 gm were placed in 12 glass beakers with three fish in each. The sublethal concentrations (0.3, 0.6 and 0.8 µg/L) were selected according to the value of LC50. The fish were placed in glass beakers as far as three fish in each with three replicates in each concentration as well as the control group. In each beaker, the fish were fed with 0.3 gram daily along the period of the test. The total volume of each concentration was 250 ml and the prepared concentrations were replaced each 48 hours for 28 days [6, 7].

At the end of chronic exposure, the treated and control fish were weighted, dissected from the ventral side, their ovaries were removed and weighted. Then, the gonadosomatic index (GSI) was calculated by the following formula [2]:

$$GSI = \frac{\text{Gonad (Ovary) weight}}{\text{Body weight}} \times 100$$

Furthermore, formalin solution (5%) was used in order to conserve the ovaries and count the ova for all groups. Then, the ovum diameter for a

sample composed of 20 ova in each fish was measured using eye micrometer. The ovum size was calculated according to the following formula [7]:

$V = 4/3\ \pi\ r^3$

Where as V = ovum size, π = constant ratio (3.1416), r = ovum radius.

Statistical analysis

The results were analyzed statistically using analysis of variance (ANOVA) applicable to a completely randomized design. Then, the significance among means was tested depending on Duncan Multiple Range Test using SPSS program [8].

Results and Discussion:

Table (1) illustrates the effect of dichlorvos pesticide on the weight of body and ovary as well as gonadosomatic index in female mosquito fish. The results showed that there was a significant ($P<0.05$) decrease in the body and ovary weights in the fish treated with sublethal concentrations of dichlorvos compared with control animals. The body weight means were 251, 234, 196 and 346 mg, for concentration 0.3, 0.6, 0.8 and control treatment respectively. While the ovary weight means were 4.62, 4.13, 3.35 and 6.39 mg, respectively in the three treated groups and the control animals. In addition, the results demonstrated that there was no significant ($P<0.05$) difference in gonadosomatic index in these treated groups with dichlorvos compared with control group. In these treated groups and the control group, the GSI means were 1.85, 1.79, 1.72 and 1.86, respectively

Table (1): Effect of dichlorvos on the means of body and ovary weights and gonadosomatic index in mosquito fish.

Treated groups Dichlorvos concentration µg/L	Body weight (mg)	Ovary weight (mg)	Gonadosomatic index (GSI)
0.3	b 251 ± 9.71	b 4.62 ± 0.14	a 1.85 ± 0.05
0.6	b 234 ± 8.97	c 4.13 ± 0.08	a 1.79 ± 0.07
0.8	c 196 ± 6.86	d 3.35 ± 0.07	a 1.72 ± 0.06
Control	a 346 ± 11.66	a 6.39 ± 0.12	a 1.86 ± 0.07

Values are means ± SE.
Similar letters indicate no significant differences and different letters indicate significant differences at $P< 0.05$.

These results in agreement with the result of [9] in that the animals lose weight and decreased appetite after continuous exposure to low doses of organophosphates pesticide.

The lose in body weight in treated fish with dichlorvos may be explained as a decrease in consumed food and inhibition of acetylcholinesterase (AchE) enzyme in the nervous system that reached more than 70% [4]. It has found that the sublethal exposure of the perch to the organophosphorus insecticide DDVP resulted in the decreased ability of fish to consume oxygen at low concentration in water and decrease in the brain acetylcholinesterase activity as well [10].

Concerning the decrease in ovary weight in the treated groups with dichlorvos, it may be explained as a decrease in protein content. The depletion of tissue protein was due to diversification of energy to meet the impending energy demand under toxic stress and altered enzyme activities [11].

Regarding the number of ova and ovum size, the results in Table (2) indicated that there was a significant

(P<0.05) decrease in the means of ova number and ovum size in the three treated groups with sub lethal concentrations of dichlorvos compared with control animals. The mean number of ova was 43.50, 40.60, 35.70 and 54 ovum/ovary, respectively in the treated groups with the three concentrations of dichlorvos and the control group. Concerning the ovum size means, they were 0.30, 0.21, 0.16 and 0.41 mm^3, respectively in the three treated groups and the control group.

Table (2): Effect of dichlorvos on the means of ova number and size in mosquito fish.

Treated groups Dichlorvos concentration µg/L	No. of ova (ovum/ovary)	Ovum size (mm^3)
0.3	b 43.50 ± 0.76	b 0.30 ± 0.01
0.6	c 40.60 ± 0.94	c 0.21 ± 0.008
0.8	d 35.70 ± 0.70	c 0.16 ± 0.01
Control	a 54.00 ± 0.87	a 0.41 ± 0.03

Values are means ± SE.
Similar letters indicate no significant differences and different letters indicate significant differences at P< 0.05.

The present result is corresponded with the result of [9] in that little concentrations of organophosphorous pesticide produce a decrease in gametes production as well as produce reproductive problems in animals. Different pesticides, including organophosphates (OPs), have reported to induce oxidative stress due to generation of free radicals and alteration in antioxidant defense mechanisms [5]. Stress has deleterious effects on gonads, produce bad gametes, reduce ovum size in female, reduce the ability of reproduction and reduce the survival of offspring [12].

As known, the process of oocytes growth occurrs through vitellogenesis. The yolk granules and proteins are synthesized in the liver and secreted into blood, then transported into ovary and integrated with oocytes. The ability of hepatocytes to synthesize vitellogenin is stimulated by estrogens. Therefore, the reduction of estrogens stimulation to hepatocytes as insecticide action might be led to vitellogenesis inhibition in fish [2]. It is revealed that oocytes and ovarian follicles at their different stages of maturation get affected differently at various concentrations of organophosphorous insecticides. This may be contributed to ovarian compositional and structural changes [7]. Furthermore, stressed oocytes frequently contain less yolky granules, ruptured oocyte walls, damaged yolky vesicles, nucleolar and cytoplasmic changes [13]. In addition, the organophosphorous pesticides reduce the level of 3 –hydroxysteroid dehydrogenase; an essential enzyme in steroid synthesis [2]. Thus, the reduction in the synthesis of the sex hormones especially estrogens is produced and may affected on ovary weight and ovum size [2, 10, 13].

This study added evidence on the ability of insecticide dichlorvos in inhibition of ovary growth and reduction of ova size and numbers in treated fish. It is obvious that these results has returned on economic fish production with regard to reduction in productive offspring and their survival. Therefore, it is necessary to perform a general survey to the aquatic environment to control the used pesticides in order to increase in fish production economically.

Concerning the high toxicity of this pesticide especially to reproductive organs of fish [2] and the fact that high temperature is made it highly toxic about 5 – 150 time [3], therefore, it is advised not to spray it directly on aquatic environments especially in Summer season.

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تأثير مبيد الداي كلوفوس في مبايض أسماك البعوض *Gambusia affinis* (Baird and Girard)

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الخلاصة:

اختبرت سمية المبيد الحشري الداي كلورفوس (90%) في مبايض أسماك البعوض وذلك من خلال استخدام ثلاث تراكيز تحت مميتة منه وهي 0.3 و 0.6 و 0.8 مايكروغرام/ لتر ودراسة تأثيراتها في معدلات أوزان الجسم والمبيض والدليل الجسدي المنسلي وحجم البيضة وتعداد البيوض.

أظهرت النتائج بأن هناك انخفاضا معنويا ($P<0.05$) في معدلات وزن الجسم والمبيض وحجم البيضة وتعداد البيوض فيما لم يظهر هناك فرقا معنويا في معدل الدليل الجسدي المنسلي للمجاميع المعاملة بالتراكيز الثلاثة من مبيد الداي كلورفوس مقارنة بمجموعة السيطرة.

لقد أضافت هذه الدراسة دليلا على السمية المبيضية لمبيد الداي كلورفوس من ناحية تأثيره على إنتاج الأسماك واختزال في معدلات الصغار المنتجة ومعيشتها.