

**Protein and transaminases activities modulation by
sublethal concentrations of pirimiphos-methyl in blood
serum of boliti fish *Oreochromus niloticus***

By

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ABSTRACT

The acute toxicity of pirimiphos-methyl on boliti fish *Oreochromus niloticus* had been evaluated, and the 96-hr LC_{50} was determined (3.2 ppm). The exposure of fish to sublethal concentration of pirimiphos-methyl was utilized to investigate the non acute effects on the modulation of protein and transaminases in blood serum of boliti fish.

The results illustrated that pirimiphos-methyl reduced the protein level in blood serum and the process of protein reduction was correlated to the increase of tested concentration. Such decrease slowed down with the increase of exposure period to reach the normal level. With regard to the results of transaminases; glutamic-oxaloacetic-transaminase (GOT) and glutamic-pyruvic-transaminase (GPT) modulation in blood serum of fish at low concentration 0.32 ppm (one tenth of 96-hr LC_{50}) of pirimiphos-methyl, the activities of both enzymes were decreased in comparison to the same enzymes in untreated fish during the first 2 days of exposure and then increased over that of control. On the contrary, at the highest concentrations 0.4, 0.8 and 1.0 of the 96-hr LC_{50} of pirimiphos methyl, a stimulatory action on GPT and GOT activities was observed spontaneously during the exposure period.

These results suggest that the protein levels and transaminases activities of fish blood serum could be used as biomarkers for aquatic pollution caused by insecticides.

INTRODUCTION

Many insecticides are broad spectrum biocide and are considered as toxic not only to target arthropods but also to vertebrates. Widespread contamination of aquatic ecosystems by a whole range of pesticidal chemicals has been responsible for the hulking fish kills and is recognized as a potential human health hazard as well (Lefferts 1988, Ochumba 1988, Huckle and Millbun 1990, Ismail, *et al.*, 1995, Sekine *et al.* 1996 and Ismail *et al.*, 1997).

Pesticides are regularly used at Ismailia province during vegetable production to protect the crops from losses incurred by numerous pests. Pirimiphos-methyl {O- (diethylamino) 6- methyl 4- pyrimidinyl } O.O dimethyl phosphorothioate} is used on common beans (*Phaseolus vulgaris*) to control aphids *Aphis craccivora* and white fly (*Bemisia tabaci*). The spray drift or run off of such insecticide may affect aquatic life including Fish (Saunders, 1969 and Grande *et al.* 1994).

Fish has long been used as a biomarker of aquatic pollution caused by insecticides (Johnson 1968, Holden 1973, Shakoori *et al.*, 1992, Tejada, 1996 and Ismail *et al.*, 1997).

The present study was carried out to explore the possibility of using blood serum protein and transaminases activities modulation of treated Bolti fish by sublethal concentrations of pirimiphos-methyl as a more viable and specific biomarker tool of aquatic pollution with such insecticide.

MATERIALS AND METHODS

Tested compound :

Pirimiphos-methyl (Actellic 50% EC)

(O - (2- (diethylamino) 6- methyl 4- pyrimidinyl) O,O dimethyl phosphorothioate), ICI company, UK .

Tested Animals :

The boliti fish *Oreochromus niloticus* was obtained from fish research center , Suez Canal University . The fish was kept in large tank with aerated tap water and left to acclimate to laboratory conditions for about two weeks before the experiment began . During rearing , standard fish diet was introduced .

1- Toxicological studies :

Acute toxicity of pirimiphos-methyl to boli fish

The experiments were performed with Nile *Tilapia* each healthy male of approximately 45 ± 3 gm weight each and 15 ± 2 cm length . After acclimatization , the fish were transferred into 60 L glass aquaria at $23 \pm 1^\circ\text{C}$ with aerated tap water where bioassays for determination of 96-hr LC_{50} were performed. The water used in the experiment had the following mean values for the water quality characteristics : pH 7.5 total hardness as CaCO_3 105 mg/L, dissolved oxygen 4.6 mg/L and 3.6 alkalinity value . Feeding was stopped 48 hr before treatment and during the 96hr test period. The median lethal concentration of pirimiphos-methyl was measured using series of concentrations. Ten fish were used in each replicate and three replicates for each treatment . Mortality was recorded after 96hr . The fish toxicity data were subjected to the probit analysis method of Finney (1971) to determine the 96-hr LC_{50} .

2- The Longterm effect of sublethal concentrations of pirimiphos-methyl on boliti fish :

a - Effect on blood serum protein and transaminases

Groups each of 10 fish were exposed to a sublethal concentration 0.32 ppm (one- tenth of the 96-hr LC_{50} values) for 24, 48 , 72 and 96-hr . Other groups each of 10 fish were exposed to a sublethal concentration series of 0.32 , 1.28 , 2.56 and 3.2 ppm values (0.1 , 0.4 , 0.8 and 1.0 of the 96-hr LC_{50}) for 24-hr . Controls were prepared under identical conditions with the same number of fish . The caudal peduncle was cut off

with a sharp razor blade and then the free flowing blood was collected in sterile disposable glass test tubes (Okawa *et al* 1987) .

The blood samples were allowed to stand for about one hr. and within that period the clotting was completed. These samples were centrifuged for 15 min at 3600 rpm to separate the serum from the clotted cells (Booke , 1965) . The serum without any blood corpuscles was transferred to a sterile test tube . The tubes were sealed , labeled , and frozen at -20 °C .

b: Protein assay

The protein concentrations of blood serum were determined by the dye binding method “ Bio- Rad protein assay “ according to the method of Bradford (1976) .

c: Transaminases assay :

The activities of glutamic oxaloacetic transaminases (GOT) and glutamic pyruvic transaminases (GPT) in the tested samples of blood serum were determined spectrophotometrically using the Reitman and Frankel method (1957) . The function of enzyme activity was expressed as extinction coefficient of yellow colour (2,4- dinitrophenyl hydrazone) produced from the reaction .

ketoglutarate + aspartate $\xrightarrow{\text{GOT}}$ oxaloacetate + glutamate or
 α -ketoglutarate + alanine $\xrightarrow{\text{GPT}}$ pyruvate + glutamate in the presence of
dinitrophenylhydrazine .

The increase in the optical density of yellow colour is proportional to the formed oxaloacetate for (GOT) or pyruvate for GPT . Then the base line of enzyme activities was calculated as specific activity ($\mu\text{M mol}$ of oxaloacetate or pyruvate = 0. D/min/mg/protein) .

RESULTS AND DISCUSSION

The toxicity of pirimiphos-methyl to the boliti fish *Oreochromis niloticus* was evaluated . The results manifested a regression line of the toxicity with 3.09 slope value and 96-hr LC_{50} value of 3.2 ppm .

Table (1) Effect of ($LC_{50} \times 0.1$) pirimiphos-methyl on blood serum protein of boliti fish *O. niloticus* at different exposure intervals.

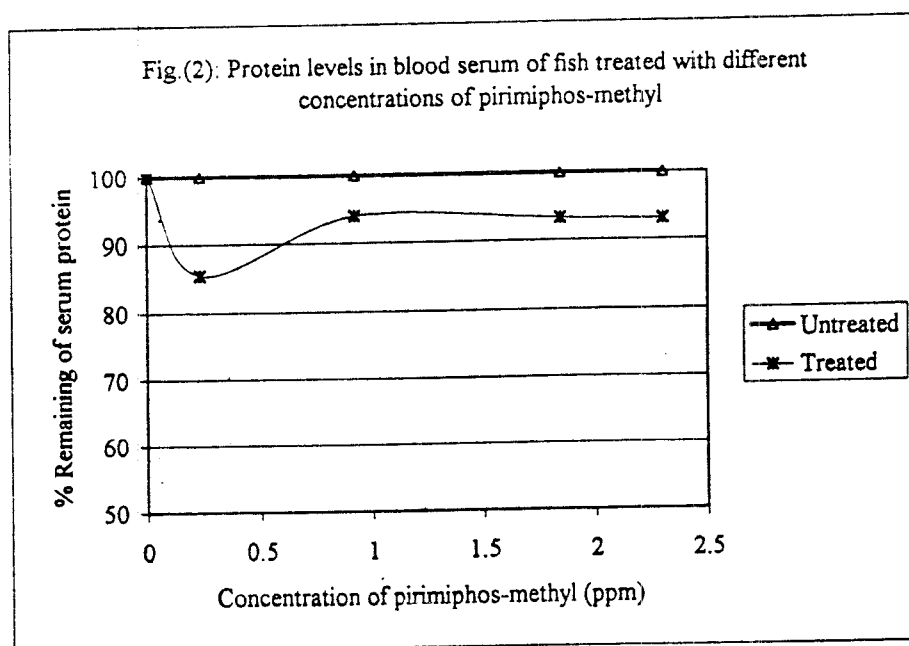
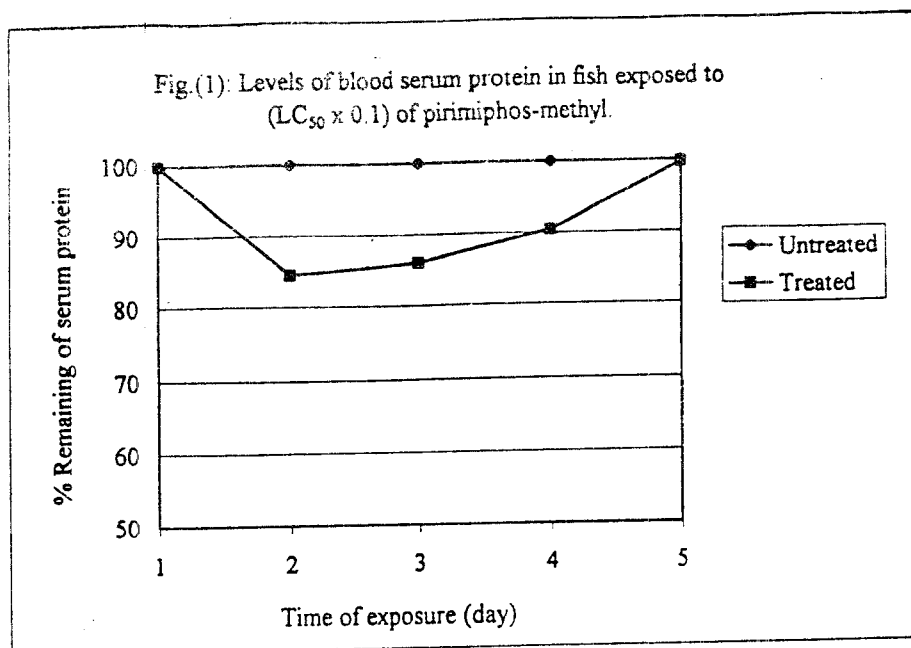
Treatment	Levels of blood serum protein (mg/100ml)*			
	Time of exposure (day)			
	1	2	3	4
Untreated fish	2.824 ± 0.085	2.83 ± 0.113	2.82 ± 0.112	2.822 ± 0.141
Treated fish	2.384 ± 0.119	2.422 ± 0.05	2.550 ± 0.102	2.81 ± 0.056
% Reduction of protein	15.58	14.165	9.57	0.356

*Results are expressed as mean $\pm$ standard deviation (SD), n=6.

Table (2) :Variation of blood serum protein of boliti fish *O. niloticus* treated with different concentrations of pirimiphos-methyl for one day.

Treatment	Levels of blood serum protein (mg/100ml)*			
	0.32 ppm	1.28ppm	2.56 ppm	3.2 ppm
Untreated fish	3.384 ± 0.135	3.382 ± 0.169	3.380 ± 0.135	3.386 ± 0.169
Treated fish	2.874 ± 0.087	2.836 ± 0.141	2.280 ± 0.113	2.810 ± 0.084
% Reduction of protein	13.888	16.1	16.81	16.97

*Results are expressed as mean $\pm$ standard deviation (SD), n=6.



Protein levels in pirimiphos-methyl treated fish :

The results in Table 1 indicated that the highest reduction of protein (15.58%) in blood serum of *Oreochromis niloticus* exposed to one tenth of 96-hr LC_{50} (0.32 ppm) was recorded after one day of exposure. Such reduction of protein was found to be 14.165, 9.57 and 0.35610 percentage after 2, 3 and 4 days of exposure respectively. The pattern of remaining protein in blood serum during the four-day exposure period to one tenth of 96-hr LC_{50} of pirimiphos-methyl is shown in Figure (1).

The results shown in Table (1) and Figure (1) illustrated that pirimiphos-methyl reduced protein level in the blood serum and the process of protein decrease slowed down with the increase of exposure period. When the *O. niloticus* was exposed to different sublethal concentrations of pirimiphos-methyl for one day the results (Table (2), and Figure (2) demonstrated that there is a correlation between the increase of pirimiphos-methyl concentration and the percent of protein reduction in blood serum. These observations (Tables 1, 2 and figures 1,2) concerning the behavioural response of *O. niloticus* after sublethal exposure to pirimiphos-methyl are in agreement with those of Carter, 1971 and Wildish *et al.*, 1971 who exposed *L. catenatus* and *Salm. salar* fish to organophosphorus insecticides. Similar results were obtained when *Cirrhinus mrigala* was exposed to Trebon (Shakoori *et al.*, 1992).

In the light of these investigations, it seems logic to say that pesticides could stimulate proteolysis by activating protease enzymes. Protein depletion in serum plays a role of compensatory mechanism under the pesticidal stress. Under proteolysis enhanced breakdown dominates over synthesis, while in the case anabolic process, increased synthesis dominates the protein breakdown (Harber *et al.*, 1977).

Serum Transaminases Activities of pirimiphos-methyl treated fish .

It is known that the disruption of transaminases from the normal values denotes biochemical impairment of tissues and cellular functions as they are involved in the detoxification processes, metabolism, and biosynthesis of energetic macromolecules of different essential functions (Tordior and van Heemstra - Lequin, 1980). El Gendy *et al.* (1990) found that pyrazophos and glyphosate caused spontaneous activation of liver

GOT and muscle GPT and caused significant inhibition of brain GOT in common carp (*Cyprinus caprio* L.) Habiba and Ismail (1992) studied GOT and GPT levels in the New Zealand white rabbit fed on clover contaminated with prophenophos . They found that brain muscle and kidney GOT were inhibited over the 8 days of the test period .

Table (3) The *in vivo* activity of blood serum transaminases in fish- treated with one-tenth of 96-hr LC₅₀ of pirimiphos-methyl at different exposure intervals.

Type of transaminase	Transaminase activities ^a at different intervals (day).			
	1	2	3	4
GOT				
Untreatment fish	36±1.44	36.18±1.1	35.98±1.62	36.13±2.1
Treated fish	29.33±1.6	32.14±1.9	34.6±2.42	42.15±2.5
%Change of control	+18.53	-11.17	-3.84	+16.66
GPT				
Untreatment fish	6±0.3	6.05±0.42	6.02±0.3	5.99±0.36
Treated fish	5.66±0.25	5.49±0.29	6.34±	8.06±0.29
%Change of control	-5.67	-9.26	+5.32	+34.5
GOT/GPT				
Untreatment fish	6.00	5.98	5.97	6.03
Treated fish	5.18	5.85	5.46	5.23

^a Transaminase activity is expressed at unit / 100ml

Results are expressed as mean ± standard deviation (SD), n=6.

In the present results, the change of GOT and GPT activities in blood serum of fish exposed to one - tenth of 96-hr LC₅₀ for four days are presented in Table (3) and Figure (3).

Table (4) Determination of blood serum Transaminase in pirimiphos-methyl treated fish after one day exposure period.

Type of transaminase	Level of transaminase activities ^a in treated fish with different concentrations of pirimiphos-methyl (ppm)			
	0.32	1.28	2.56	3.2
GOT				
Untreatment fish	58.72	59.22	58.51	59.01
	±2.99	±3.55	±2.75	±3.19
Treated fish	47.80	63.53	73.48	77.41
	±2.15	±2.61	±2.64	±3.25
%Change of GOT	-18.6	+7.2	+25.58	+31.18
GPT				
Untreatment fish	9.85±0.69	9.89±0.53	10.0±0.45	10.1±0.67
Treated fish	9.44	10.53	10.95	12.08
	±0.52	±0.82	±0.64	±0.41
%Change of GPT	-4.16	+6.87	+9.5	+19.6
GOT/GPT				
Untreatment fish	5.96	5.98	5.85	5.9
Treated fish	5.06	6.03	6.71	6.4

^a Transaminase activity is expressed at unit / 100ml

Results are expressed as mean ± standard deviation (SD), n=6.

Fig (3): Activities of GOT and GPT in blood serum of pirimiphos-methyl- treated fish during 4- day exposure period.

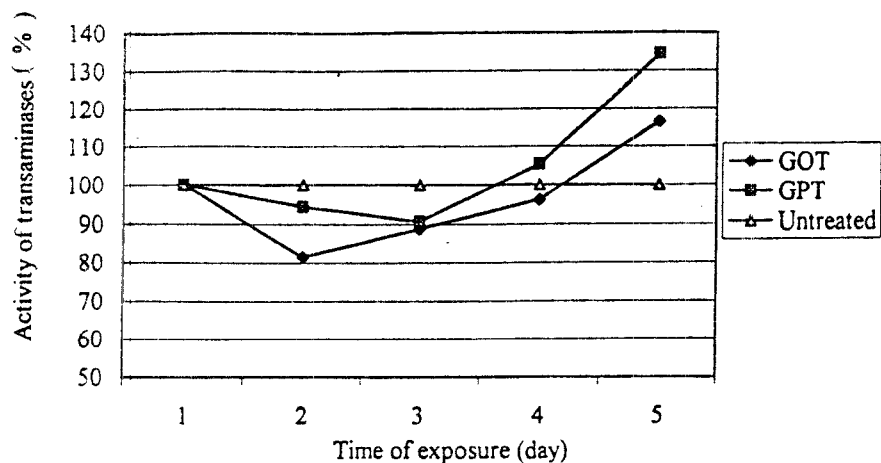
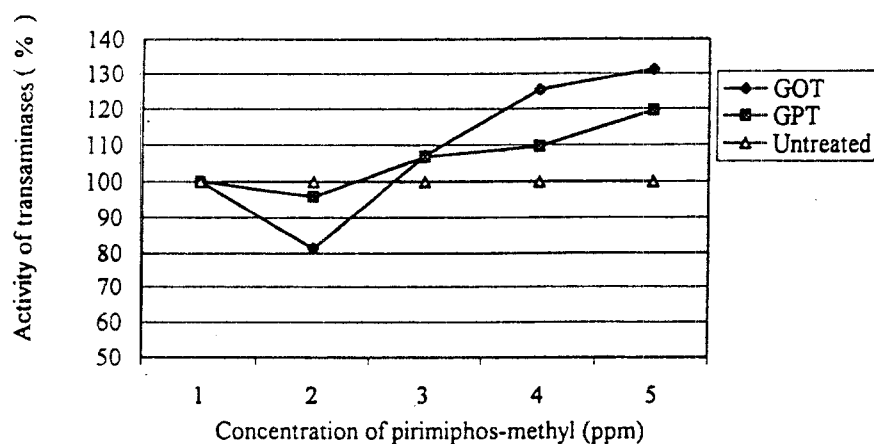


Fig (4): Activities of GOT and GPT in blood serum of Pirimiphos-methyl- treated fish during 4 days of exposure.



The results showed that the activities of both transaminases were decreased in pirimiphos-methyl treated fish in comparison to the untreated fish after 1, 2 and 3 days for GOT and after 1 and 2 days of exposure for GPT, and then the activities of the two enzymes recovered and increased over that of the control. Such stimulatory action on GPT was time-dependent over the last two days of exposure period.

Activities of GOT and GPT were observed in fish exposed to concentrations over the one tenth of 96-hrLC₅₀ (1.28, 2.56 and 3.2 ppm) for one day (Table 4 and figure 4). These observations are in agreement with that obtained by Gill *et al* (1990) and Ismail *et al* (1997).

From the above results it can be deduced that the low concentration of pirimiphos-methyl decreased protein of fish serum and decreased the activities of transaminases (GOT and GPT). With time, and because the toxicant pirimiphos-methyl dose not persist, the fish serum protein regain their normal level and the transaminases are stimulated. The present study suggests that the blood serum protein and transaminases (GOT and GPT) activities in fish could be used as a non specific biomarker tool for aquatic pollution caused by insecticides.

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الملخص العربي

تغير نظام البروتين و نشاط الانزيمات لناقلة للمجموعة الاميدية في سيرم دم اسماك البلطي المعامل بمبيد البريموفوس ميثايل بجرعت تحت المميتة

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تم تقدير الجرعة المتوسطة لمبيد Primiphos- methyl على سمك البلطي ووجد انه ٣٢ مجم/التر (جزء فى المليون). ثم تم اجراء تجارب تعريض البلطي للجرعات الاقل من المميتة لدراسة مدى تأثيرها على معدل بناء البروتينات وكذا نشاط الانزيمات الناقلة للامينات.

واوضحت النتائج ان المبيد الحشرى المختبر بالتركيزات الاقل من القاتلة تحدث انخفاضاً فى معدل بناء البروتين فى سيرم دم الاسماك. ويتناسب مقدار الانخفاض فى معدل بناء البروتين طردياً مع زيادة التركيز المستخدم من المبيد. كما أن معدل الانخفاض يقل مع زيادة زمن التعريض للمبيد. اما انزيمات GOT , GPT فى سيرم دم الاسماك - عند ١٠/١ من التركيز المتوسط للموت فان نشاط الانزيمات قد تم خفضها مقارنة بالكونترول وبزيادة تركيز المبيد يتضاعف انخفاض نشاط تلك الانزيمات.