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Full Length Research Paper

Acute toxicity and haematological changes in common carp (*Cyprinus carpio)* caused by diazinon exposure

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The common carp (*Cyprinus carpio*) was exposed to diazinon to determine the lethal concentration (LC_{50}) values at different times and effects of sub-lethal concentrations on haematological and biochemical parameters. The LC_{50} values at varying times were recorded as high as 18.57 mg/l (24 h), 14.87 mg/l (48 h), 12.29 mg/l (72 h) and 9.76 mg/l (96 h). Fishes exposed to sub-lethal concentrations (0.5, 1.0 and 2.0 mg/l) for four weeks revealed that the pesticide causes alterations in various blood parameters. Red blood cell (RBC) counts, haemoglobin concentration and haematocrit values increased whereas white blood cell (WBC) count decreased. Plasma glucose level, glutamate-oxaloacetate transminase (PGOT) and plasma glutamate pyruvate transminase (PGPT) activities increased in the fish exposed to diazinon. Protein level in plasma and glycogen in liver and muscles were found to decrease in treated fish with diazinon.

keyword: Bioassay, Biochemical and Haematological Changes, Diazinon, *Cyprinus carpio.*

INTRODUCTION

Toxic discharge to the environment from industrial processes, mining, and agricultural developments may have detrimental effects on aquatic animals (Beijer and Jornelo, 1979; Hamilton and Mehrle, 1986). Pesticides are one of the pollutants causing great harm to aquatic animals including fishes. Diazinon (0,0-diethyl 0-[6-methyl-2(1-methylethyl)-4-pyrimidinyl], an organo-phosphate insecticide, has agricultural and commercial uses (Abass et al., 2011). This pesticide is used to control a variety of insects including aphids, beetles, scales and pill bugs, primarily in household environment and agriculture crops (Cox, 2000; Cong et al., 2009). Similar to other insecticides, diazinon is toxic because it inhibits the activity of enzymes (Fulton and Key, 2001; Oruce and Usta, 2007). Most of several studies have already demonstrated the affect of diazinon for fish (Dutta and Meijer, 2003; Aydin and Koprocu, 2005; Lecoeur et al., 2006; Uner et al., 2006). Bananee et al. (2008, 2011) have performed an extensive work on the effects of diazinon on haematological and biochemical parameters of *Cyprinus carpio* and *Onchorhynchus mykiss*. Effects of diazinon on the liver function and immunological system of Nile tilapia was studied by Giron-Pirez et al. (2007).

Diazinon is widely used in agricultural field,date palm trees and homes for the control of different types of pests in Saudi Arabia (Personal Obs.). The published report shows the presence of diazinon in Saudi soil as high as 0.024 mg/kg (Al-Wabel et al., 2011). Its concentration in natural groundwater is reported to range from 0.32 to 3.286 µM (Mohamed et al., 2009). The concentrations (0.002-0.03 µg/kg of wet weight) of diazinon in the muscles of different fish cultured in Riyadh region were reported by El-Saeid (2010). The author is not aware of any published report regarding its concentration in surface water of Riyadh area.

Fish appear to possess the same biochemical path-ways to deal with the toxic effects of endogenous and exogenous agents as do mammalian species (Lackner, 1998). It is important to examine the toxic effects of pesticides on fish since they constitute an important link in food chain and their contamination by pesticides imbalances the aquatic system. Fish form an important part of human food (Oruce and Usta, 2007). Pollutants like pesticides affect the fish in various ways mostly by damaging different vital organs. Gills are the first organs to be exposed to water-born contaminants (Gallagher and Digiulio, 1992). Kidney plays a vital role in the maintenance of an organism's internal environment being the key to the regulation of extracellular fluid volume and composition as well as acid-base balance. It is also a

target of toxic chemicals which can disrupt its functions, and cause temporary or permanent derangement of homeostasis (Miller et al., 2002).

Blood parameters are often measured when clinical diagnosis of fish physiology is applied to determine the sub-chronic effects of pollutants (Wedemeyer and Yasutake, 1977).

The use of haematological parameters (haemoglobin, haematocrit, blood cell counts, glycemia and ion concentrations), can indicate a physiological response to a contaminated environment (Dethloff et al., 2001). Moreover, the change in plasma glutamate-oxaloacetate transaminase (PGOT) and plasma glutamate pyruvate transaminase (PGPT) activities can also indicate the impacts of water pollution on fish (Bucher and Hofer, 1990). *C. carpio* is an economically important freshwater fish and commonly cultured in many parts of the world. In this study, an attempt was made to investigate the toxicity of diazinon to common carp (*C. carpio*) as it is increasingly used to control pests in agricultural field and homes; measured by its effects on mortality and changes in haemoglobin concentration, cell counts, haematocrit values, glucose and glycogen content and enzymes (PGOT and PGPT) activities.

MATERIALS AND METHODS

Specimens of common carp were procured from a fish farm located west of Riyadh, Saudi Arabia. The length and weight ranged from 12 to14 cm and 55 to 60 g, respectively. They were kept in glass aquaria for two weeks to get acclimatized to laboratory conditions. During the period of acclimation, the fish were fed a commercial fish food twice daily to satiety. The water conditions like temperature, pH, dissolved oxygen and hardness analyzed weekly were 22.5±1.5°C, 7.6±0.5, 6.6±0.4 mg/l and 235.5±4.5 mg/l as calcium carbonate ($CaCO_3$), respectively.

After two weeks, when the acclimation time was over, 30 aquaria each containing 30 L of water were stocked with ten fish per aquarium. Different concentrations (5, 7, 9, 11, 13, 15, 17, 19, and 21 mg/l) of diazinon (DIADEM®600EC, 600 g/L active ingredient diazinon, purchased from Atrachem, Al-Khober, Saudi arabia) were prepared by adding the required volume from the stock solution. A control set was run with the same number of fish and the same volume of water but without diazinon. The experiment was run in triplicates. The water was aerated and the feeding was stopped. Dead specimens were removed immediately and their numbers were recorded. The water in the aquaria was renewed daily. The lethal concentration (LC_{50}) for 24, 48, 72 and 96 h was computed by the probit method (Finney, 1971).

After finding the LC_{50}, the fish were exposed to three different sub-lethal concentrations (0.5, 1.0, and 2.0 mg/l, selection based on the 96 h LC_{50}) for four weeks. A control group was also run for the same time but without diazinon. The experiment was run in triplicates. The fish were fed once daily to satiety. Two fish from each aquarium were removed after every week during the experimental period. Blood samples were collected in heparinized vials (Crescent Diagnostics, Jeddah, Saudi Arabia) by cutting the caudal peduncle. Blood of two fishes was pooled to get enough quantity of blood. Samples of clotted blood were discarded. Haemoglobin was estimated by the cyano-methemoglobin method using a diagnostic hemoglobin kit (Crescent Diagnostics, Jeddah, Saudi Arabia). Hematocrit values were determined by using a micro-hematocrit centrifuge. RBC and WBC count was determined by using Neubar haemocytometer after diluting the blood with Dace's solution and Turk's solution, respectively.

The remaining blood was centrifuged at 6000 rpm for 10 min at 4°C and the collected plasma was stored at -20°C till analyzed. Glucose, total protein, calcium (Ca) and magnesium were analyzed using their respective kits (BIOMERIEUX, France). Ca ion reacts with methylthymol blue (MTB) and form Ca-MTB complex. The colour intensity of this complex is proportional to the quantity of Ca ions in the sample. Similarly, the magnesium ion reacts with calmagite and form a complex. The quantity of magnesium ion is measured from the colour intensity of this complex which is proportional to the quantity of magnesium ions in the sample. A spectrometer was used to measure the enzyme activities. The activities of PGOT and PGPT in plasma were estimated using a Diagnostic Kit (BIOMERIEUX, France) and the results were expressed as IU/l. Liver and muscle glycogen was extracted by the method of Ashman and Seed (1973) and measured by the method of Montgomery (1957).

For statistical analysis, the one-way analysis of variance (ANOVA) was applied to test the significance of difference among the different values (Minitab, V-10.0). P values less than 0.05 were considered statistically significant.

RESULTS AND DISCUSSION

Mortality as a function of diazinon is given in Table 1. The LC_{50} values computed from the graph figure 1. made between probit of kill and $\log_{10}$ concentrations were 18.57 mg/l for 24 h, 14.87 mg/l for 48 h, 12.29 mg/l for 72 h and finally 9.76 mg/l for 96 h. The value recorded in this study was higher than the value recorded (0.72×10^{-4} ppm) for the fry of this fish by Dembele et al. (2000). Office of Pesticide Programs (2000) reported 96 h LC_{50} values of diazinon for sheephead minnow (*Cyrinodon virginica*) as 1.47 mg/l and for fathead minnow (*Pimephales minnow*) as 7.8 mg/l. Keizer et al. (1991) estimated 96 h LC_{50} value of diazinon for guppy (*Poecilia reticulate*) as 0.8 mg/l and for zebrafish (*Brachydanio rerio*) as 8.0 mg/l. The LC_{50} value for fingerling European catfish was estimated as 4.14 mg/l by Koprucu et al. (2006). This difference in the values may be attributed to the factors like hardness of water, pH and susceptibility of the test animals. The difference in the toxic potential of diazinon to different species can be related to the differences in susceptibility and tolerance related to its accumulation, biotransfor-mation and excretion. Differences in metabolic pathways among species may result in varied patterns of bio-transformation, leading to more or less toxic metabolites (Johnson and Toledo, 1993). The magnitude of toxic effects of pesticides also depends on length and weight, corporal surface/body weight ratio and breathing rate (Singh and Narain, 1982; Murty, 1986). Oh et al. (1991) reported three factors causing the selective toxicity of diazinon for various fish species: varied inhibition of acetylcholinesterase, detoxification and absorption. In general the toxicity varied with respect to species, size of fish and duration of exposure (Oh et al. 1991; Dutta et al., 1995).

Blood parameters, generally, of fish are suitable tool for

Table 1. Number and percentage (in parentheses) of dead fish in different concentrations of diazinon at different time.

Concentration (mg/l)	Time (h)			
	24	48	72	96
5.0	-	-	-	4 (13.33)
7.0	-	-	5 (16.67)	10 (33.33)
9.0	-	5 (16.67)	9 (30.0)	16 (53.33)
11.0	2 (6.67)	9 (30.0)	16 (53.33)	20 (66.67)
13.0	5 (16.67)	14 (46.67)	19 (63.33)	26 (86.67)
15.0	9 (30.0)	17 (56.67)	25 (83.33)	30 (100)
17.0	16 (53.33)	24 (80.0)	30 (100)	ND
19.0	24 (80.0)	3 (100)0	ND	ND
21.0	30 (100)	ND	ND	ND

evaluating the effects of chemicals (Roche and Boge, 1996). Some investigators have also identified changes in several haematological parameters as indicators of metal exposure (Cyriac et al., 1989). However, these findings indicate that in *C. carpio,* sub-lethal chronic exposure to diazinon altered the blood parameters.

The fish exposed to different concentrations of diazinon indicated an increase in the RBC count, haemoglobin concentrations and haematocrit values compared to the control fish (Table 2). It is documented that under stress condition, fish become hyper active perhaps to get out of the stressful medium and would require an increased amount of oxygen to meet their energy requirement. Secondly, the fish secreted an increased amount of mucus to coat the body especially gills to get relief from the irritating effects of toxicant. This in turn reduces the gaseous exchange through the gill. Thus, an increased utilization of oxygen and reduced supply of it may cause a hypoxic condition in fish (Pandey et al., 1979 and Alkahem et al., 1998). In such hypoxic condition, there is a stress-mediated synthesis of more haemoglobin and release of new erythrocytes from the erythropoietic organs to improve the oxygen carrying capacity of blood (Schindler and DeVries, 1986; Murad and Mustafa, 1989; Alkahem, 1993; Al-Ghanim et al., 2008; Al-Akel et al., 2010). Majewski and Giles (1981) have attributed the increased hemoglobin in cadmium exposed fish to the toxicant induced hyperactivity and impaired gill function. Contrary to the result in this study, a decrease in the number of RBC, hemoglobin and hematocrit values of diazinon exposed fish was reported by Bananee et al. (2008, 2011) and related it to destruction of cells and/or decrease in size of cells due to the adverse effects of pesticide.

The total leukocyte count was decreased, which might be due to malfunctioning of the haematopoietic system caused by exposure to diazinon. Changes in the leukocyte system manifest in the form of leukocytosis with heterophilia and lymphopenia, which are characteristics of leukocytic response in animals exhibiting stress. Al-Kahem (1995) reported reduction in the WBC count of fish exposed to chromium and noted it to be a consequence of significant decline in the number of lymphocytes and thrombocytes. Reduction in the number of lymphocytes in trichlorfon exposed *O. niloticus* was attributed to fall in the delivery of these cells to the circulation because of reduced production or alternatively an increased rate of removal from circulation and subsequent rapid destruction of cells (Al-Kahem et al., 1998). Svoboda et al. (2001) and Jaffar Ali and Rani (2009) reported decreased leukocyte count in carp exposed to diazinon- based pesticide and tilapia exposed to phosalone, respectively.

Blood cell indices like mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) seem to be changes that are more sensitive and can cause reversible changes in the homeostatic system of fish. Fluctuations in these indices correspond with values of RBC count, hemoglobin concentration and packed cell volume. In the study a significant increase in these indices was noticed in *C. carpio* after exposure to diazinon. A similar response was noted in common carp and other freshwater fish exposed to acute toxic level of pesticides (Svoboda et al., 2001; Rao, 2010). Reduction in the concentration of Ca and magnesium ions were registered in the fish exposed to high dose of diazinon and in the last period of exposure. The pesticide may change the functions of vital organs like liver and kidneys, disrupting the homeostatic condition of the body. Similar observations were reported by Al-Akel et al. (2010) in the same species of fish after the exposure of dietary copper.

Depletion of glycogen content in the liver and muscles of diazinon exposed fish is presented in Table 2. Pronounced effects were registered in the fish at higher concentration and in the last period of exposure. Previous studies have shown that due to physical disturbance of fish (Nakano and Tomlinson, 1967), and stress of various toxicants and hypoxia (Al-Akel, 1994; Alkahem, 1996; Al-Ghanim et al., 2008), the glycogen level in liver and

Table 2. Effects of diazinon exposure on hematological parameters and biochemical composition of *C. carpio*.

Parameter	Concentration (mg/l)	Exposure time (week)			
		1st	2nd	3rd	4th
Erythrocytes (Cellx10^6/mm^3)	Control (0.0)	1.56±0.05^a	1.61±0.06^a	1.65±0.05^a	1.58±0.05^a
	0.5	2.01±0.05^a	2.12±0.04^a	2.14±0.04^a	2.43±0.06^b
	1.0	2.12±0.05^a	2.20±0.05^a	2.27±0.06^b	2.43±0.06^b
	2.0	2.15±0.06^b	2.21±0.06^a	2.45±0.05^b	2.51±0.05^b
Leucocytes (Cellx10^3/mm^3)	Control (0.0)	35.51±0.51^a	36.23±0.62^a	37.21±0.71^a	36.54±0.56^a
	0.5	35.02±0.51^a	34.56±0.58^a	35.65±0.68^a	35.01±0.64^a
	1.0	33.56±0.54^a	32.89±0.57^a	32.12±0.59^b	32.01±0.64^b
	2.0	32.45±0.61^b	32.01±0.43^b	31.25±0.59^b	30.54±0.24^b
Hematocrit (%)	Control (0.0)	33.06±0.75^a	34.76±0.54^a	33.44±1.02^a	34.21±0.62^a
	0.5	35.54±0.61^a	36.21±0.89^a	35.23±0.56^a	35.80±0.96^a
	1.0	36.65±0.96^a	37.35±1.12^b	38.21±1.05^b	37.65±1.10^b
	2.0	37.45±0.98^b	37.85±0.99^b	38.42±1.20^b	39.25±1.09^b
Hemoglobin (g/dl)	Control (0.0)	5.65±0.09^a	6.01±0.12^a	5.95±0.10^a	6.12±0.11^a
	0.5	6.11±0.11^a	6.15±0.13^a	6.35±0.10^a	6.56±0.09^a
	1.0	6.54±0.14^a	6.64±0.13^a	6.66±0.09^b	6.54±0.11^a
	2.0	6.94±0.09^a	6.89±0.08^a	7.24±0.12^b	7.54±0.12^b
MCV (fl/cell)	Control (0.0)	211.92±3.85^a	215.96±4.21^a	202.67±3.58^a	216.65±5.12^a
	0.5	176.54±3.54^b	170.65±4.21^b	164.45±4.23^b	148.64±3.56^b
	1.0	172.58±4.25^b	169.65±3.56^b	168.85±5.21^b	154.65±4.25^b
	2.0	174.19±4.23^b	171.75±4.25^b	156.86±5.65^b	156.75±4.95^b
MCH (Pg/cell)	Control (0.0)	36.24±1.85^a	37.33±1.25^a	36.06±2.01^a	38.74±1.75^a
	0.5	30.40±1.54^a	29.01±2.13^a	29.67±1.75^a	27.11±2.15^a
	1.0	30.85±2.15^a	30.18±2.35^a	29.34±1.85^a	26.91±2.15^a
	2.0	32.28±2.12^a	31.18±1.45^a	29.55±2.65^b	30.041.56^b
MCHC (%)	Control (0.0)	17.09±1.45^a	17.29±1.56^a	17.79±0.95^a	17.89±1.01^a
	0.5	17.19±0.85^a	16.47±0.65^a	18.02±1.25^a	18.23±0.98^a
	1.0	17.84±1.05^a	17.78±0.96^a	17.42±0.85^a	17.37±0.75^a
	2.0	18.52±1.25^b	18.20±1.35^a	18.84±1.65^a	19.21±1.45^b
Total Protein (g/dl)	Control (0.0)	18.75±0.78^a	19.35±0.87^a	19.65±0.89^a	19.85±1.05^a
	0.5	18.65±0.86^a	19.65±0.68^a	20.25±1.22^a	20.56±0.96^a
	1.0	17.98±0.98^a	20.65±1.02^a	21.05±0.95^b	22.85±0.75^b
	2.0	19.05±0.88^a	20.85±0.75^a	21.25±0.86^b	23.60±0.85^b
Ca (mg/dl)	Control (0.0)	185.45±12.3^a	190.25±10.2^a	203.45±11.2^a	210.45±09.6^a
	0.5	175.45±10.1^a	170.25±11.2^a	178.95±12.3^a	204.65±12.4^a
	1.0	165.23±10.2^a	162.45±12.3^a	160.25±10.6^b	165.25±12.3^a
	2.0	155.65±13.1^b	160.35±09.6^a	158.65±08.9^b	162.35±10.8^b
Mg (mg/dl)	Control (0.0)	105.25±3.12^a	110.03±2.15^a	112.32±3.25^a	118.45±4.25^a
	0.5	98.45±2.05^a	105.24±3.25^a	115.24±4.25^a	120.54±3.45^a
	1.0	108.24±5.21^a	124.45±5.21^b	120.45±4.23^a	135.24±4.56^a
	2.0	106.23±5.21^a	122.56±3.45^b	125.45±5.24^b	140.25±4.75^b
PGOT (IU/l)	Control (0.0)	190.25±15.2^a	195.32±13.5 ^{a}	185.26±17.4^a	180.65±14.5^a
	0.5	195.25±13.3^a	201.25±16.2^a	198.23±15.2^a	201.35±15.3^a
	1.0	197.25±16.2^a	205.35±14.3^b	208.45±13.5^b	205.65±15.2^b
	2.0	201.25±14.2^a	210.25±16.2^b	225.35±14.2^b	235.12±14.2^b
PGPT (IU/l)	Control (0.0)	115.12±5.66^a	108.22±8.11^a	118.21±6.84^a	120.21±7.66^a
	0.5	135.21±8.55^a	125.44±6.88^b	132.23±8.25^b	140.25±8.25^a
	1.0	145.25±11.3^b	135.25±10.3^b	143.25±11.2^b	142.25±12.1^b
	2.0	159.25±11.2^b	142.35±12.1^b	149.25±11.1^b	154.23±11.3^b

Table 2. Continues.

Glucose (mg/100ml)	Control (0.0)	65.25±6.25[a]	68.35±5.68[a]	66.52±5.88[a]	65.95±6.88[a]
	0.5	75.25±6.55[a]	78.25±8.25[a]	75.55±5.85[a]	80.25±7.88[b]
	1.0	85.25±7.68[b]	84.65±8.25[b]	88.54±7.75[b]	90.25±8.52[b]
	2.0	90.25±8.56[b]	92.25±8.54[b]	100.25±9.25[b]	105.25±8.25[b]
Liver glycogen (mg/g)	Control (0.0)	8.183±0.12[a]	7.920±0.21[a]	7.900±0.17[a]	7.913±0.18[a]
	0.5	7.756±0.17[a]	7.565±0.18[a]	7.456±0.16[a]	7.443±0.18[a]
	1.0	7.245±0.16 [b]	7.195±0.19[b]	7.265±0.16 [b]	7.125±0.18[b]
	2.0	7.126±0.15[b]	7.012±0.17[b]	7.105±0.15[b]	6.865±0.18[b]
Muscle glycogen (mg/g)	Control (0.0)	2.465±0.08[a]	2.375±0.06[a]	2.375±0.06[a]	2.381±0.05[a]
	0.5	2.235±0.06[a]	2.205±0.06[a]	2.107±0.07[b]	2.174±0.05[a]
	1.0	2.155±0.06[b]	2.124±0.05[b]	2.105±0.05[b]	2.100±0.05[b]
	2.0	2.085±0.05[b]	2.065±0.05[b]	1.962±0.06[b]	1.955±0.05[b]

Treated values in columns with different letters show significant ($P < 0.05$) difference from control.

muscle are rapidly depleted. Glycolysis in the fish exposed to different toxicants may be expected to meet the energy requirements of the animal for the increased level of physical activities and increased physiological processes for metabolizing and eliminating the toxicant. The increased secretion of adrenocorticosteroids and catecholamines in stressful condition may also be one of the causes for reduction in the carbohydrate levels (glycogen). The significant elevation of glucose level in the blood of exposed fish may be due to the break down of glycogen into glucose. It is a well-established fact that stress stimuli elicit rapid secretion of glucocorticoids and catecholamines from adrenal tissue of the fish. Both hormones are known to produce hyperglycemia in animals. The hyperglycemic condition in this study may be related to increased secretion of these hormones which causes glycolysis in the liver and muscles of diazinon exposed fish.

PGOT and PGPT are important diagnostic tools in medicine and are used to detect the toxic effects of various pollutants (Nelson and Cox, 2000). Our results show that diazinon exposure significantly ($P<0.05$) increase the activity of both these enzymes of *C. carpio* after two weeks at higher dose (1.0, 2.0 mg/l) and after four weeks in all exposed groups. Metal exposure at various concentrations increases the activity of these enzymes as shown by McKim et al. (1970), Suttles and Mills (1966). Jeney et al. (1991) reported an increased level of these two enzymes [serum glutamic-oxaloacetic transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT)] in the serum of fish exposed to toxicant (ammonia). They mentioned that SGPT is very sensitive to any change in the environment. Similarly, a significantly higher value of SGOT activities were recorded by Lemair et al. (1992) in fish fed a diet without docosahexaenoic acid, whereas SGPT did not show any change. They found that hepatic parenchyma develop into generalized massive steatosis, exhibiting necrosis centers with docosahexaenoic acid free diet. Sastry and Sharma (1980) and Agrahari et al. (2007) observed an increase in SGOT and SGPT in the blood of *C. punctatus* following treatment with mercuric chloride and monocrotophos, respectively. Vaglio and Landriscina (1999), Gupta and Paul (1978) and Palanivelu et al. (2005) suggested that liver is rich in SGOT and SGPT, and damage to it could result in liberation of large quantities of these enzymes into the blood. An increase in these enzymes after exposure to pollutants is a sensitive indicator of cellular damage (Van-Dar et al., 2003; Palanivelu et al., 2005). Therefore, higher activities of these enzymes registered in this investigation may be ascribed to damage caused by diazinon to liver.

Conclusion

From the results obtained, it can be concluded that the diazinon is moderately toxic to *C. carpio*. The 96 h LC_{50} value recorded in this study falls well within the range reported in previous investigations for other species of fish. Exposure to chronic sub-lethal concentrations of diazinon resulted in significant haematological and biochemical alterations. These changes suggest that the treated fish are faced with a serious metabolic crisis. The elevated values of RBC count, hemoglobin concentration and hematocrit values in the exposed fish are indicative of stress mediated production of RBC and haemoglobin by the fish. The results clearly indicate that the usage of the pesticides in the fields may be a threat to both aquatic fauna and flora as well as humans.

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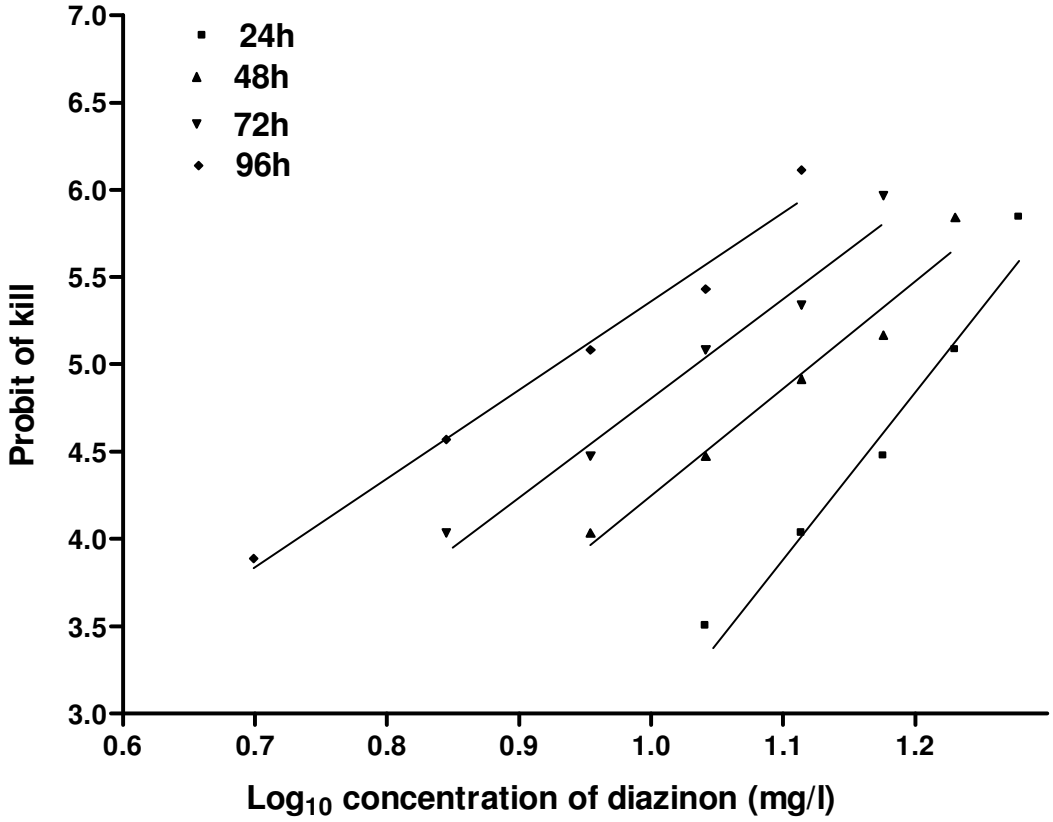

Figure 1. Relationship between probit of kill and $\log_{10}$ concentration of diazinon to find the LC_{50}.

Research Center.

Abbreviations

LC_{50}, Lethal concentration; **RBC**, red blood cell; **WBC**, white blood cell; **PGOT**, plasma glutamic-oxaloacetic transaminase; **PGPT**, plasma glutamate pyruvate transminase; **MTB**, methylthymol blue; **MCV**, mean corpuscular volume; **MCH**, mean corpuscular hemoglobin; **MCHC**, mean corpuscular hemoglobin concentration; **SGOT**, serum glutamic-oxaloacetic transaminase; **SGPT**, serum glutamate pyruvate transaminase.

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