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ACUTE AND CHRONIC TOXIC EFFECTS OF DIAZINON PESTICIDE EXPOSURE ON GILLS TISSUES OF *LIZA ABU* (HECKEL, 1843)

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ABSTRACT

Liza abu fish were exposed to different concentrations of the Diazinon pesticide (Organophosphate pesticide) (diethyl 2-isopropyl -6- methyl -4- pyrimidinyl phosphoro -thionate) in the acute exposure (96) hour and chronic exposure (14) days ,

Histopathological examination showed the hyperplasia of the gill tissue, some engorgement with blood of the secondary lamellae, epithelial separation , necrosis , clubbing of epithelial cells and fusion of adjacent secondary lamellae. These changes different according to the pesticide concentration and it's exposure time.

INTRODUCTION

In recent years, a large number of pesticides have been produced and discharged into the environment. Despite of it's useful in agriculture and forestry, but they create problems for aquatic life. Pesticide reach the aquatic systems by direct application spray drift, aerial spraying, washing from the atmosphere by precipitation erosion and run-off from agricultural land , by discharge of effluents from factories, and in sewage (NAPIAP,1993). If they are not judiciously used they will pose threat to aquatic life. Many pesticides reduce survival, growth and reproduction of fish (Mckim *etal.*,1975).Therefore, it is important to examine the toxic effects of pesticide on fish, which form an important part of human food.

The respiratory system provides the most extensive interface of a fish with the aquatic environment. Because of this it is frequently the first system affected by pollutants , and when death occurs as a result of acute exposure, it is often due to failure of respiratory homeostasis (Sharma *etal.*,2001) .When fish exposure to Pesticides many of histopathological changes observed in different organs, but the gills were the most organs damaged(Ortiz *etal*,2003). Histopathological changes of various pesticide by fish's gills have been reported by several investigators (Kumaraguru *etal.*,1982 ; Richmonds and Dutta,1989; Nowak,1992; Bhatnagar *etal*, 1992; Pandey *etal.*, 1993 ; Sharma *etal.*,2001). These changes include hyperplasia, epithelial separation, necrosis, clubbing of epithelial cells or hyperplasia and fusion of adjacent secondary lamellae. But there are still many pesticides that should be studied. Further the same changes on the other species of fish are necessary to exactly evaluate these pesticides effects.

The main of present study were determine of toxic effect of Diazinon pesticide on *L. abu* fish's gills because these fishes are a good bioindicator and very sensitive to pollutants (Balasem *etal.*,1999) and pesticides (Abd Al-Rezzaq, 2002).

MATERIALS AND METHODS

Liza abu , body length 9-11 cm, weight 11.5-8.5 g, obtained from fish farm of Marine Science Center, University of Basrah. Fish were transferred to fiber-glass tanks containing 350 L of well aerated, dechlorinated tap water at 20 °C (± 2), mean pH 7.85 (± 0.3) ,and dissolved oxygen 8.1 (±0.4), the water was changed every two day to

remove accumulated fecal materials. They were then left one week to acclimate to their new surrounding. Feeding was stopped prior 24 h and during the experimentation.

In the acute exposure (96)h, fish were exposed to sublethal concentrations of Diazinon pesticide that kill (% 10, %25, %50) of fish (LC10=0.48, LC25=0.7, LC50=1.08) ppm respectively (this concentrations taken from precedent study (Abd Al-Rezzaq, 2002), while fish exposed to (LC10=0.48, LC25=0.7) ppm in the chronic exposure (14)day . Plastic aquariums (30) L use to this purpose, each one containing 10 fish, four replicate for each concentration comprised control treatment.

Fish were killed and gills from the control and experimental fish were isolated. The gills were cleaned in physiological saline and gill arches excised and fixed by use the Bouin's fixative for 24h. Gill tissues were dehydrated in ethanol, cleared in xylene, and embedded in paraffin wax. Section were cut at (6-7 μ) and stained with hematoxylin and eosin (Humason, 1972 and Baker and Silverton, 1976).

RESULTS AND DISCUSSION

The control fish gill (Figure 1) is made up of filaments or primary lamellae arranged in double rows. Secondary lamella is lined by a squamous epithelium. Inside this epithelium are lamellar blood sinuses separated by pillar cells. At the tip of the secondary lamella is a marginal blood sinus lined by a thick stratified epithelium. This region contains the mucous cells and chloride cells.

Several pathological changes showed and their frequencies increased with increasing of concentration.

A summary of histological changes were evident among fish exposed to (0.48, 0.7, 1.08) ppm of Diazinon pesticide after acute exposure and (0.48, 0.7) ppm after chronic exposure (Table 1). In the acute exposure fish showed different distortions of the secondary lamella and their frequencies increased with increasing of concentration. The separation and sloughing of epithelial layers, Edema and mucus secretion (Fig. 2). Lamellar aneurysms of many filaments were observed.

Table 1: Histopathological changes in *Liza abu* gills exposed to sublethal concentrations of Diazinon pesticide

Time exposure	Concentration ppm	Epithelial separation	Sloughing epithelium & Necrosis	Edema	Fusion of lamellae	Clavate or clubbing lamellae	Hyperplasia	Aneurysms
96 hours acute exposure	control	None	None	None	None	None	None	None
	0.48	++	+	++	None	None	+	++
	0.7	+++	÷	+++	None	None	+	++
	1.08	+++	+++	++++	+	+	+	++
14 days chronic exposure	control	None	None	None	None	None	None	None
	0.48	+	+	None	++	+++	+++	None
	0.7	+	++	None	+++	+++	++++	None

÷ = mid ++ = moderate +++ = sever ++++ = very sever

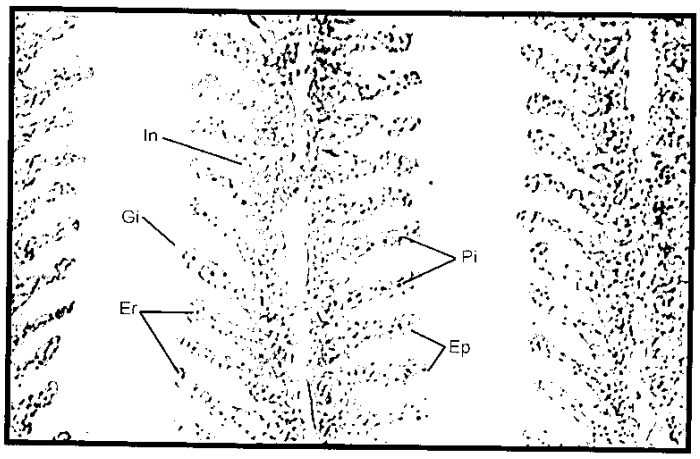

Figure 1: Section of control fish gill. showingIn = Inter lamellar cells. ; Gi = Gill lamella.; Pi = Pillar cell.; Ep = Epithelial cell., Er = Erythrocyte., (Hematoxylin & Eosin X350).

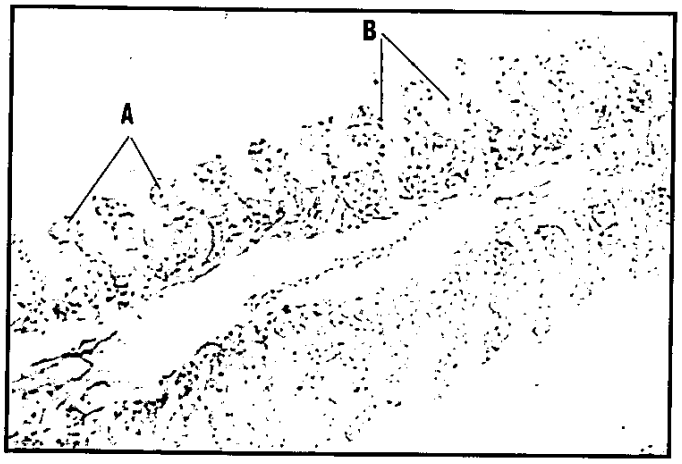

Figure 2: Section through secondary lamellae after acute exposure (96 hours) to 0.7 ppm of Diazinon showing: A = Edema ; B = Epithelial separation , (Hematoxylin & Eosin X350).

Figure 3: Section through secondary lamellae after acute exposure (96 hours) to 1.08 ppm of Diazinon showing: A = Aneurysms , (Hematoxylin & Eosin X350).

Figure 4: Section through secondary lamellae after chronic exposure (14 day) to 0.48 ppm of Diazinon showing: A = Clubbing lamellae ; B = Epithelial separation ; C = Sloughing epithelium ; D = Fusion of lamellae. , (Hematoxylin & Eosin X350).

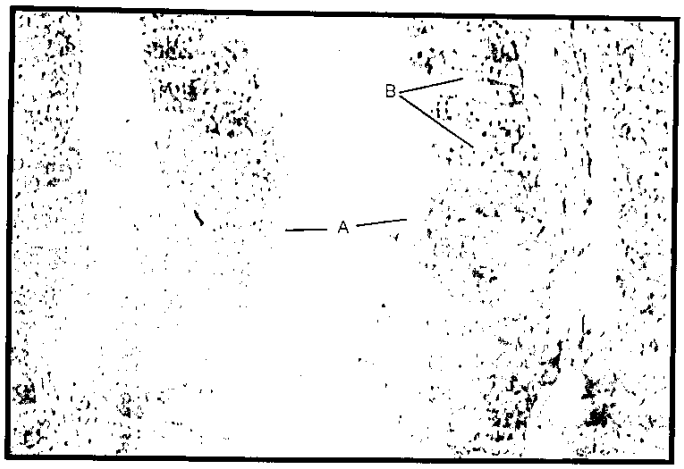

Figure 5: Section through secondary lamellae after chronic exposure (14 day) to 0.7 ppm of Diazinon showing: A = Hyperplasia and Fusion of lamellae, B = Epithelial separation (Hematoxylin & Eosin X350)

Lamellae adjacent to aneurismal lamellae often had necrotic pillar cell. The filament tissue between adjacent lamellae was commonly separated from its anchorage. Numerous necrotic cells were found within these separated interlamellar zones. The damage was more pronounced after acute exposure of 1.08 ppm, but it was less severe in the 0.48 and 0.7 ppm exposure. The chronic exposure of Diazinon pesticide resulted in a pathological condition called the bulbing of the secondary lamella or clavate lamella (Fig. 4), with thickness of the primary lamellar epithelium (Fig. 4). Hyperplasias of the gill lamella were observed, fusion of adjacent secondary lamellae as a result of hyperplasia. Further some mild degenerative changes were seen in the interlamellar region, lifting up of the epithelium. Sloughing and excessive mucus secretions were observed in contaminated fish (Fig. 4).

Similar reports on gill damages were observed in *Cyprinus carpio* exposure to dipterex by Chinabut *etal.* (1987). *Rivulus marmoratus* was exposed to diethylnitrosamine by Thiyagarajah and Grizzle (1985), *Clarias garipinus* was exposed to inorganic fertilizers by Ufodike and Onusiriuka ,(1990). a Glyphosate Herbicide, on Nile tilapia (*Oreochromis niloticus*) by Jiraungkoorskula *etal.*(2002) and in *Mugil* sp., *Cyprinus carpio* and *Barbus* sp. was exposed to lindane by Ortiz, *et.al.* (2003).

General observation showed evidence of proliferative lesions in the gill epithelium and mutilations of gill rakers. Gill lesions can be divided in to two groups, the direct deleterious effects of the irritants and the defense responses of the fish (Temmink *etal.*, 1983). The observed epithelial necrosis and rupture of the gill epithelium are direct responses induced by the action of Diazinon. The defense responses notice are excessive mucus secretion , lifting up of the epithelium , lamellar fusion and clavate lamella. Hyperplasia may in some situations represent an adaptation by the fish to protect underlying tissues from any irritant (Kumaraguru *etal.*,1982). The lifting of the epithelium increases the distance through which the toxicant has to travel to reach the blood stream and lamellar fusion could be protective in that it diminishes the amount of vulnerable gill surface area (Richmonds and Dutta, 1989 and Mallatt ,1985) . Branchial responses that serve to slow the entry of toxicant have the undesirable effect of

threatening to suffocate the fish (Leion,*etal.*1987). Omoregie and Ufodike,(1991) noted increase of opercular ventilation or hyperventilation indicated the damage being caused by the insecticide on the gills and as such , the fish increased its ventilation rates in an attempt to take up more oxygen, but finally gets exhausted and increased pesticide concentration in the gill. Sharma *et.al.*, (2001) found the histopathological changes include necrosis, rupture of the gill's epithelium contributes to problems with respiration and ionic and acid base balance.

Hyperplasia , would not only decrease the surface area available for oxygen extraction but also would increase the oxygen diffusion distance between water and blood . Thus, while hyperplasia may indeed be having a protective function it may also be hindering the respiratory , secretory and excretory function of the gills (Kumaraguru *etal.*,1982). The hyperplasia was accompanied by a marked increase in mitotic figures. Changes did not involve all gill filaments and were not localized to specific areas of the branchial arches .Lymphocytic infiltration, indicative of an inflammatory response, of the interlamellar filamental epithelium was evident in all animals with exposure beyond 20 hr.(Campbell,1997) The lifting of the epithelium increases the distance through which the toxicant has to travel to reach the blood stream. Lamellar fusion could be protective in that it diminishes the amount of vulnerable gill surface area (Ortiz, *et.al.* 2003).

التاثيرات السمية الحادة والمزمنة لمبيد الديازينون في أنسجة غلاصم أسماك الخشني *Liza abu* (Heckel, 1843)

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الخلاصة

عرضت اسماك الخشني *Liza abu* الى تراكيز مختلفة من مبيد الديازينون (مبيد عضوي فوسفوري diethyl 2-isopropyl -6- methyl -4- pyrimidinyl phosphoro -thionate) عند التعرض الحاد (٩٦) ساعة والتعرض المزمن (١٤) يوما.

أظهرت الفحوصات النسيجية المرضية فرط التنسج في انسجة الغلاصم وبعض حالات الأحتقان الدموي في الصفائح الثانوية، انفصال طبقة النسيج الطلائي، موت نسيجي، ظهور حالات الشكل البصلي او هراوي في خلايا الطبقة الطلائية والتحام الصفائح الثانوية المتجاورة، هذه التغيرات اختلفت حسب تركيز المبيد وزمن التعرض له.

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