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COMPARATIVE TOXICITY OF SOME PESTICIDES TO COMMON CARP AND THEIR EFFECTS ON BIOCHEMICAL TARGETS IN LIVING TISSUES

BY

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ABSTRACT

Four pesticides were evaluated in laboratory studies to determine acute toxicity (96-hr) to common carp (Cyprinus carpio L).LC_{50} values of pyrazophos, glyphosate, fenvalerate and copper sulphat were 6.11, 5.89,3.98 x 10^{-3} and 11.75 mg/l respectively .

The activity of acetyl cholinesterase (AChE), acid phosphatase (APase) glutathione S-transferase (GST) and transaminases (glutamate axaloacetate transaminase (GOT) and glutamate pyruvate transaminase (GPT)were measured in brain, liver, kidney, gill and muscle in a comparative study.
An in vivo effects of half LC_{50} and LC_{50} levels of tested compounds on different enzymes in different organs were studied. Pyrazophos and glyphosate caused the highest inhibition of brain AChE. Liver and kidney APases were stimulated by the tested compounds. GST was inhibited in brain and gill while it was stimulated in liver of treated fish. All tested compounds caused spontaneous activation of liver GOT and muscle GPT, while caused significant inhibition of brain GOT .

INTRODUCTION

One of the most important nontarget organisms in the ecosystem is fish. Pesticides are introduced into water system by direct application, spray drift, agricultural run-off and industrial effluents. Pesticides are uptaken by aquatic organisms from water through gills and skin during respiration, and orally when feeding. Pesticides are then distributed through various organs, metabolized and finally excreted partially back to the water system.

Many authors found that most pesticides were toxic to aquatic invertebrates and fish e.g. Folmar et al. (1979), Schimmel et al. (1983) and Rattner and Franson (1984).

The increased production and use of pesticides might cause adverse effect to fish production. Many pesticides increase the level of enzymes: acid phosphatase [Sexena and Sarin (1980) and Abou-Donia et al. (1986)]; glutathione S-transferase [Oeschet, et al. (1982) and EL-Gendy (1990)]; and transaminases[Bogusz (1986) and EL-Toweissy (1986)] in living organisms. Most organophosphorus compounds acutely inhibit acetylcholinesterase (AChE) O'Brien (1969), and Aldridge (1971), causing neurological effects.

The aim of the present work was to evaluate the relative susceptibility of common carp to four commonly used pesticides: Pyrazophos, an organophosphorus fungicide; glyphosate an organophosphorus herbicide; fenvalerate a synthetic pyrethroid insecticide and copper sulphate, a salt which is used as a fungicide, herbicide, algicide and molluscicide. In addition, the distribution pattern of the enzymes; AChE, APase, GST, GOT and GPT in different organs of fish was carried out. Besides the interaction between the acute toxicity of these pesticides and their biochemical and toxicological impact on the biological systems was investigated.

MATERIALS AND METHODS

I. MATERIALS:-

Tested Compounds:-

Pyrazophos, 30 % EC; Hoechst Company, West Germany; Glyphosate, 48 % EC.; Monsanto Company, USA; Fenvalerate, 20 % EC, Sumitomo Chemical Company, Japan and Copper Sulfate, chemical pure grade.

Tested animals:-

The common carp (Cyprinus carpio L.)was collected from Saft Khalid farm at Etay Al-Barood, Behera Governorate. The fish was kept in large tanks 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 intr-oduced.

II. METHODS:-

1 - Acute Toxicity of Tested Pesticides to Common Carp:-

The experiments were performed with healthy common carp approximately 6-10 cm length and 10-20 gm weight. Fishes were fasted 24 hours before treatment and during the 96 hours test period. The median lethal concentrations (LC_{50}'s)of the tested pesticides in their formulated form were measured using series of concentrations. Ten fishes were used in each replicate and three replicates for each treatment. Mortality was recorded after 96 hours. The fish toxicity data were treated by the probit analysis method of Finney (1971) to determine the LC_{50}'s and 95 % confidence limits, and slope of regression lines .

2 - Toxicological Studies:-

a) Distribution of some enzymes in different organs of fish:-

A pilot study was carried out to measure activity and distribution of AChE, APase, GST, GOT and GPT in brain, liver, kidney, gill and muscle of three different fishes.

b) In vivo effects of half LC_{50} and LC_{50} levels of tested compounds on some enzymes in fish:-

Experimental animals were divided into nine groups (ten fishes each). Eight groups were treated with half LC_{50} or LC_{50} of each of the tested pesticides. The ninth group was used as a control. Three fishes each from control and treatment were killed after 96 hours. Then, brain, liver, kidney, gill and muscle were removed rapidly, cleaned and weighed. Each tissue was homogenized in 10 volumes (W/V) of physiological saline solution. The homogenates were centrifuged at 6,000 x g for 30 minutes at 4°c. The supernatants were used for measurement of each enzymatic activity. The activity of AChE was measured spectrophotometrically using acetyl thiocholine iodide as substrate (Ellman et al., 1961). APase was determined according to Bessey et al.(1946). GST was assayed by the method of Vessey and Boyer (1984). Activities of GOT and GPT were assayed according to Reitman and Frankel method (1957) using a commercially available kit from BioMerieux . Protein was determined according to Lowry et al. (1951).

RESULTS AND DISCUSSION

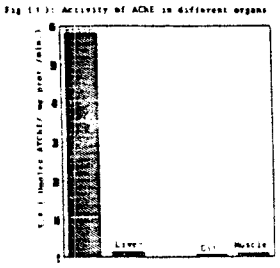
I . Comparative Toxicity of Four Pesticides to Common Carp:-

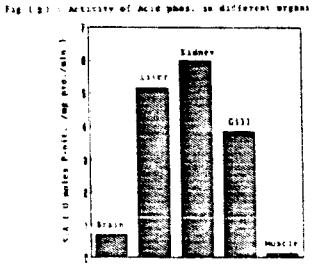
In the present investigation four formulated pesticides were tested against common carp (Cyprinus carpio L.) to determine the toxicity of these compounds (table 1). The LC_{50} values of pyrazophos, glyphosate, fenvalerate and copper sulfate were 6.1°, 5.89, 3.98 x 10^{-3} and 11.75 ppm, respectively. It is clear that fenvalerate was the most toxic compound tested. It is well known that pyrethroid insecticides, including fenvalerate, are highly toxic to many aquatic biota; Khan (1983) and Schimmel et al. (1983). Possible explanations for the high toxicity of pyrethroids to fish include; sensitivity at the site (s) of action, highly efficient uptake of insecticides across the gill, and inefficient metabolism and elimination.

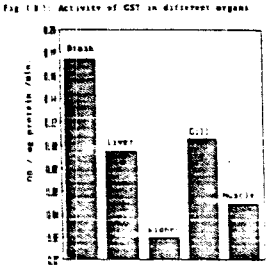
Table (1): Toxicity of tested compounds on common carp (Cyprins carpio L)after 96 hr. according to Finney methods of analysis.

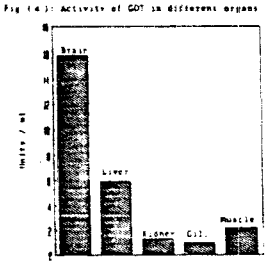
Pesticides	Regression of response (y) on log dose (X)	Calculated LC_{50} (ppm)	LC_{50} (95% con limits) (ppm)	M LC_{50} x 10^{3} *
Pyrazophos	y = 177.465 + 232.21 X	6.106	6.092 --- 6.123	16.4
Glyphosate	y = - 5.7 + 13.9 X	5.89	5.68 --- 6.105	34.8
Fenvalerate	y = - 4.37 + 15.77 X	3.98×10^{-3}	(3.82 - 4.15) x 10^{-3}	2.94×10^{-2}
$CuSO_4$	y = 0.195 + 4.51 X	11.75	10.36 --- 12.57	47.1

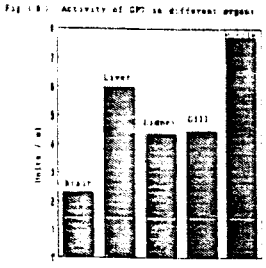
* M LC_{50} : Molar LC_{50} = LC_{50} /molecular weight of compound.











Glyphosate was less toxic to common carp than pyrazophos by about 2.1 times when comparing the molar LC_{50}. Most organophosphorus compounds showed their toxic effect against the aquatic system; Folmar et al. (1979) and Cleveland et al. (1986).

Copper sulfate was considered the least toxic compound when compared with the other tested pesticides against common carp. Pyrazophos, glyphosate and fenvalerate were 1.9 , 2.0 and 2.952 times, respectively more toxic to common carp than Cu SO_4. Torres et al.(1987) found that acute copper toxicity to dogfish (S. canicula) after 24 and 48 hours were 16 and 4 mg/L respectively.

II. Toxicological Studies:-

1. Cholinesterase

Cholinesterase activity was surveyed in different organs of fish. The results detected that brain contained extremely high activity (58.08 µ moles acetyl thiocholine iodide hydrolyzed/mg protein/minute) in comparison with other organs that contains almost negligible activity (Fig 1).
The two tested organophosphorus compounds; pyrazophos and glyphosate caused 78.36 % and 49.71 % inhibition by half LC_{50} and 80.73 % and 40.5 % inhibition by LC_{50}, respectively (table 2 and 3) . On the other hand, the other two pesticides (Fenvalerate and Cu SO_4) caused much less inhibition. It is well known that organophsphorus compounds produce their acute toxic action by inhibiting cholinesterase (O'Brien, 1969 and Aldridge, 1971). The data agrees with the results of Rattner and Franson (1984).
In conclusion, this study pointed out that inhibition of AChE in fish exposed to organophosphorus compounds may serve as indicator of the hazard due to application of these chemicals in the environment.

2. Acid Phosphatase:-

It is quite clear from figure 2 that kidney and liver contained the highest activities of acid phosphatase. Table 2 and 3 show that both concentrations (½ LC_{50} and LC_{50}) of all tested compounds stimulated acid phosphatase activity in kidney and liver. And the higher the concentration of pyrazophos and glyphosate the higher the activity of APase and vise versa in the case of fenvalerate and Cu SO_4. Also, the induction of APase in the liver was higher than that in kidney. These finding are supported by similar observations,in other studies (Sexena and Sarin, 1980 and El-Sebae, et al., 1987). Other investigators found that pollutants did not affect or decrease the activity of APase Micol, et al. 1980) . It is known that the detoxification of the toxic material which enter the body occurs mainly in the liver, Hinderer and Menzer 1976. The toxic effects of the four tested pesticides could

Table 2- *In vivo* effects of half LC_{50} of tested pesticides on different enzymes in different organs of common carp (*Cyprinus carpio* L.) after 96 hr.

Enzymes	Organs	Specific activity (mean± S.D)				
		Control	Pyrazophos	Glyphosate	Fenvalerate	$CuSO_4$
AChE	Brain	58.0800±2.6000	12.5800±1.1000**	29.2170±4.5050**	48.0830±4.8250	51.3[illegible]0±2.8700
APase	Kidney	6.0000±0.5450	6.3030±0.1793	7.3300±0.1815*	6.3570±0.2150	7.6700±0.5200*
	Liver	5.1500±0.6700	5.6670±0.3000	6.8570±1.2900*	9.4030±2.7050**	7.8100±1.0850**
GST	Brain	0.1743±0.0051	0.1269±0.0100*	0.1230±0.0020*	0.0970±0.0190*	0.1217±0.0110*
	Gill	0.1060±0.0100	0.0857±0.0070	0.0768±0.0170*	0.0640±0.0040*	0.0430±0.0018**
	Liver	0.0970±0.0017	0.1210±0.0010**	0.1960±0.0280**	0.1100±0.0100	0.1660±0.0080**
GOT	Brain	15.8400±1.1800	9.3000±0.0000*	10.4700±1.0700**	10.0400±0.4400*	12.5830±0.1258*
	Liver	5.8700±0.1600	9.9400±0.7500**	10.6170±1.2850**	6.4617±0.2995	7.0830±0.4450*
GPT	Muscle	7.6970±0.2200	18.4200±1.6800	16.8000±0.2000**	12.0200±0.0200**	14.7000±0.6000**
	Liver	6.0100±0.4000	6.2500±0.1500	1.1700±0.1150	2.5470±0.1286**	1.2750±0.0750**

S.A of enzymes
AChE : Umole ATChI/mg protein/min.
APase : Umole p-nitrophenol/mg protein/min
GST : OD/mg protein/min
GOT & GPT : units/ml
* Significantly different from control ($P < 0.05$)
** Highly significant different from control ($P < 0.05$)

Table 3- *In vivo* effects of LC_{50} of tested pesticides on different enzymes in different organs of common carp (*Cyprinus carpio* L.) after 96 hr.

Enzymes	Organs	Specific activity (mean ± S.D)				
		Control	Pyrazophos	Glyphosate	Fenvalerate	$CuSO_4$
AChE	Brain	58.0800±2.6000	11.1970±0.5900**	34.5500±5.8700**	35.8100±1.8400**	47.8000±0.0000
APase	Kidney	6.0000±0.5450	6.4830±0.2550	8.9300±1.7850*	6.0530±0.0160	7.1200±0.6800
	Liver	5.1500±0.6700	7.9500±0.1600**	9.5700±1.0420**	6.9530±0.6750*	7.0983±0.1330
GST	Brain	0.1743±0.0051	0.1363±0.0010*	0.1584±0.0060	0.1391±0.0078*	0.1353±0.0045*
	Gill	0.1060±0.0100	0.0923±0.0010	0.0962±0.0040	0.0931±0.0025	0.0423±0.0042**
	Liver	0.0970±0.0017	0.1800±0.0100**	0.1100±0.0100	0.1000±0.0000	0.1740±0.0260**
GOT	Brain	15.8400±1.1800	10.1730±0.5980*	7.8570±0.2450**	8.8250±0.5750**	11.4330±2.1500*
	Liver	5.8700±0.1600	6.9930±1.0250	5.9700±0.1150	5.9850±0.0230	7.6070±.10500*
GPT	Muscle	7.6970±0.2200	16.7400±0.7400*	8.8000±0.6000	15.9900±0.4900**	17.2000±0.2000**
	Liver	6.0100±0.4400	6.7400±0.1400	1.1970±0.2040**	1.9070±0.1100**	1.5970±0.1250**

- Specific activities of these enzymes expressed by:

AChE : Umole ATChI/mg protein/min.
APase : Umole P-nitrophenol/mg protein/min.
GST : OD/mg protein/min.
GOT & GPT : Units/ml

* Significantly different from control ($P < 0.05$)
** Highly significant from control ($P < 0.05$)

affect the liver cells and consequently its function, resulting in the release of APase enzyme in the fish body.

3. Glutathione S- transferase:-

According to the preliminary study (fig.3), brain, gill and liver contained the highest level of GST. Tables 2 and 3 showed that the tested compounds in two concentrations caused inhibition of GST in brain and gill of fish. It was shown that the percent inhibition of GST with LC_{50} of the tested compound was less than that with half LC_{50}. Cu SO_4 reduced GST activity significantly in both brain and gill, but more in gill. It is quite clear that tested compounds posses stimulatory effects on liver GST. The highest activation reached about 102 % with half LC_{50} of glyphosate. Our results are in agreement with the results of Oeschet, el al. (1982). While they contradict that reported by Vessey and Boyer (1984) who found that herbicides 2, 4-D and 2,4,5-T inhibit rat liver GST.

GST is thought to play a physiological role in the detoxification and elimination of toxic and undesirable foreign compounds including pesticides and drugs. (Chasseaud, 1973). In the present study, it is found that liver GST increased in fish intoxicated with tested pesticides to protect the fish from pesticide injury (Smith, et al. 1977).

4. Transaminases:-

It was evident that the brain contained the highest activity of GOT followed by liver, while gill contained the least activity (fig 4). The activity of GOT in liver was about 5.87 fold that in gill. The highest activity of GPT was found in muscle followed by liver. Gill and kidney posses about the same level of GPT activity (fig.5) .

From all recorded data (tables 2 and 3) the common trend showed that GOT activity was significantly inhibited in brain,but showed spontaneous activation in liver after treatment with the tested pesticides. The percentage inhibition of brain GOT increased by increasing the concentration of all pesticides except pyrazophos. It is quite clear, that half LC_{50} of both pyrazophos and glyphosate caused the greatest activation of liver GOT (69.34 % and 80.86 %, respectively). It was found, that all the tested compounds caused highly significant stimulation in muscle GPT except LC_{50} of glyphosate, which caused slight stimulation (14.32 %). Glyphosate, fenvalerate and Cu SO_4 caused highly significant inhibition of liver GPT after 96 hours of exposure. The disruption of transaminases from the normal values denote biochemical impairment and lesions of tissues and cellular function because they are involved in the detoxification process, metabolism and biosynthesis of energetic macromolecules for different essential

functions (Tordior and Van Heemstra - Lequin, 1980).

In conclusion, the liver was seriously affected by the four tested pesticides. The damage of the liver cells was accompanied by an increase of APase, GOT and GPT levels.

Direct and indirect contamination of water by these pesticides may cause fish killing, reduced fish reproductivity and elevated concentration of undesirable chemicals in edible fish tissues. Therefore, there is an increasing need to minimize the adverse effects of these compounds on environmental quality. These chemicals should also be restricted to field of agriculture far from lakes and rivers.

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الملخص العربي

قدرت التركيزات من مبيدات البيرازوفوس والجليفوسات والفنفاليرات وكبريتات النحاس المسببه لموت ٥٠ / من سمك البروك بعد ٩٦ ساعه من التعرض لها وكانت كالتالى :- ٦ر١٠٦ و ٨٩٠ ر ٥ ٥ ٩٨ ر ٣ × ١٠-٣ ٧٥٥ ر ١١ ميكروجرام لكل لتر على التوالـــى ٠

وقدر نشاط انزيمات الاسيتايل كولين استريز والاسيد فوسفاتيز والجلوتاثيون أس ترانس فيراز والترنس أمينيز فى مخ وكبد وكليه وخياشيم وعضلات السمك للمقارنه كما درس تأثير التركيز المسبب لموت ٥٠ / من الافراد ونصفه من المبيدات المستخدمه على هذه النظم الانزيميه وقد وجـــد ان البيرازوفوس والجليفوسات سببا أعلى تثبيط لانزيم الاسيتايل كولين استريز فى المخ ٠ بينما حدث تنشيط لانزيم الاسيد فوسفاتيز فى الكبد والكليه بالمركبات المختبره ٠ انزيم الجلوتاثيون اس ترانس فيراز قـــل نشاطه فى المخ والخياشيم وزاد نشاطه فى الكبد للاسماك المعامله بالمركبات ٠ بينما سببت المركبـــات المختبره زياده نشاط انزيمات الترانس أمينيز فى كل من الكبد والعضلات وانخفاض النشاط فى المخ ٠