Study on the effect of diazinon on haematological indices in common carp (*Cyprinus carpio* L.)

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Abstract： The goal was to assess an effect of diazinon [0,0-diethyl 0-(2-isopropyl-6-methylpyrimidin-4yl) phosphorothioate] on common carp (*Cyprinus carpio* L.). Fish were exposed to six different concentrations of diazinon (0, 5, 10, 20, 40, 80 ppm) for 96 h in 180 L glass aquaria. LC50-96h was obtained for diazinon (20 ppm) indicates diazinon is highly toxic to *C. carpio*. The results showed that the number of leukocytes (WBC), erythrocytes (RBC), haematocrit (PCV) and hemoglobin (Hb) was significantly decreased ($P<0.05$), but the amount of MCV and MCH was increased significantly by 50 % and decreased again. Lymphocyte decreased significantly by 60 % and then increased by 80 %. There was a significant increase in neutrophils count by 55 % and then decrease by 70 %. Resulted changes in erythrocyte and leukocytes after exposing to Diazinon are due to malfunction in hemopoiesis and decrease in non-specific immune system. It has been concluded that long-term exposure to diazinon at sub-lethal concentrations induced alterations in haematological indices in common carp and offers a simple tool to evaluate toxicity derived alterations.

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1.Introduction

Producing high quality fish depends on many factors, for example is the presence of pesticides negatively may affect the early growth stages Sanchez-fortum and Barahona (2005). Pesticides and insecticides are also the main causes of toxicity even today (Far et al. 2012). Pesticides can pollute water sources in two ways: through direct applications of pesticide in aquatic system and indirect uses such as erosion from agricultural lands and agricultural waste water infiltration and eventually washed into deep water environments and ecosystem (PirizZirkoohi and Ordog, 1997; Saglam, 2003; Dutta and Arneds, 2003). Pesticides in the aquatic environment can negatively affect the aquatic environment of the aquatic organisms particularly fish (Mahboob et al. 2011). Although the aquatic environment is not the target of such pesticides, but the widespread use has of them led to some serious problems including toxic residues in gross and toxicity of non-target organisms such as mammals, birds and fish (White, 1995; Manisingh and Wilson, 1995; Far et al. 2012).

Organophosphorous pesticides have fully replaced the persistent chlorinated pesticides in the 1970's and in the beginning of 1980's. The main advantage of the organophosphorous pesticides was their low cumulative ability and short-term persistence in the environment. Although the organophosphorous pesticides have been replaced by pyrethroid-based pesticides within the last 10–15 years, there is still a very intensive utilization of organophosphates. Organophosphorous pesticides such as Diazinon (O. O-diethyl O-2-isopropyl-6 nethylpyrimidin-4-phosphorothiate) are also utilized in fish culture (mainly those based on dichlorvos and trichlorfon) in order to suppress some parasitary diseases such as monogeneoses and arthropod (Noga 1995; Schlotfeldt and Alderman 1995; Navratil et al. 2000). Nevertheless, the pesticide preparations are considered harmful to fish in most cases (Svobodova et al. 1998). Diazinon is a common active substance of organophosphorous pesticides (Roberts and Hutson 1998). Scholz et al. (2000) reported that not all of its effects to fish organism are known, despite its very intensive use. Although the aquatic environment is not the main target and the aquatic invertebrates are not the target organisms, the presence of diazinon in water was reported and its negative effect to aquatic organisms is proven (DeVlaming et al. 2000). The ELISA assay and a biological toxicity test on *Ceriodaphnia dubia* (Mansingh and Wilson 1995; Tsuda et al. 1996; Bailey et al. 2000; De-Vlaming et al. 2000; Akhtar et al. 2012) is used for monitoring of diazinon and of its metabolite diazinon in water and in running water bottom sediments. Van-Der Geest et al. (1997) described an accidental pollution of Mense River (The Netherlands) with diazinon, as well as the negative consequences of this pollution, mainly the kill of aquatic invertebrates. The mechanism of a toxic effect of diazinon is the same as of other organophosphorous substances. There is an inhibition of a whole series of enzymes and mainly of acetylcholinesterase (Goodman et al. 1979; Sastry and Sharma 1980; Ansari et al. 1987; Hamm et al. 1998; Mahboob and Ghazala, 2011; Akhtar et al. 2012). There are differences in the acute toxicity of diazinon for various fish species. The 96hLC50 values

range in tenths to several tens of mg.l-1 (Seikai 1982; Hidaka et al. 1984; Keizer et al. 1991; Oh et al. 1991; Keizer et al. 1993; Kikuchi et al. 1996; Giddings et al. 1996; Tsuda et al. 1997; Mahboob et al. 2009). In European eel (*Anguilla anguilla*) the 96hLC50 values range even in hundredths of mg.l-1 (Sancho et al. 1992ab, 1993). The different toxicity of diazinon may be demonstrated on the example of two fish species used for ecotoxicological assessment of chemical substances. The 96hLC50 value of diazinon for guppy (*Poecilia reticulata*) was found to be 0.8 mg l^{-1} but for zebrafish (*Brachydanio rerio*) it was found to be 8 mg.l-1 (Keizer et al. 1991). Several authors have investigated the effects of pesticide in fish (Anees, 1978; Rajandernath, 1990; Jyostana et al. 2003; Almeida et al. 2005). Also many works have assessed the effect of various pesticides on the behaviors and haematological responses of different species of fish (Anees, 1978; Rajandernath, 1990). Therefore, the present study was planned to evaluate the effect of diazinon on some important heamatological parameters on the *Cyprinus carpio*.

2. Materials and Methods

2.1 Collection and maintenance of Fish

Healthy and active *Cyprinus carpio* fish was procured from the Fish Farm maintained by the Fisheries Department in the Kingdom. Fingerlings of common carp (*Cyprinus carpio* L.) with 18.0 ± 2.25 g mean body weight and 97.3 ± 7.26 mm mean body length was used for the experiment. Fish were brought to the laboratory in large aerated crates, acclimated for 30 days in large fiber tanks (22 **x** 12 **x** 5 feet) and fed with commercial dry feed pellets.

In the laboratory, the fish were held in 180 L glass aquaria (120 cm **x** 45 cm **x** 80 cm) containing dechlorinated tap water for acclimatization (20 days) at 25±1°C. The physico-chemical characteristics of the tap water were analyzed following the methods mentioned in APHA (1998). Water was renewed every day and a 12-12 h photoperiod will be maintained during acclimatization and test periods. The fish were fed regularly with commercial fish food pellets during acclimatization and test tenures, but feeding was stopped 2 days prior to the exposure to test medium for acute toxicity test.

2.2 Acute toxicity

The effect of acute toxicity of the agricultural toxicant diazinon (60 EM) was determined as LC50 (96 h). For this purpose, 6 treatments including control were set up to test toxicity; each treatment was replicated with 10 fish per tank with 180 liters water capacity. Nominal concentrations of active ingredient tested were 0, 5, 10, 20, 40 and 80 ppm for diazinon. After obtaining the final results, the information was analyzed statistically with Probit program version 1.5 (USEPA, 1985), and mortality was recorded at 24, 48, 72, and 96 h after the start and dead fishes were removed immediately from the tank. The LC1, LC10, LC30, LC50, LC70, LC90 and LC99 values at 24 48, 72 and 96 h; the maximum allowable concentration (MAC) value (96h LC50 divided by 10) (TRC, 1984); and the degree of toxicity were determined Table 3). The second stage of experiments consisted of five treatments: Median lethal concentrations of 30%, 60% and 90% of LC50 were tested and brood stocks of *C. carpio* was treated with these concentrations for 45 days. An experiment was carried out under static conditions based on the standard TRC, (1984) method for a period of 45 days.

2.3 Haematology

After the biometric test period, brood stocks were anesthetized, blood samples were obtained through tail vein puncture and blood factors were measured using different experimental techniques in the laboratory. The blood samples were transferred to heparinized and non-heparinized tubes. At the time of blood sampling, the appropriate smears were prepared for Giemsa staining. The smears were air-dried, fixed in 96% ethanol for 30 minutes and stained with Giemsa staining in 30 minutes. The smears were examined for the leukocyte differential count under a compound microscope (Klont, 1994). The haematological parameters examined were erythrocyte count (RBC), haematocrit (PCV), haemoglobin (Hb), mean corpuscular haemoglobin (MCH), mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC), leucocyte count (WBC) and differential leucocyte count (Klont, 1994).

Red Blood Cell Count: The number of red blood cells in a cubic millimeter of blood volume was calculated using the slide hemositometr by the following formula according to (Simons, 1997).

RBC = N × 10000

White Blood Cell Count: The differential diagnosis of white blood cells, blood vessels expand and develop their counting method was followed of Simons (1997).

MCV: Was calculated using the following formula according to Stoskopf (1993):

MCV = Hct x10 / RBC ; MCH: Was calculated by the following formula according to Stoskopf (1993):

MCH = Hb x10/RBC ; MCHC: Was calculated using the following formula according to Stoskopf (1993).

MCHC = Hb x100 /Hct

2.4 Statistical analysis:

The data obtained from this study was subjected to various statistical tools. The differences in the means (±SEM) between groups were assessed using Independent Samples-t test, adjusted to 95% confidence limits some important. SPSS program version 15.0 was used for statistical analysis and Excel 2010 used for drawing charts.

3. Results and Discussion

The physio-chemical characteristics of test water are listed in Table 1. The temperature ranged from 34 to 39 ^{0}C during experimentation. The pH of the water ranged from 7.8 to 8.1, which was slightly higher than neutral. Dissolved oxygen ranged from 7.2 to 8.3 mg/L. The body weight of diazinon exposed fish showed a slight but progressive decrease in the time course when compared with normal fish (Table 1), suggestive of the loss of somebody constituents. Since the loss of weight was probably associated with the susceptibility to pesticides (Gish and Chure, 1970), the prolonged exposure of fish to the same concentration of diazinon may prove to be fatal. Studies with other organophosphorus compound like methyl parathion on the fish *Tilapia mossambica* and malathion and lindane on the same species (Basha, 1980) showed a decrease in the body weight. Since symptoms of pesticide toxicity normally involve respiratory distress (Ferguson and Goodyear, 1967; AlGhanim, 2012), the decreased oxygen consumption of the diazinon exposed fish is probably due to the absorbance of more pesticide through the gills.

There was no recorded mortality in fish during the acclimation period before exposure and in the control group during acute toxicity tests. The mortality of *C. Capo* for diazinon examined during the exposure times in 24, 48, 72 and 96 h are presented in Table 2. For diazinon there was 100% mortality at 80 ppm within the 96h after exposure (Table 2).

Hematological Effect:

Mean values of haematological analysis under control and treated groups with diazinon are given in Table 4. RBC, WBC, PCV and Hb in control test results and treatments 30, 60 and 90% of LC50 shows the highest average rate as compared to control. RBC experiments of fish between treatments have statistically significant difference ($P \leq 0.05$). RBC, WBC, PCV and Hb levels were reduced after exposure to the pesticide Diazinon. Significant ($P>0.05$) difference for MCH and MCV under groups treated with different concentrations of diazinon. The highest amount of MCH and MCV were recorded in the group treated with 90 % of LC%0 of Dawson. In the blood of fish MCHC in the present study showed non-significant difference in the groups treated with 30, 60 and 90 % of LC50 of diazinon which indicated that the pesticide has no poisoning effect on the fish for this parameter. The variations of the mean values for the red blood cell parameters after exposure are shown in Table 4, which demonstrated a significant ($P < 0.05$) decrease in RBC count and Hb for the groups exposed to diazinon. Compared to the control specimens, fish after an acute exposure to diazinon had a lower erythrocyte count ($p < 0.01$), haemoglobin content ($p < 0.01$) and lower haematocrit value ($p < 0.01$). Haematological parameters of fish are highly variable between and within species and seasons (Luskova, 1997), with the values of individual indicators differing relative to temperature, season, sex, food, and the type of culture (Sopi*ƒ*ska 1985, Thomas et al. 1999). Haematological indices are varied under the influence of various environmental factors and chemicals as reported by Sanchez-Fortum and Barahona (2005). Studies have shown that when water quality is affected by toxicants any physiological changes will be appeared as a change in the values of various blood parameters (Alkahem, 1994; Al-Akel et al. 2010; AlGhanim, 2012). Thus water quality is one of the major factors, responsible for individual variations in haematology of fish as they are sensitive to slight fluctuation (Far et al. 2012). It has been reported by a number of investigators that high concentration of pesticides or long term exposure to sub lethal concentration of diazinon usually decreases erythrocyte indices (Al-Akel et al. 2010). Blood parameters may also show within-population differences (Allen 1993; Thomas et al. 1999), which explain wide variations within the control during the experiment. The values of the hematological parameters recorded in the control were close to those typical of the healthy carp (Singh et al. 2010). Neutrophils in the blood of *C. carpio* showed significant difference in all the treated groups (Table 4). The neutrophil increased in the group treated with 30 % of LC50 by 60 percent. But neutrophils decreased with increase in concentration by 90 % of the LC50 of diazinon in the present study. Lymphocytes in the treated groups increased significantly ($P<0.05$) as compared to control group. Maximum lymphocytes were recorded in the group treated with 90 % of diazinon. The decreased number of white blood cells (leucopoenia) may be the result of bioconcentration of the tested metal in the kidney and liver. Other authors have associated the cause to hindering of granulopoiesis or lymphpoiesis, induced by primary or secondary changes in haematopoietic organs (Tomaszewski, 1997; Al-Akel et al. 2010). In the present study the values obtained for the hematological indices, no significant change was recorded in the mean corpuscular volume (MCV) and mean corpuscular hemoglobin content (MCHC). It has been observed there was a significant change in the mean corpuscular hemoglobin (MCH) especially at higher concentrations (that is, 40 and 80 ppm). However, slight fluctuations were recorded in the MCV and MCHC when compared with the control. Ololade and Oginni (2010) reported that cell released from the spleen, which is an erythropoietic organ would have the lower MCV values when compared with the control. A similar observation was made for *Cyprinus carpio* after cadmium exposure (Al-Akel et al. 2010; Singh et al. 2010). The significant change ($P < 0.05$) in the MCH of the experimental fish when compared with

the control may be due to the reduction in cellular blood iron. These results were upheld by the findings of Hodson et al. (1978). Oh et al. (1991) mentioned that one of the factors that affects the aquatic toxicity, is time. When fish are exposed to fixed concentrations of the toxin, the resistance of fish over time is dwindling. The impact of toxin in fish will decrease. In these cases toxins accumulate in fish tissues and also increase its adverse effects on the fish. The role of blood parameters in the assessment of the health status of fish is emphasized by Cyriac et al. (1989). The findings of the present study are in line with the above mentioned workers.

Table 1: Physicochemical characteristics of water samples used(n=10 each concentration) exposed to acute diazinon

Parameter	Unit	Minimum	Maximum
Temperature	^{o} C	34	39
pH	-	7.8	8.1
Dissolved oxygen	mg O_2 L^{-1}	7.2	8.3
Chemical oxygen demand	mg O_2 L^{-1}	82	117
Biochemical oxygen demand	mg O_2 L^{-1}	5.9	8.7
Total dissolved solids	g L^{-1}	52.5	57.7
Chloride	g L^{-1}	15.3	18.6
Salinity	g L^{-1}	34.2	36.7
Total hardness	mg L^{-1} as CaCO3	344	377
Total alkalinity	mg L^{-1}	166	198
Ammonia nitrogen	mgN L^{-1}	0.19	0.86
Nitrite nitrogen	mgN L^{-1}	0.14	0.17
Nitrate nitrogen	mgN L^{-1}	0.13	0.18

Table 2: Cumulative mortality of *Cyprinus carpio*in the experiment

No. of mortality				
Concentration (ppm)	24h	48h	72h	96h
0	0	0	0	0
5	0	1	1	1
10	0	1	2	2
20	1	3	4	5
40	2	4	5	7
80	5	7	10	10

Table 3: Lethal Concentrations (LC1-99) of diazinon (mean ± standard error) depending on time (24-96h) for *C. carpio*

Concentration (ppm) (95 % of confidence limits)				
Point	24h	48h	72h	96h
LC_1	6.78±0.44	-	-	-
LC_{10}	38.6±1.11	19.2±0.88	7.18±0.55	9.20±1.11
LC_{30}	96.7±2.26	47.3±1.28	37.6±1.05	17.1±0.95
LC_{50}	106.8±1.88	67.5±1.34	42.1±1.11	20.3±0.87
LC_{70}	111.9±1.23	78.8±1.55	60.6±1.62	27.4±0.71
LC_{90}	123.6±2.32	82.7±2.29	65.1±1.43	34.9±1.07
LC_{99}	136.81.±1.94	91.6±1.66	71.5±1.83	40.2±1.42

Table 4: Mean values of haematological analysis control and exposed group of *C. carpio*

Exposed group				
	Control	30%	60%	90%
Indices	Mean SEM±	Mean SEM±	Mean SEM±	Mean SEM±
RBC(m3)	1270000 ± 146000a	92600 ± 4100d	790000 ± 37000b	710000 ± 38000c
WBC(m3)	13880 ± 887b	10230 ± 755c	23700 ± 1300a	8200 ± 80d
PVC (%)	44.5 ± 2.7a	37.6 ± 2.2b	33.5 ± 2.7c	29.6.4 ± 1.8d
Hb (%)	8.4 ± 0.77a	6.5 ± 0.55b	5.7 ± 0.63c	4.4 ± 0.41d
MCH (pg)	60.2 ± 3.4c	77.1 ± 2.2b	80.3 ± 3.6b	97.9 ± 4.7a
MCV (fl)	377.8 ± 7.9d	440.5 ± 8.7c	528.6 ± 7.3b	554.8 ± 9.4a
MCHC (g/l)	67.1 ± 3.4b	70.9 ± 3.6b	73.2 ± 3.4b	77.2 ± 4.1a
Neutrophil (%)	18.3 ± 2.4d	53.2 ± 3.1b	63.7 ± 5.2a	23.7 ± 2.8c
Lymphocyte(%)	87.5 ± 5.6b	60.2± 4.6c	36.6 ± 5.9d	136.1 ± 6.2a

Values are expressed as the mean ± S.E. Means in the same horizontal column followed by different superscript are significantly different (≤= 0.05) according to Duncan's new multiple range test.

Conclusion

It has been concluded that reduction of non-specific immunity in *C. Cope* was probably induced by reduction in the number of leukocytes, lymphocytes and neutrophils due to stress caused by diazinon. It is further concluded that the assessment of the blood parameters is important to know the health status of fish under controlled and wild habitat.

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