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THE EFFECT OF PESTICIDES EXPOSURE ON CERTAIN DIGESTIVE ENZYMES IN THE INTESTINAL TRACT OF FRESH WATER FISH (*Tilapia Nilotica*)

I. A. El-Elaimy, and E. M. El-Habibi

Zoology Depts., Faculty of Science, Monoufia & Mansoura Universities.

ABSTRACT

The activity of some digestive enzymes of intestinal tract was studied in control and in fish (Tilapia nilotica) under the stress of exposure to pesticides. Two pesticides; diazinon (organophosphate) and neopybuthrin (pyrethroid) were selected. The enzymes studied were; trypsin, Amylase, lipase and pepsin. Two experiments were conducted. In the 1st experiment fish groups were exposed in aquaria to an initial concentration of 96 hr. LC_{50} and then batches were decapitated at intervals of 1, 3, 6, 12 and 24 hr of exposure time. In the 2nd one, fish groups were exposed to different concentrations of each pesticide (1 / 1000, 1 / 100, 1/ 10 and 1 x 96 hr. LC_{50}). The treated groups together with control one were decapitated 6 hrs after exposure.

The data obtained revealed that the digestive enzymes exhibited variable reductions in their activities depending on the enzyme, exposure time, pesticide tested and its concentration. The maximum inhibition recorded after 6 hrs of exposure. The most sensitive enzyme was amylase followed by pepsin, lipase and trypsin. In all cases, diazinon showed a more potential effect than neopybuthrin. These results were discussed in the light of physio-chemical properties of such pesticides.

INTRODUCTION

Interest in the water pollution with chemical pesticides, has led to numerous studies concerning the effect of these compounds on the different metabolic activities in fish. Thus, impairment in the protein metabolism (Gabr, 1986, El-Elaimy, *et . al..*, 1987); lipid metabolism (Bansal *et. al..* 1979; Rao & Rao,

1981; El-Elaimy *et. al.*, 1987) and carbohydrate metabolism (Grant, 1976, Sastry and Siddique, 1984; El-Elaimy & El-Habibi, 1990) has been reported to occur in fishes after exposure to a variety of insecticides.

Besides skin and gills; intestine is considered as the most common route through which pesticides absorption may occured (Hutson & Roberts, 1985). The effect of insecticides on the intestine of different fishes has been described by many authors (Sastry & Malik, 1979; Sastry & Sharma, 1979; Gupta & Singh, 1982 and El-Elaimy *et. al.*, 1990 a, b.). In these investigations numerous morphological and structural changes have been reported. Such alterations may affect directly and / or indirectly the absorption as well as secretory functions of intestine. For testing this suggestion, the present investigation was performed. It is concerned with studying the effect of exposure to diazinon (OP) and neopybuthrin (pyrethroid) on some digestive enzymes secreted into the intestinal tract of fresh water fish (***Tilapia nilotica***).

MATERIALS AND METHODS

Pesticides used :

Two pesticides represonting two major insecticidal groups were used in the present investigation namely;

1. Diazinon (60 %) belongs to organophosphates.
2. Neopybuthrin (40 %) belongs to pyrethroids.

The commercial formulation of these pesticides were used. They were diluted with water to give the desired concentration toxicity; the lethal concentrations (LC_{50}) of the two pesticides were deduced from the dose mortality curves previously constructed in our laboratory for ***Tilapia nilotica.*** The 96 hr. LC_{50}'s were; (20 mg / L) for diazinon and (2.1 mg / L) for neopybuthrin.

Fishes :

Fresh water teleost fish, ***Tilapia nilotica*** (family cichlidae) were used. Such fish are considered as the most common species living in river Nile. Alive healthy fish individuals were collected from the Nile and transported to large laboratory aquaria.

They were acclimatized to the laboratory conditions for about one week. The aquaria were well aerated and their temperature ranged between 27 - 30 °C during experimentation period. Fish individuals, weighing 80 - 100 g, were fed twice a day with suitable fish pellets during acclimatization period. However, they were starved without food during pesticides exposure.

Pesticides Exposure :

Two experiments were carried out in the present study

- Experiment I. In this experiment a number of 75 individual fishes were divided equally into three groups; 25 fish were exposed in aquaria to fresh untreated dechlorinated water (control group); 25 fish were stored in aquaria containing an initial concentrtion of diazinon ($^1/_2$ 96 hr LC_{50}) and the other 25 fish were stored in aquaria containing the same level of neopybuthrin ($^1/_2$ 96 hr. LC_{50}). Throughout the exposure (24 hr.), batches (five fish) were taken from each group and decapitated at intervals of 1, 3, 6, 12 and 24 hr.

Experiment II. A number of 45 fish were divided into nine equal batches (Five fish for each). One batch was exposed to fresh dechlorinated tap water (control). Four batches were stored in four different aquaria containing 1 / 1000, 1 / 100, 1 / 10 and 1 96 hr LC_{50} of diazinon. The other four batches were exposed in four aquaria to the same concentration levels of neopybuthrin. All batches were removed and killed, after 6 hrs of exposure.

Immediately after killing the fish individuals, they were dissected. The gastric and intestinal contents were collected, centrifuged (1000 r. p. m. for 15

min.). The supernatant fluid was used for determination of the enzymes levels.

Enzymatic Analysis :

The gastric and intestinal juice; were diluted with distilled water in the proportion suitable for the methods used, which were as follows :-

For determination of pepsin activity, 2% of haemoglobin - HCl solution (pH 1.8) was used as substrate. The method is fully described by Reimer (1982). The activity of amylase was determined by a method modified by Hofer (1979). Trypsin activity was determined using 0.5 mμ solution of p - tosyl - L arginine methylester - HCl (in this - HCl - buffer, pH 8.2) as substrate, (the method described by Reimer, 1982). As for lipase, the test combination No. 263346 of Boehringer Mannheim was used. In this test, the decrease in the turbidity of a stabilized triolein emulsion at pH 9.2 is measured in a spectrophotometer at 340 nm. The experimental temperature was 27° ± 1°C.

The significance between means of treated and control groups was determined using "t" Student test.

RESULTS

In the first experiment the fish individuals were exposed to an initial concentration of $^1/_2$ 96 hr LC_{50} of diazinon or neopybuthrin. The fish groups were followed up throughout an observation period of 24 hr. The data obtained for enzyme activities are represented in figure (1). Evidently, the results showed that the digestive enzymes (trypsin, amylase; lipase and pepsin) exhibited gradual reduction in their activities with the time of exposure except after one hour of exposure, whereas such enzymes showed slight to moderate elevation in their activities. The maximum reductions were recorded after 6 hr. of initial exposure. The percentage inhibitions recorded were, 20%, 50%, 33% and 37% (in case of diazinon) and were 13%, 29%, 255 % and 32% (in case of neopybuthrin) for trypsin, amylase, lipase and pepsin respectively. As indicated from this experiment, the most sensitive enzyme was amylase, followed by pepsin, lipase and trypsin.

In the second experiment, the fish groups were exposed to gradual increasing concentration of both pesticides (1 / 1000, 1 / 100, 1 / 10 and 1 of 96 hr. LC_{50}) in separate aquaria. The exposure time was 6 hrs. (Time at which the maximum inhibition was attained for all enzymes, in the first experiment). The data recorded in this experiment (figure 1) indicated that there are inhibitions in all enzymes activities of both diazinon and neopybuthrin poisoned fish. The magnitued of inhibition (in %) depended on the concentration of an insecticide used. However, in all cases diazinon was more effective than neopybuthrin.

DISCUSSION

The primary aim of the present investigation is to study the influence of pesticide exposure on the digestive function of gut in fresh water teleost model (***Tilapia nilotica***). For this purpose the activities of some digestive enzymes from the intestinal tract of control and pesticides stressed fish were determined.

Evidently, the data obtained herein, revealed that exposure to organophosphate (diazinon) or pyrethroid (neopybuthrin) at concentration levels of $^1/_2$ 96 hr LC_{50}, caused inhibitions in the activities of all digestive enzymes (Trypsin, Amylase, lipase and Pepsin) with different degrees, depending on the type of insecticide and the exposure time. However, the maximum inhibitions in all enzymes levels were recorded after 6 hrs of exposure. It seems that this time is required by pesticide to produce its actual action. Most pesticides suffered a metabolic biotransformation in vivo to give more active metabolites (Huston and Roberts, 1985), which are able to produce the gross signs of intoxication. The inactivation of the digestive enzymes in the gut of tilapia is preceded by slight to moderate activation (one hour after exposur to pesticides). This activation may be an adaptive response of the secretory intestinal cells to the pesticide intoxication.

Earlier investigations, dealing with the effect of chemical insecticides on digestive enzymes showed strong action of organophosphates on such. Thus, Jansen *et. al.,* (1949) found that concentration of diisopropyl flourophosphate of 4×10^4M caused 80% inhibition of trypsin, while a concentration of 8×10^6M caused 50% inhibition to chymotrypsin. Using the same insecticide, similar

inhibitions were recorded for α , β chymotrypsin as well as trypsin (Jansen & Balls, 1952) and for crystalline trypsin and chymotrypsin (Mounter, *et. al.*, 1957, 1963).

Applying increasing concentrations of the two pesticides used in the present investigation (1 / 1000, 1 / 100, 1 / 10 and 1 x 96 hr LC_{50}), brought about concentration dependent inhibitions in the four enzymes activities. Although the lower concentrations of such pesticides (1 / 1000, 1 / 100 96 hr LC_{50}) were not so effective. In all cases diazinon showed higher potency than neopybuthrin. Amylase showed to be the most sensitive enzyme followed by pepsin, lipase and trypsin.

In this respect we may recall the work done by Gabr *et. al.*, (1972). The authors studied the effect of some insecticides (DDT, lindane and malathion) at two dose levels (1 / 10, 1 / 100 of lethal dose) on the digestive enzymes (pepsin, trypsinogen + enterokinase, salivary amylase and pancreatic amylase and lipase) from white rat (***Rattus rattus***). They recorded different inhibitions in all enzymes activities, depending on the sensitivity of the enzymes to the insecticide used. They also reported that the effectiveness of each insecticide depended on the dose applied. These results agree with that recorded in the present investigation.

Being the primary site responsible for the terminal events of digestion and absorption, gut and the brush border of the intestinal tract were subjected to extensive histopathological studies. Microscopic examination of the intestine of diazinon or neopybuthrin exposed tilapia revealed many pathological alterations including; degeneration of muscular layer, vacuolation of the epithelial cells (El-Elaimy *et. al.*, 1990 a, b). Similar histological examinations were observed in intestine brown trout exposed to DDT (King, 1962), ***Channa punctatus*** exposed to dimecron (Sastry & Milk, 1979) and in ***Clarias batrachus*** poisoned with sumithion (Mandel & Kulshrestha, 1980). The degeneration of the intestinal layer under the effect of pesticides exposure was contributed to the liberation of acid hydrolyses from the lysosomes (Sastry & Malik, 1979). The autolysis of secretory epithelium in the intestinal tract of autolysis of secretory epithelium in the

intestinal tract of fish may lead to leakage of enzyme secretion and thus, lower concentrations of such enzymes may be occurred in the gut lumen under the stress of exposure to pesticides.

One might notice from the data presented herein, the higher potency of diazinon (organophosphate) over that of neopybuthrin (pyrethroid). The factors influencing such potentialities are still questionable and controversial, however, El-Elaimy *et. al.,* (1988) attributed the lower toxicity of pyrethroids to their rapid in vivo biodegradability (Huston, 1979; Chambers, 1980); Oxidative hydrolysis to give less toxic metabolites (White *et. al.,* 1976, Casida *et. al.,* 1983) and lower rate of skin penetration or intestinal absorption (Wang *et. al.,* 1981). Moreover, pyrethroids have lower photostability (Elliot *et. al.,* 1973). These suggestions may explain the lower potency of neopybuthrin as compared to the organophosphate diazinon.

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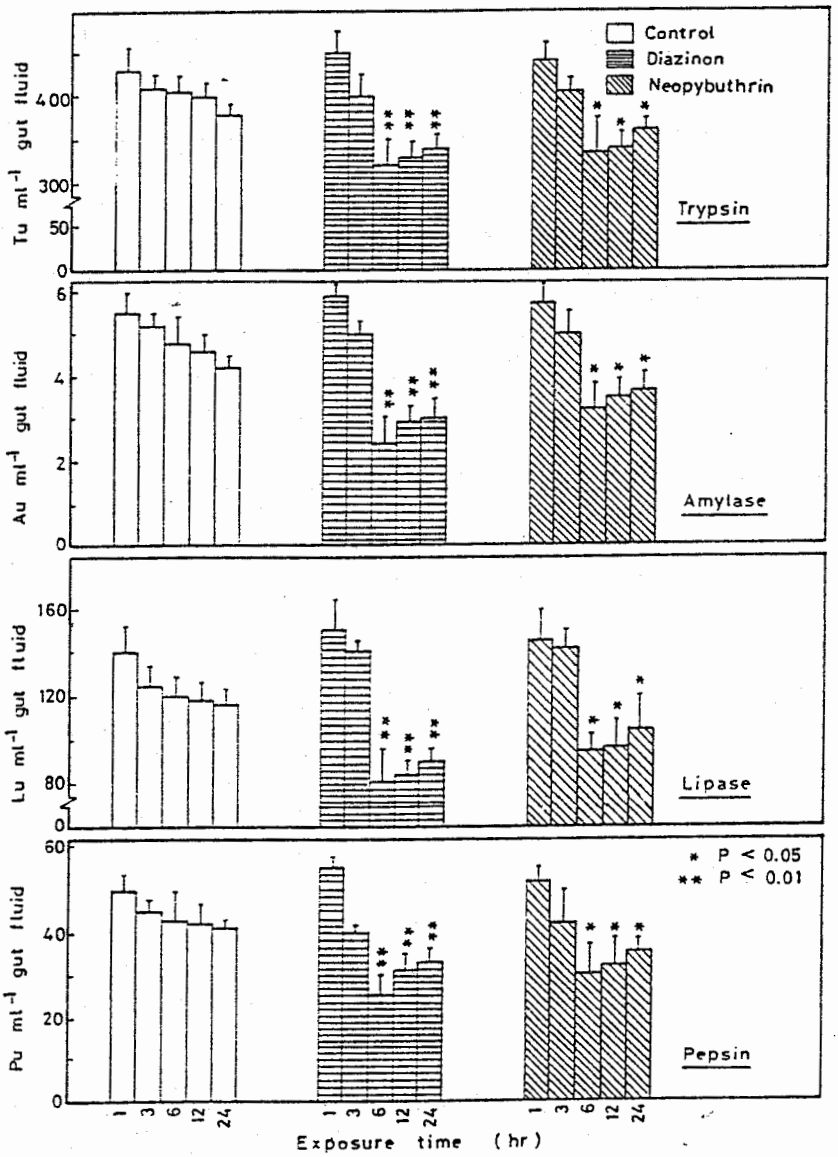

Figure 1. The levels of some digestive enzymes in the gut of freshwater fish (Tilapia nilotica) during exposure to 1/2 96 hr LC_{50} of diazinon or neopybuthrin.

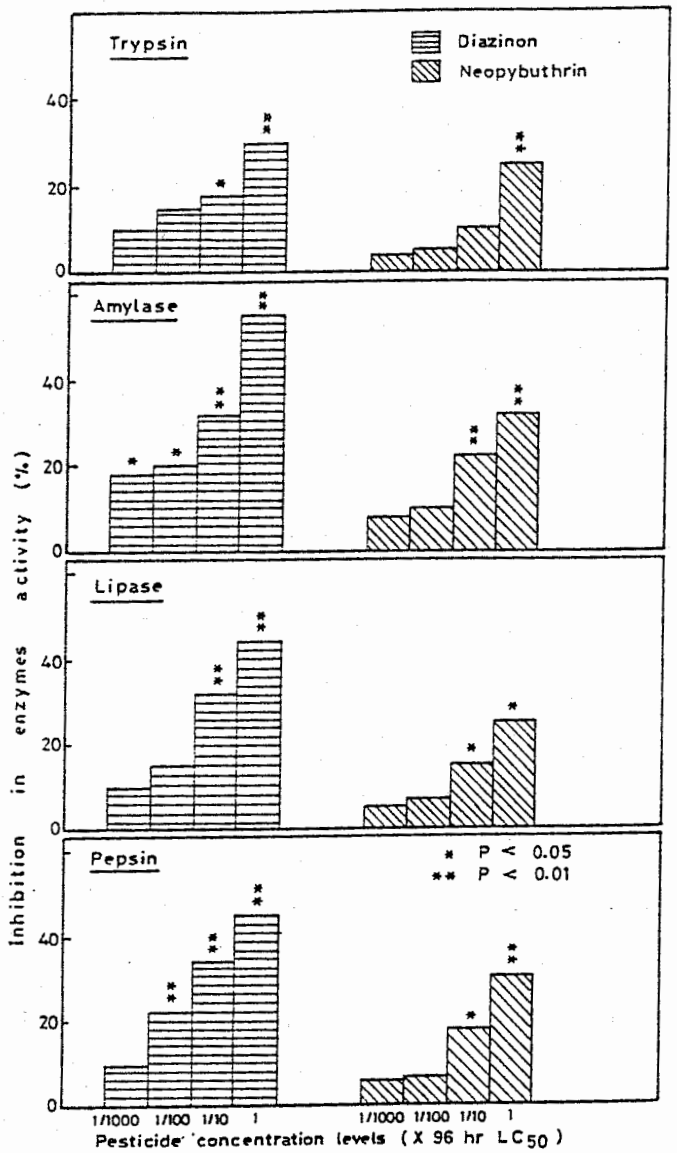

Figure 2. Inhibition of digestive enzymes in the gut of freshwater fish (Tilapia nilotica) under the stress of exposure to different concentrations of diazinon or neopybuthrin.

دراسة على تأثير التعرض للمبيدات الحشرية على بعض انزيمات الهضم فى أمعاء أسماك المياة العذبة (التيلابيا نيلوتيكا)

" ملخص البحث "

يهدف هذا البحث الى دراسة بعض أنزيمات الهضم فى أمعاء أسماك المياة العذبة (البلطى النيلى) وذلك تحت تأثير تعرض هذه الأسماك للمبيدات الحشرية فى المياة.

إستخدم لذلك مبيدين هما الدايازينون (مبيد فسفورى عضوى) ومبيد الثيوبيوثرين (مبيد بيرفيرويدى)

تم قياس نشاط انزيمات الأمليز ، الليبيز ، البسين وكذلك التربسين فى أمعاء ومعى هذه الأسماك.

أجرى لهذا الغرض تجربتين فى التجربة الأولى : تم تعريض مجموعات من الأسماك تحت الدراسة لتركيزات تعادل ($^1/_2$ 96 hr LC_{50}) من كل المبيدين على هذه ثم ذبحت مجموعات من هذه الأسماك على فترات ١، ٣، ٦، ١٢، ٢٤ ساعة من التعرض لهذه التركيزات.

وفى التجربة الثانية : تم التعرض مجموعات من الأسماك لتركيزات مختلفة من كل من المبيدات وهذه التركيزات كانت تعادل ($^1/_{1000}$ ، $^1/_{100}$ ، $^1/_{10}$ ، ١ من الجرعة القاتلة للنصف (96 hr LC_{50}) وذبحت جميع مع المجموعة القياسية بعد ٦ ساعات من التعرض.

وأضحت النتائج احباطات فى نشاط هذه الأنزيمات بنسب مختلفة وتعتمد هذه النسب على نوع الأنزيم ، نوع المبيد ، فترة التعرض لهذا المبيد وكذلك تركيزه فى المياة.

وقد أظهر أنزيم الأميلز أعلى حساسية يلية أنزيمات البسين والليبيز والتربيسن.
وفى كل الحالات كان الدربازيتون أكثر فاعلية من الثيربيوثرين.
وقد نوقشت هذه النتائج فى ضوء الخواص الفسيوكيميائية لهذه المبيدات.