Scientific Research and Essays Vol. 7(16), pp. 1674-1680, 30 April, 2012
Available online at http://www.academicjournals.org/SRE
DOI: 10.5897/SRE12.039
ISSN 1992-2248 © 2012 Academic Journals

Full Length Research Paper

Acute toxicity and effects of sub-lethal malathion exposure on biochemical and haematological parameters of *Oreochromis niloticus*

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Accepted 22 March, 2012

The specimens of *Oreochromis niloticus* were exposed to Malathion to determine the lethal concentration (LC_{50}) value and effects of sub-lethal concentrations on haematological and biochemical parameters. The LC_{50} value was registered as 1.06 mg/l. Fishes exposed to sub-lethal concentrations (0.12, 0.23 and 0.46 mg/l) for 6 weeks revealed that the pesticide causes alterations in various blood parameters. Red blood cell (RBC) and white blood cell (WBC) counts, haemoglobin concentration and haematocrit values were decreased. Plasma glucose and cholesterol level was elevated whereas plasma protein was decreased in exposed fish. Alanine amino-trasferase (ALT) activity was increased in the fish exposed to Malathion.

Key word: Malathion, bioassay, biochemical and haematological changes, *Oreochromis niloticus*.

INTRODUCTION

The use of pesticides is amplified many folds in recent years to enhance crop production and improve human health by controlling/eradicating unwanted insects, plants, animals as well as disease vectors (Prakasam et al., 2001). In United States of America, approximately 2 billion kg of pesticides are applied annually to forests, gardens, homes and agricultural lands (Aspelin and Grube, 1999). Most of the chlorinated compounds used in the past are replaced by organophosphorus compounds (OPS) because the persistence of latter in the environment is short. Malathion (O-dimethyl-S1-2-di (ethoxycarbonyl) - ethylphosphorodithioate) is an organophosphorus insecticide widely used in agriculture and houses to control variety of insects including aphids, beetles, scales and pill bugs. Apart from target specimens, non-target animals including fish are greatly affected by these pesticides (Al-Akel et al., 2010; Alkahem Al-Balawi, 2011). It is believed that the fish posses the same biochemical pathways to deal with the toxic effects of endogenous and exogenous agents as do mammalian species (Lackner, 1998; Al-akel et al., 2010; Ahmad, 2011). Therefore, 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 imbalance the aquatic system. Survey of literature indicates that only few investigations (Osman et al., 2010, 2011) describing the concentration of this pesticide in the green vegetables (Qaseem region) has been published from Saudi Arabia. Degradation of malathion in aqueous solution has been worked out by Mohamed et al. (2009) and Zhang et al. (2010). Author is not aware of any literature pertaining to the contamination of Arabian surface and ground water with malathion. Its effects on animals other than fish were described by Relyea (2004), Uzun et al. (2009), Bakry et al. (2011) and Moore et al. (2011).

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; Venakataramana et al., 2006). The haematological parameters like haemoglobin, haematocrit, blood cell counts, glycemia and ion concentrations can be used to find physiological response of a contaminated environment (Dethloff et al.,

Table 1. Number of dead fish in different concentrations of Malathion at different time.

Concentration (mg/l)	Time (h)			
	24	48	72	96
Control (0.0)	-	-	-	-
0.75	-	-	2	5
1.00	-	3	5	13
1.25	-	2	9	18
1.50	3	6	14	23
1.75	3	9	18	26

2001). Alanine amino-transferase (ALT) is normally found within the cells of liver, heart, gills and kidneys (Shalaby, 2009) but its increase in plasma indicates the tissue injury or organ dysfunction (Wells et al., 1996). However, enzymatic, biochemical and haematological changes in fish exposed to different pollutants have been documented (Bucher and Hofer, 1990; Al-Attar, 2005; Ogueji and Auta, 2007; Aker et al., 2008; Cristina et al., 2008; Adedeji et al., 2009; Shalaby, 2009; Abalaka et al., 2011; Ahmad, 2011; Al-Kahem Al-Balawi et al., 2011).

Oreochromis niloticus is an economically important freshwater fish and commonly cultured in Saudi Arabia. In the present study, an attempt was made to investigate the toxicity of malathion to tilapia, *O. niloticus*, as measured by its effects on mortality and changes in haemoglobin concentration, cell counts, haematocrit values, glucose, cholesterol, protein content and enzyme (ALT) activity.

MATERIALS AND METHODS

Specimens of *O. niloticus* were procured from a fish farm located at Al-Kharj, south of Riyadh. The length and weight ranges from 12 to 14 cm and 55 to 60 g, respectively. They were kept in glass aquaria (160 × 55 × 60 cm) for 2 weeks to get acclimatized to laboratory conditions. During the period of acclimation, the fish were fed with a commercial fish food twice daily to satiety. The water conditions like temperature, pH, dissolved oxygen and hardness analyzed weekly were 23.5±1.5°C, 7.8±0.5, 7.5±0.4 ppm and 230.5±4.5 ppm as $CaCO_3$, respectively.

After 2 weeks, 10 fishes were transferred to each aquarium (55 × 30 × 35 cm) containing 30 L of water. Different concentrations (0.75, 1.00, 1.25, 1.50 and 1.75 mg/l) of malathion were prepared by adding required volume from the stock solution. Commercial grade malathion with 57% active ingredient was obtained from Delta company, Riyadh. A control set was run with same number of fish and same volume of water. The experiment was run in triplicates. The feeding was stopped and water was aerated with mechanical pump. Dead specimens were removed immediately after death and their numbers registered. The medium of aquaria was renewed daily. The 96 h lethal concentration (LC_{50}) was computed by the method of Finney (1971).

After finding the LC_{50}, the fish were exposed to three different sub-lethal concentrations (0.12, 0.23 and 0.46 mg/l) for 6 weeks in triplicates. A control set was also run for the same time but without malathion. Two fish from each aquarium (three replicates) were removed after every 2 weeks during whole experimental period. Blood samples were obtained separately in heparinized vials by cutting the caudal peduncle. In case of insufficient quantity, blood of two or more fishes was pooled. Total 6 samples for each concentration were analyzed. Samples of clotted blood were discarded. Haemoglobin was estimated by the cyano-methemoglobin method using a diagnostic hemoglobin kit. Hematocrit values were determined by using a micro-hematocrit centrifuge. The red blood cell (RBC) and white blood cell (WBC) counts were determined by using neubar haemocytometer after diluting the blood with Dace's solution and Turk's solution, respectively.

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, triglyceride, cholesterol and ALT were analyzed using their respective kits (BIOMERIEUX, France).

The one way analysis of variance (ANOVA) was applied to test the significance of difference among the control and treated values. P values less than 0.05 were considered statistically significant.

RESULTS

Mortality of fish as a function of malathion is shown in Tables 1 and 2. The LC_{50} value computed from the graph (Figure 1) made between log_{10} concentrations and probit of kill was 1.06 mg/l**.** The present findings indicate that sub-lethal chronic exposure to malathion has altered the blood parameters of *O. niloticus*. The fish exposed to different concentrations of malathion indicated a decrease in the RBC and WBC counts, haemoglobin concentration and haematocrit values compared to control fish (Table 3). In the present study, a significant increase in different indices like mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) was noticed in *O. niloticus* after exposure of malathion. Hyperglycemia and hypoproteinaemia was evident in the blood of exposed fish. The level of cholesterol and triglyceride was significantly elevated in the fish exposed to malathion. Exposure of malathion markedly elevated the ALT activity in the fish (Table 4). The results were more pronounced in higher dose and in the last period of investigation.

Table 2. Per cent mortality of fish at various time intervals in different concentrations of Malathion.

Concentration (mg/l)	Time (h)			
	24	48	72	96
Control (0.0)	-	-	-	-
0.75	-	-	6.66	16.66
1.00	-	10.00	16.66	43.33
1.25	-	6.66	30.00	60.00
1.50	10.00	20.00	46.66	76.66
1.75	10.00	30.00	60.00	86.66

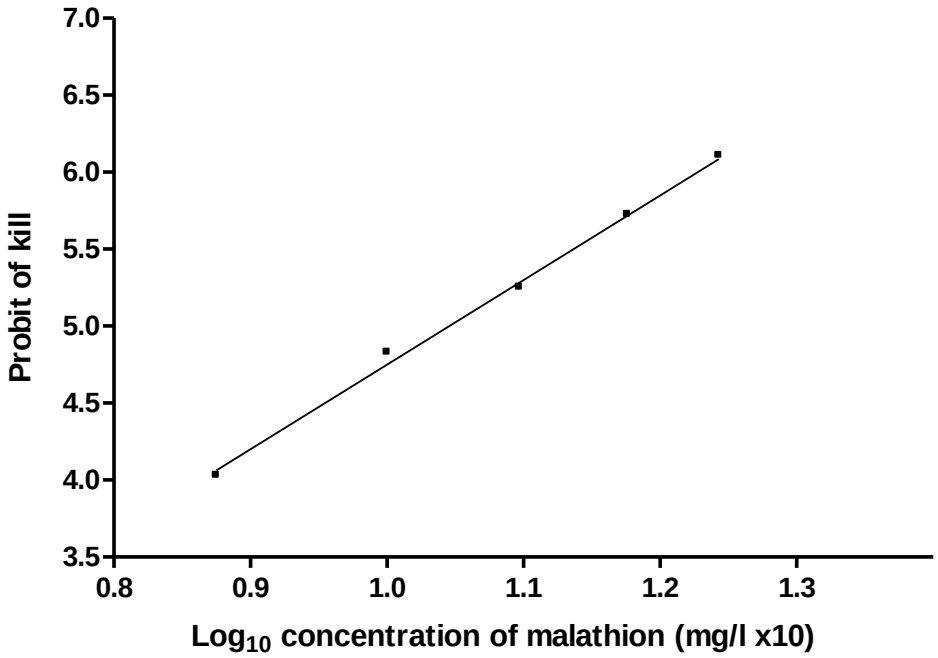

Figure 1. The relationship between probit of kill and $\log_{10}$ concentration of malathion to find the LC_{50}.

DISCUSSION

The LC_{50} value registered in the present investigation is quite less than the value (2.2 ppm) reported for this fish by Pathiratne and George (1998). Newhart (2006) listed the LC_{50} values of malathion for different species of fish which ranges from 0.06 to 7620 µg/l. Ivan et al. (2007) reported higher LC_{50} value (7.83 mg/l) of diazinon for same species *(O. niloticus)*. Malathion is highly toxic to fry of *Labeo rohita* (LC_{50} value 9 µl) as reported by Patil and David (2008). Similarly, Pugazhvendan et al. *(*2009) found that *Opheocephalus punctatus* is also sensitive to malathion (LC_{50} 16 µg/l). This difference in the toxic potential of the pesticide may be attributed to the factors like hardness of water, pH and susceptibility of the test animals. The difference in the toxic potential of malathion can be related to the differences in susceptibility and tolerance related to its accumulation, biotransformation and excretion. Differences in metabolic pathways among species may result in varied patterns of bio-transformation, leading to more or less toxic metabolites (Johnsson 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 pesticides 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 3. Effect of different concentration of malathion on blood parameters of *O. niloticus* at different time.

Time (weeks)	parameters	Concentration of malathion (mg/l)				P<0.05
		Control (0.0)	0.12	0.23	0.46	
2	RBC (Cell × 10ˆ6/MMˆ3)	1.75 ± 0.17	1.57 ± 0.25	1.34 ± 0.23	1.21 ± 0.57	***
	WBC (Cell × 10ˆ3/MMˆ3)	85.10 ± 2.69	87.70 ± 2.12	75.27 ± 7.27	68.25 ± 26.37	***
	Hb (g/100 ml)	8.55 ± 0.92	7.65 ± 1.34	6.73 ± 1.19	6.15 ± 2.33	***
	HCT (%)	24.70 ± 3.54	21.65 ± 4.88	19.20 ± 3.68	16.05±9.26	***
	MCV (µmˆ3)	140.75 ± 6.86	137.40 ± 8.91	143.50 ± 2.69	128.65 ± 16.62	***
	MCH (Pg)	48.85 ± 0.49	48.65 ± 0.64	50.57 ± 5.24	52.00 ± 5.09	***
	MCHC (g/dl)	34.70 ± 1.27	35.55 ± 1.77	35.30±3.80	40.95 ± 9.12	***
4	RBC (Cell × 10ˆ6/MMˆ3)	1.61 ± 0.19	1.47 ± 0.09	1.56 ± 0.20	1.50 ± 0.08	***
	WBC (Cell × 10ˆ3/MMˆ3)	86.30 ± 2.29	83.90 ± 5.20	80.17 ± 4.96	81.43 ± 6.71	***
	Hb (g/100 ml)	7.77 ± 0.92	7.40 ± 0.66	7.37 ± 1.35	6.13 ± 0.51	***
	HCT (%)	22.83 ± 1.53	21.00 ± 1.65	20.00 ± 3.10	20.03 ± 1.11	***
	MCV (µmˆ3)	142.10 ± 8.0	142.87 ± 4.7	147.30 ± 2.4	147.33 ± 0.64	***
	MCH (Pg)	48.17 ± 2.40	50.40 ± 1.92	47.27 ± 2.61	47.97 ± 0.89	***
	MCHC (g/dl)	34.00 ± 2.85	35.23 ± 2.12	35.07 ± 1.37	38.57 ± 0.55	***
6	RBC (Cell × 10ˆ6/MMˆ3)	1.79 ± 0.33	1.69 ± 0.18	1.34 ± 0.15	1.39 ± 0.42	***
	WBC (Cell ×10ˆ3/MMˆ3)	79.3 ± 16.97	79.25 ± 15.20	70.6 ± 1.13	70.5±8.34	***
	Hb (g/100 ml)	8.25±1.77	7.03 ± 0.31	7.00 ± 0.92	6.25±2.19	***
	HCT (%)	21.65 ± 4.03	22.9 ± 2.36	18.2±1.56	20.35 ± 5.86	***
	MCV (µmˆ3)	121.25 ± 0.35	135.27 ± 5.73	136.5 ± 3.95	138.35 ± 0.63	***
	MCH (Pg)	46.10 ± 1.27	47.7 ± 3.86	49.00 ± 1.41	48.85 ± 0.92	***
	MCHC (g/dl)	34.0 ± 1.13	35.23 ± 2.38	35.9 ± 1.98	35.25 ± 0.49	***

Values are mean± standard error.

evaluating the effects of chemicals (Roche and Boge, 1996). Past investigators have also identified changes in several haematological parameters as indicators of metal exposure (Cyriac et al., 1989). However, the changes in blood variables suggested that there was osmotic disturbances and change of oxygen carrying capacity during the exposure of pesticide. Similar to present results, a decrease in the number of RBC, hemoglobin and hematocrit values of diazinon (an organophosphate pesticide) exposed fish was reported by Banaee 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. Misra and Srivastava (1983) and Zaki et al. (2009) reported a decreased RBC count, heamoglobin concentration and packed cell volume (PCV) values in the fish exposed to Malathion. Adeyemo (2007) reported decreased haemoglobin, RBC count and haematorit values in *Clarias gariepinus* exposed to lead nitrate. Toxicants might cause an adverse effect on the haematopoietic organs which reduces the supply of RBC either due to less production and/or increased rate of removal from circulation. Fall in the level of haemoglobin may be the consequence of toxic effects of Malathion on the synthesis of this molecule. The insecticide may disrupt the synthetic pathway by affecting the activity of enzymes involved in the synthesis of haemoglobin. Consequently, reduces the level of haemoglobin of exposed fish.

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 MCV, MCH and MCHC seem to cause changes that are more sensitive and can cause reversible changes in the homeostatic system of fish.

Fluctuations in these indices correspond with values of

Table 4. Plasma biochemical composition of *O. niloticus* exposed to malathion.

Time (weeks)	Plasma	Concentration of malathion (mg/l)				P<0.05
		Control (0.0)	0.12	0.23	0.46	
2	Total protein (g/dl)	6.50 ± 1.52	5.60 ± 1.32	5.20 ± 1.65	5.00 ± 1.45	***
	Triglyceride (mg/dl)	135.45 ± 15.50	325.05 ± 30.50	370.45 ± 25.25	380.45 ± 31.45	***
	Cholesterol (mg/dl)	175.45 ± 15.45	305.50 ± 20.43	350.45 ± 20.43	360.45 ± 22.45	***
	Glucose (mg/dl0	52.45 ± 4.30	60.42 ± 3.56	62.35 ± 4.50	67.37± 5.20	***
	ALT (U/l0	45.45 ± 3.40	48.45 ± 3.21	55.46 ± 3.50	62.45 ± 4.25	***
4	Total Protein (g/dl0	6.81 ± 1.21	6.12 ± 1.02	5.82 ± 1.20	5.86 ± 1.02	***
	Triglyceride (mg/dl)	138.35 ± 10.25	326.22 ± 15.21	340.25 ± 18.25	360.35 ± 18.29	***
	Cholesterol (mg/dl0	179.35 ± 10.25	310.26 ± 15.25	340.25 ± 19.25	355.45 ± 14.86	***
	Glucose (mg/dl0	55.25 ± 5.25	60.35 ± 2.56	66.25 ± 13.45	65.45± 2.56	***
	ALT (U/l)	55.25 ± 3.65	65.25 ± 3.43	63.45 ± 4.25	67.45 ± 3.62	***
6	Total Protein (g/dl)	6.00 ± 1.41	5.00 ± 0.00	5.00 ± 0.00	4.50 ± 0.71	***
	Triglyceride (mg/dl)	133.00 ± 20.13	338.50 ± 26.16	355.00 ± 53.74	364.00 ± 14.14	***
	Cholesterol (mg/dl)	175.00 ± 14.61	316.50 ± 3.53	344.00 ± 15.97	485.50 ± 29.63	***
	Glucose (mg/dl)	49.50 ± 4.95	42.00 ± 2.83	41.00 ± 1.41	38.50 ± 2.12	***
	ALT (U/l)	48.17 ± 0.76	59.16 ± 0.75	61.33 ± 0.11	71.33 ± 0.76	***

Values are mean ± standard error.

RBC count, hemoglobin concentration and PCV. 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).

The significant elevation of glucose level (Table 4) in the blood of exposed fish may be due to increased demand for energy resulting from the mobilization of glycogen in glucose. It is well established fact that stress stimuli elicit rapid secretion of glucocorticoids and catecholamines from adrenal tissue of the fish (Pickering, 1981). Both hormones are known to produce hyperglycemia in animals. Such elevation may also be due to enhanced gluconeogenesis response of stressed fish in their attempt to satisfy their new energy demands (Winkalar et al., 2007). This study agrees with the findings of Abalaka et al. (2011), Ahmad (2011) and Alkahem-Al-Balawi et al. (2011). The hyperglycemic condition observed in present study may be related to increased secretion of these hormones which enhance the glycolysis in the fish exposed to malathion. A significant hypoproteinaemia was recorded in the fish exposed to malathion specially at higher concentration and in the last period of exposure. Similar results were reported by Omoniyi et al. (2002) and Shalaby (2009). They attributed this reduction to the cellular destruction or necrosis with subsequent impairment of protein synthesis machineries (Bradbury et al., 1987) or due to pathological alterations in kidney leading to excessive loss of proteins (Salah El-Deen et al., 1996). However, the hypoproteinaemia in the present study may be ascribed to aforementioned factors. In contrast to present findings, a hyperprotenaemia was reported by Al-Attar (2005), Omitoyin (2007) and Abalaka et al. (2011). It is obvious that fish exposed to malathion utilize glucose, glycogen and proteins for the fulfillment of increased energy requirements and fish try to spare the existing amount of cholesterol and triglyceride and/or synthesize more quantity of these compounds for future use.

Zaki et al. (2009) and Abalaka et al. (2011) have registered an elevated level of ALT activity in the fish exposed to malathion and extracts of Porkiabiglosa pods, respectively. They opined that this increased activity in the exposed fish are suggestive of hepatic damages leading to their leakage into circulation (Mousa et al., 2008) and/or increased synthesis of enzyme in the liver. Contrary to this, Sadhu et al. (1985), Sunmonu and Oloyede (2006), Okechukwu and Auta (2007) and Hedayati et al. (2010) found that there was inhibition of ALT activity in the fish exposed to different pollutants. These workers attributed the reduction in the enzyme activity to liver necrosis caused by toxicants and a possible damage to hepaptocytes or low sub-lethal doses of toxicants used to expose the fish.

Conclusion

Malathion seems to be moderately toxic to *O. niloticus.* The 96 h LC_{50} (1.06 mg/l) registered in the present investigation falls well within the values obtained for other fish species. It is an addition to the existing knowledge of biochemical and haematological alterations in fish due to

chronic sub-lethal exposure of malathion. The results obtained clearly indicated that the metabolism of macromolecule and haematopoietic organs of fish was adversely affected by malathion. The use of pesticide in the field may be a threat to human health and fauna and flora of the environment.

ACKNOWLEDGMENTS

The author gratefully acknowledge Research Centre, College of Sciences and Humanity Studies, Salman bin Abdul-Aziz University, Al-Kharj, Kingdom of Saudi Arabia for providing financial assistance to carry out the present research work.

REFERENCES

Abalaka SE, Esievo KAN, Shoyinka SVO (2011). Evaluation of biochemical changes in *Clarias gariepinus* adults exposed to aqueous and ethanolic extracts of *Parkia biglobosa* pods. Afr. J. Biotechnol., 10: 234-240.

Adedeji OB, Adeyemo OK, Agbede SA (2009). Effects of diazinon on blood parameters in the catfish (*Clarias gariepinus*). Afr. J. Biotechnol., 8: 3940-3946.

Adeyemo OK (2007). Haematological profile of *Clarias gariepinus* (Burchell, 1822) exposed to lead. Turkish J. Fish. Aquat. Sci. 7: 163-169.

Ahmad Z (2011). Toxicity bioassay and haematological changes induced by diazinon in common carp, *Cyprinus carpio.* Afr. J. Biotechnol., 10: 13852-13859.

Aker WG, Hu X, Wang P, Hwang HM (2008). Comparing the relative toxicity of Malathion and malaoxon in blue catfish Ictalurus furcatus. Environ. Toxicol., 23: 548-554.

Al-Akel AS, Alkahem-Al-Balawi HF, Al-Misned F, Mahboob S, Ahmad Z, Suliman EM (2010). Effects of dietary copper exposure on accumulation, growth, and haematological parameters in *Cyprinus carpio*. Toxicol. Environ. Chem., 92: 1865-1878.

Al-Attar AM (2005). Biochemical effects of short-term cadmium exposure on the freshwater fish, *Oreochromis niloticus*. J. Biol. Sci., 5: 260-265.

Alkahem HF (1995). Behavioral responses and changes in some haematological parameters of the cichlid fish, *Oreochromis niloticus*, exposed to trivalent chromium. J. King Abdul Aziz Univ. Sci., 7: 5-13.

Alkahem HF, Ahmad Z, Al-Akel AS, Shamsi MJK (1998). Toxicity bioassay and changes in haematological parameters of *Oreochromis niloticus* induced by trichlorfon. Arab Gulf J. Scient. Res., 16: 581-593.

Alkahem Al-Balawi HF, Ahmad Z**,** Al-Akel AS, Al-Misned F, Suliman EM, Al-Ghanim KA (2011). Toxicity bioassay of lead acetate and effects of sub-lethal exposure on growth, haematological parameters and reproduction in *Clarias gariepinus*. Afr. J. Biotechnol., 10: 11039-11047.

Aspelin AL, Grube AH (1999). Pesticide industry sales and usage 1996 and 1997 market estimates, EPA 733-R-99-001, Office of pesticide programs, Washington, DC.

Bakry FA, Hasheesh WS, Hamdi SAH (2011). Biological biochemical and molecular parameters of *Helisoma duryi* snails exposed to the pesticides Malathion and deltamethrin. Pest. Biochem. Physiol., 101: 86-92.

Banaee M, Mirvaghefi AR, Rafei GR, Majazi Amiri B (2008). Effects of sub-lethal diazinon concentrations on blood plasma biochemistry of common carp. Int. J. Environ. Res., 2: 189-198.

Banaee M, Sureda A, Mirvaghefi AR, Ahmadi K (2011). Effects of diazinon on biochemical parameters of blood in rainbow trout (*Onchorhynchus mykiss)*. Pest. Biochem. Physiol., 99: 1-6.

Bradbury SP, Symonic DM, Coats JR, Atchison GJ (1987). Toxicology of fenvalerete and its constituent isomers to the fathead minnow (*Piephales promeos*) and blue gill *(Lepomis macrochirus*). Bull. Environ. Cont. Toxicol. 38: 727-735.

Bucher F, Hofer R (1990). Effect of domestic wastewater on serum enzyme activities of brown trout (*Salmo truta*). Comp. Biochem. Physiol., 97: 85-390.

Cristina SA, Cristina MM, Anca D (2008). Malathion induced histological modifications in gills and kidney of *Carassius auratus gibelio*. Zootehnie Biotehnologie, 41: 448-453.

Cyriac PJ, Antony A, Nambison PNK (1989). Hemoglobin and hematocrit values in the fish *Oreochromis mossambicus* (Peters) after short term exposure to copper and mercury. Bull. Environ. Conta. Toxicol., 43: 315-320.

Dethloff GM, Bailey HC, Maier KJ (2001). Effect of dissolved copper on selected haematological, biochemical and immunological parameters of wild rainbow trout (*Oncorhynchus mykiss*). Archi. Environ. Conta. Toxicol., 40: 371-380.

Dutta HM, Munshi JSD, Dutta GR, Singh NK, Adhikari S, Richmonds CR (1995). Age related differences in the inhibition of brain acetylcholinesterase activity of *Heteropneustes fossilis* (Bloch) by malathion. Comp. Biochem. Physiol., 111A: 331-334.

Finney DJ (1971). Probit analysis. S. Chand and Company Ltd. Ram Nagar, Delhi.

Hedayati A, Safahieh A, Savari A, Marammazi JG (2010). Assessment of aminotransferase enzymes in yellow fin sea bream (*Acanthopagrus latus*) under experimental condition as biomarkers of mercury pollution. World J. Fish Mar. Sci., 2: 186-192.

Ivan GPM, Anne S, Fabiola GJ, Josefina CS, Marcela HC, Jorge PS, Akiro T, Galina Z (2007). Immunotoxicity and hepatic function evaluation in Nile tilapia (*Oreochromis niloticus*) exposed to diazinon. Fish Shellfish Immunol., 23: 760-769.

Jaffer Ali HA, Rani VJ (2009). Effect of phosalone on haematological indices in the tilapia, *Oreochromis mossambicus*. Turk. J. Vet. Anim. Sci., 33: 407-411.

Johnsson CM, Toledo MCF (1993). Acute toxicity of endosulfan to the fish *Hyphessobrycon bifasciatus* and *Brachydanio rerio*. Archiv Environ. Conta. Toxicol., 24: 151-155.

Lackner R (1998). Oxidative stress in fish by environmental pollutants. Ecotoxicol., pp. 203-224.

Misra J, Srivastava AK (1983). Malathion induced hematological and biochemical changes in the Indian catfish *heteropneustes fossilis*. Environ. Res., 30: 393-398.

Mohamed KA, Basfar AA, Al-Kahtani HA, Al-Hamad KS (2009). Radiolytic degradation of malathion and lindane in aqueous solutions. Rad. Phys. Chem., 78: 994-1000.

Moore PD, Patlolla AK, Tchounwou PB (2011). Cytogenetic evaluation of malathion-induced toxicity in Sprague-Dawley rats. Mut. Res. Gen. Toxicol. Environ. Mutag., 725: 78-82.

Mousa MMA, El-Ashram AMM, Hamed M (2008). Effects of Neem leaf extract on freshwater fishes and zooplankton community. 8th International symposium on tilapia in aquaculture. The Central Laboratory for Aquaculture Research, Cairo, Egypt. Oct. 12-14.

Murty AS (1986). Toxicity of pesticides to fish. CRC Press Inc Boca Raton. FL, p. 143.

Newhart KL, (2006). Environmental fate of malathion. California Environmental protection Agency. p. 20.

Ogueji EO, Auta J (2007). Investigations of biochemical effects of acute concentrations of Lamda-cyhalothrin on African catfish, *Clarias gariepinus* –Teugels. J. Fish. Int., 2: 86-90.

Oh HS, Lee SK, Kim YH, Roh JK (1991). Mechanism of selective toxicity of diazinon to killifish (*Oryzias latipes*) and loach (*Misgurnus anguillicaudatus*). Aquat. Toxicol. Risk Assess, 14: 343-353.

Okechukwu EO, Auta J (2007). The effects of sub-lethal doses of Lambda- cyhalothrin on some biochemical characteristic of the African catfish, *Clarias gariepinus.* J. Biol. Sci., 7: 1473-1477.

Omitoyin BO (2007). Plasma biochemistry changes in *Clarias gariepinus* (Buchell, 1822) fed poultry litter. Asian J. Anim. Sci., 7: 45-52.

Omoniyi I, Agbon AO, Sodunke SA (2002). Effects of lethal and sub-lethal concentrations of Tobacco (*Nicotiana tobaccum)* leaf dust extract on weight and haematological changes in *Clarias gariepinus* (Burchell) J. Appl. Sci. Environ. Manage., 6: 37-41.

Osman KA, Al-Humaid AM, Al-Rehiayani SM, Al-Redhaiman KN (2010). Monitoring of pesticide residues in vegetables marketed in Al-Qasim region, Saudi Arabia. Ecotoxicol. Environ. Saf., 73: 1433-1439.

Osman KA, Al-Humaid AM, Al-Rehiayani SM, Al-Redhaiman KN (2011). Estimated daily intake of pesticide residues exposure by vegetables grown in green houses in AL-Qssim region, Saudi Arabia. Food Control, 22: 947-953.

Pathiratne A, George SG (1998). Toxicity of Malathion to Nile tilapia, *Oreochromis niloticus* and modulation by other environmental contaminants. Aquat. Toxicol., 43: 261-271.

Patil VK, David M (2008). Behaviour and respiratory dysfunction as an index of Malathion toxicity in the freshwater fish *Labeo rohita* (Hamilton). Turkish J. Fish. Aquat. Sci., 8: 233-237.

Pickering AD (1981). Stress and compensation in teleostean fishes: Response to social and physical factors. In: Pickering AD (ed.) Stress and fish. Academic Press, New York, USA, p. 295-322.

Prakasam A, Sethupathy S, Lalitha S (2001). Plasma and RBCs antioxidant status in occupational male pesticide sprayers. Clin. Chem. Acta, 310: 107-112.

Pugazhvendan SR, Narendiran NH, Kumaran RG, Kumaran S, Alagapan KM (2009). Effect of malathion toxicity in the freshwater fish *Opheocephalus punctatus*-A histological and histochemical study. World J. Fish Mar. Sci., 1: 218-224.

Rao DS (2010). Carbaryl induced changes in the haematological, serum biochemical and immunological responses of common carp, *Cyprinus carpio,* (L.) with special emphasis on herbal extracts as immunomodulators. Ph. D. Thesis, Andhra University, India. p. 235.

Relyea RA (2004). Synergistic impacts of Malathion and predatory stress on six species of North American tadpoles. Environ. Toxicol. Chem., 23: 1080-1084.

Roche H, Boge G (1996). Fish blood parameters as a potential tool for identification of stress caused by environmental factors and chemical intoxication. Mar. Environ. Res., 41: 27-43.

Sadhu KA, Chowdhury DK, Mukhopadhyay PK (1985). Relationship between serum enzymes, histological features and enzymes in hepatopancrease after sub-lethal exposure to malathion and phophamidon in the murrel, *Channon straitus* (B.L.) Int. J. Environ. Stud., 24: 35-41.

Salah El-Deen MA, Sharada HI, Abu-El-Ella SM (1996). Some metabolic alteration in grass carp (*Ctenopharyngodon idella*) induced by exposure to cadmium. J. Egypt. Ger. Soc. Zool., 21: 441-457.

Shalaby ME (2009). The opposing effects of ascorbic acid (Vitamin C) on ochratoxin toxicity in Nile tilapia (*Oreochromis niloticus*). http://www.ag.arizona.edu/ista/ista6web/pdf/209.pdf. Rterieved:05-04-09.

Singh BB, Narain AS (1982). Acute toxicity of Thiodan to catfish (*Heteropneustes fossilis*). Bull. Environ. Conta. Tocxicol., 28: 122-127.

Sunmonu TO, Oloyede, OB (2006). Changes in liver enzyme activities in African catfish (*Clarias gariepinus*) exposed to crude oil. Asian Fish. Sci., 19: 107-112.

Svoboda M, Luskova V, Drastichova J, Zlabek V (2001). The effect of diazinon on haematological indices of common carp (*Cyprinus carpio* L.). Acta Vet. Brno., 70: 457-465.

Uzun FG, Kalender S, Durak D. Demir F. Kalender Y (2009). Malathion –induced testicular toxicity in male rats and the protective effect of vitamins C and E. Food Chem. Toxicol., 47: 1903-1908.

Venakataramana GV, Sandhya Rani PN, Murthy PS (2006). Impact of malathion on the biochemical parameters of gobiid fish, *Glossogobius giuris* (Ham). J. Environ. Boil., 27: 119-122.

Wedemeyer GA, Yasutake WT (1977). Clinical methods for the assessment of the effects of environmental stress on the fish health. United States technical papers and United States fish Wildlife services, 89: 1-18.

Wells RM, McIntyre RH, Morgan AK, Davis PS (1996). Physiological stress responses in big gamefish after exposure: Observations on plasma chemistry and blood factors. Comp. Biochem. Physiol., 84: 565-571.

Winkalar EU, Santosh TRM, Machdo-Neto JG, Martinez CBR (2007). Acute lethal and sub-lethal effects of neem leaf extracts on neotrapical freshwater fish, *Prochilodus lineatus* . Comp. Biochem. Physiol. Partc, 145: 236-244.

Zaki MS, Mostafa SO, Nasr S, Noor El-Deen AI, Ata NS, Awad IM (2009). Biochemical, clinicopathological and microbial changes in *Clarias gariepinus* exposed to pesticide malathion and climate changes. Reports Opin., 6-11.

Zhang Y, Xiao Z, Chen F, Ge Y, Wu J, Hu X (2010). Degradation behavior and products of Malathion and chlorpyrifos spiked in apple juice by ultrasonic treatment. Ultra. Sonochem., 17: 72-77.