Toxicological evaluation and effects of organophosphate compounds on hematological profile of juvenile common carps (Cyprinus carpio var. Communis)

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ABSTRACT

Present study was carried out to investigate the alterations in the haematological profile of juvenile common carp (Cyprinus carpio var. communis) against acute toxicity of organophosphorous compounds, dimethoate and chlorpyrifos. The fish were divided into 5 different experimental groups for range finding tests on the basis of which the doses for definitive tests were selected. The acute bioassay toxicity tests were carried out in triplicates for 96 hours with a control group run parallel to the experiment. Data obtained was analyzed as per Finney’s probit to determine LC_{50} values. The mean lethal concentration was found 1.1ppm and 3.8ppb respectively for dimethoate and chlorpyrifos. The samples investigated for various haematological parameters such as haemoglobin concentration (Hb), Red blood cell (TEC) and White blood cell (TLC & DLC) counts the hematocrit (Ht) level, the mean corpuscular volume (MCV) and the mean corpuscular haemoglobin (MCH). Packed cell volume (PCV) and Erythrocyte sedimentation rate (ESR) showed a negative impact of pesticide treated fishes when compared with control. Hb, TEC and Ht were found lower while as ESR and TLC were recorded higher than the control specimens. MCV and MCH showed statistically insignificant differences when compared with control. In DLC, monocyte lymphocyte, neutrophil and basophils were found increased while as eosinophil count was found unaffected than the normal. Studies suggested that the pesticides are potential toxicants for common carps among which chlorpyrifos was found to elicit profound changes intense than dimethoate.

Key words: Common carp, Differential leukocyte count, Haematology, LC_{50}, Pesticides.

INTRODUCTION

Fishes live in close proximity with the environment and reflect changes occurring around in their surroundings. Presence of environmental stressors, congenial conditions for disease occurrence and xenobiotic exposure are the potent factors which can lead to patho-physiological changes in fishes. However, in addition to the hazards caused by these factors individually, they can work synergistically to elicit profound responses in fishes. Among such health hazards, hematological alterations are known as the immediately observed responses in fishes and well reported (Qayoom, 2015). Hematological alterations are usually the first detectable and quantifiable responses to environmental change (Wendelaar, 1997). Change in the hemogram are one of the important facets of assessing the patho-physiological conditions of fishes; indicative of stress, disease or toxicity and mostly attributed to the presence of contaminants in their environments. Analysis of these parameters represent a valuable approach for monitoring the health status of farmed animals (Celik 2004; Asadi et al., 2007) and in diagnosing the structural and functional status of fish exposed to xenobiotics (Sancho et al. 2000). Hematological studies also help in understanding the relationship of blood characteristics to the habitat and adaptability of the species to the environment (Fazio et al., 2016). These parameters are therefore potentially used as biomarkers for toxicological studies.

Dimethoate [O, O-dimethyl S-methylcarbamoylmethyl phosphorodithioate] and chlorpyrifos [O, O-diethyl-O-(3, 5, 6-trichloro-2-pyridyl) phosphorothionate] are the broad spectrum cholinergic organophosphates known as acetylcholinesterase (Ache) inhibitors. Despite being characterised as an organophosphate compound, chlorpyrifos is typically known to have lipophillic properties due to the presence of three chlorine atoms in the parent benzene ring. It is for this reason that it depicts the lipophillic properties more or less like chlorinated hydrocarbons. Persistence of chlorpyrifos (36.9 days) has been found similar to that of endosulfan (alpha endosulfan = 20.4 days; beta endosulfan = 63.6 days) in a water/sediment microcosm study under tropical conditions (Laabs et al., 2007) and therefore both regarded as moderately persistent compounds. Dimethoate, on the other hand, although highly water soluble (39, 8000 ppm at 21°C) (Extoxnet, 1996) is highly toxic to fishes and other aquatic invertebrates (Abdel-Megeed and El-Nakieb, 2008).

Hematological evaluation of fishes subjected to bioassays give insight of induction of toxicity in them pro

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vide reliable information on metabolic disorders, deficiencies and chronic stress status before they are present in a clinical setting (Bahmani et al. 2001). Alteration in erythrocytic indices had been reported in fishes subjected to environmental stressors like pesticides and heavy metals (Gill and Pant, 1987; Bhatia et al, 2004; Johal and Grewal, 2004). It is with this view that this study was carried out to investigate the comparative toxic effects of dimethoate and chlorpyrifos on the hematological indices of juvenile Cyprinus carpio var. communis.

MATERIALS AND METHODS

Experimental Animals: Juveniles Cyprinus carpio var. communis were collected from the hatchery of Faculty of Fisheries SKUAST-K, and brought to the laboratory with adequate water and oxygen supply, avoiding any injury to them. The test organisms weighing 10±2 g were taken only. Care was taken that the length of the largest fish was not more than 1.5 times the length of the smallest fish. In laboratory, fishes were immediately bathed in 0.05% KMnO₄ solution for two minutes for disinfection. Glass aquaria measuring 60x30x40 cm were also washed with 0.05% KMnO₄ solution and used for range finding and definitive bioassays. Fishes were acclimatized to laboratory conditions for two weeks and fed with artificial diet (wheat bran 25%, rice bran 24%, mustard oil cake 50% and agrimin forte: a multivitamin 1% during that period.

Toxicity Tests: The static bioassay was carried out for 96 hours (Reish and Oshida, 1987) for which the technical grades of dimethoate (90.0 % purity) and chlorpyrifos (95.0 % purity) were used. The stock solution was prepared in methanol and subsequent concentrations in deionised water. Different test concentrations for range finding tests were used. To determine range finding test, concentrations with spacing factor of 10 was used (Rahman et al., 2002). For definitive tests, the final concentrations of dimethoate (0.5, 1.0, 2.0, 4.0 and 8.0 ppm) and chlorpyrifos (4.0, 8.0, 16.0, 32.0 and 64.0 ppb) were used to determine median lethal concentration (LC₅₀) values of both the pesticides.

Water quality of aquaria: Various physio-chemical parameters of aquaria during bioassay were monitored using the standard methodology (APHA, 2005). Water temperature (°C) and pH was measured by thermometer and pH meter (Japan) respectively. Dissolved oxygen was determined by Winkler’s Method, Carbon dioxide by Titrimetric method, and Total dissolved solids (TDS) by the method of Adoni et al., (1985).

Hematological studies: Blood was collected from the fishes at the termination of bioassay by direct heart puncturing (Lucky, 1977) using sterile disposable plastic syringe with a 22-gauge needle or by ablation of caudal peduncle (Schaperclaus, 1991). Heparin sodium (1%) was used as an anticoagulant (Svobodova et al, 2001). The collected blood samples were immediately subjected to hematological analysis. The blood was diluted with appropriated diluting fluids for RBC and WBC counts were determined using improved Neubauer haemocytometer and calculated (Blaxhall and Daisley 1973). Sahli’s haemoglobinometer was used to estimate haemoglobin (HB) percentage (HB %). Haematocrit (HCT) was determined using micro haematocrit capillaries filled with blood and centrifuged at 8,700 x g for 5 min and expressed as percentage of total blood volume (Wintrobe 1978). Mean corpuscular volume (MCV) and mean cell haemoglobin (MCH) were calculated from the average values of HB%. For the determination of erythrocyte sedimentation rate (ESR), the blood was mixed well and 200 mm was drawn into a Westurgen tube. The tubes were placed vertically and were left undisturbed for 60 min; after that, the level of the column of sediment was noted as ESR. For Differential Leukocyte count (DLC) the methodology of Hudson and Hay (1991) was adopted.

Statistical Analysis: For the determination of median lethal concentration values (LC₅₀), Finney’s probit method of regression was used (Finney, 1971). Differences in hematological parameters between the groups (dimethoate and chlorpyrifos treated and the control specimens) were statistically compared by Mnann-whitney non-parametric test of significance. All these statistical analyses were performed using the SPSS statistical software (Version 20.0 for Windows XP, SPSS, and Chicago, IL, USA). Descriptive statistics was performed using PAST statistical (Version 3.0 for Windows XP) wherever necessary.

RESULTS AND DISCUSSION

The results of acute toxicity of dimethoate and chlorpyrifos are presented in Table 1. The median lethal concentration (LC₅₀) of dimethoate and chlorpyrifos was found 1.1±0.053 ppm and 3.8±0.1 ppb respectively for juvenile common carps. The regression lines and coefficient of determination (R²) of three range finding tests (R₁, R₂, and R₃) of dimethoate suggest a strong co-relation between dose of the toxicant given and response elicited by fishes (Figs. 1-3). Similarly, a positive co-relation in chlorpyrifos treated fishes was observed supporting the dose – response relationship (Figs 4-6). The Small LC₅₀ values recorded in this study are attributed to small size of fishes (10±2 g) which have potentially weak immune system for biotransformation and elimination of toxicants from the body. Moreover, the rapid distribution of pesticides in the body of small sized fishes leads to faster alterations in behaviour than the normal for the uptake of a toxicant is directly dependent on the size of fishes illustrated by dose response relationships. Similar results of LC₅₀ have been reported by Campagna et al., (2006) on 3 day old Prochilodus lineatus larva (10.44 µgL⁻¹), Ganeshwade (2011) and Ganeshwade et al. (2012) in Punctius ticto (5.012 ppm). Chlorpyrifos was found much toxic than dimethoate since its LC₅₀ values were found very
Table 1: Acute toxicity of dimethoate and chlorpyrifos in *Cyprinus carpio* var. *communis*.

<table>
<thead>
<tr>
<th>Pesticide</th>
<th>LC50 values from different range-finding tests</th>
<th>Mean± S.D. of LC50</th>
<th>Variance</th>
<th>Std. Error</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R1</td>
<td>R2</td>
<td>R3</td>
<td></td>
</tr>
<tr>
<td>Dimethoate</td>
<td>1.08</td>
<td>1.16</td>
<td>1.06</td>
<td>1.1±0.053</td>
</tr>
<tr>
<td>Chlorpyrifos</td>
<td>3.7</td>
<td>3.9</td>
<td>3.8</td>
<td>3.8±0.1</td>
</tr>
</tbody>
</table>

Fig 1: Regression line of range-finding (first replicate, R1) of dimethoate.

Fig 2: Regression line of range-finding (first replicate, R2) of dimethoate.

Fig 3: Regression line of range-finding (first replicate, R3) of dimethoate.
less as compared to dimethoate (Table 1) indicative of its potential toxicity than dimethoate to carps. Various workers have reported small LC$_{50}$ values of chlorpyrifos to different fishes which support our results (Hallappa and David, 2009; Okechukwu et al., 2013; Verma and Saxena, 2013 and Wast et al., 2015).

The laboratory and water temperature did not depict profound deviation during the entire bioassay because all the trials of range finding and definitive tests were carried out under similar environmental conditions set in the laboratory. Constant values obtained for carbon-dioxide in all the treatments of chlorpyrifos and dimethoate (2.00±0.00 mg/L) is indicative of non suflicicive conditions in the aquarium. It is inferred from the data that mortality of fishes in the aquarium has occurred due to the exposure of toxicant only and not anoxia. Similar indications can be derived from data of pH which is not indicative of and acidic or alkaline conditions in aquarium for both pesticides (Table 2).

**Haematological responses:** Chlorpyrifos was found to elicit profound effects on all haematological indices of common carps than dimethoate, although the later was found to affect the fishes in the same way, but with less toxicity comparatively. Hb, TEC and PCV were found significantly (P=0.05) decreased from the control while as TLC, ESR was found significantly (P=0.05) increased from the control specimens. MCV and MCH were not found significantly altered from the control (Table 3). The trend of alteration in the haematological parameters was found same in both the pesticides; however the increased deviation in chlorpyrifos treated fishes indicated its potential toxicity to common carps than dimethoate.

Significant reduction (p≤0.05) in Hb and TEC is attributed to the destruction of RBC’S due to pesticide exposure which leads to haemato-toxicity in fishes. Lower haemoglobin level might decrease the ability of the fish to enhance its activity in order to meet occasional demands like seeking food and escape. Decrease in Hb has also been associated with the reduction of oxygen-carrying capacity in fish as reported by Karatas (2016). Decrease in erythrocyte counts could be due to haemo-dilution resulting from impaired osmoregulation across the gill epithelium. The organophosphate pesticides are known to induce changes which give evidence for decreased haematopoiesis followed by anaemia induction in fish (Sovoboda et al., 2003). A decrease in Total Erythrocyte Count and Hb content were reported in H. fossilis (Nath and Banerjee, 1996) and in common carp (Svobodova et al., 2001) after their treatment with cypermethrin. Our findings are supported by the results obtained by Das and Mukherjee (2001), Körprüci et al. (2006), Banee et al. (2008), Ali and Rani (2009), Deka and Dutta (2012), Shamoushaki et al. (2012), Lakshman et al. (2013) and Al-Ghaini (2013) who reported significant decrease in Hb and TEC contents in fishes after exposure to organophosphorous pesticides.

In this study, MCV was found fluctuating in dimethoate treated fishes but the variation calculated was statistically insignificant as compared to the control while in chlorpyrifos treated samples, statistically significant difference (P= 0.05) between treated fishes and control specimens was observed (Table 3). In both the experimental specimens, normochromic normocytic anaemia was observed with unusual occurrence of hypochromy in the RBCs of chlorpyrifos treated fishes. Our findings are supported by...
Zorriezahra et al. (2010) who reported hypochromic microcytic anemia in *Oncorhynchus mykiss*. Sharmin et al. (2014) and Köprücü et al. (2006) reported decreasing trend of MCV values in *Cyprinus carpio* exposed to malathion and *Silurus glanis* exposed to diazinon respectively. Occurrence of normochromic normocytic anaemia is also supported by the calculated values of MCH which did not show any significant difference (P= 0.05) between control and treated groups for both the pesticides. Unlike our study, different results are reported by Banee et al. (2008) and Sachar and Raina (2014) who reported increase in MCV values in *Cyprinus carpio* and *Aspidoparia morar* exposed to acute concentrations of diazinon and lindane respectively. ESR was found significantly (p=0.5) raised in the study. The sharp increase in ESR was symptomatic of toxicity in fishes which was reflected high in chlorpyrifos treated ones. The line in the wintrobe’s tube was not well demarcated due to hemolysis. This explained the drastic reduction in Hb, TEC and other hematological indices in fishes. Similar observations were reported by Janardana and Vineela (2013) while exposing *Catla catla* to azodrin. Ali and Rani (2009) reported increase in ESR of *Oreochromis mossambicus* exposed to phosalone.

Table 4: Differential leukocyte count of common carp exposed to dimethoate and chlorpyrifos.

<table>
<thead>
<tr>
<th>DLC Parameter</th>
<th>Dimethoate</th>
<th>Chlorpyrifos</th>
<th>P-value</th>
<th>Dimethoate</th>
<th>Chlorpyrifos</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monocyte (%)</td>
<td>2.33±1.154</td>
<td>5.00 ±1.73</td>
<td>0.072</td>
<td>2.33±1.154</td>
<td>5.00±1.73</td>
<td>0.822</td>
</tr>
<tr>
<td>Lymphocyte (%)</td>
<td>76.00±4.00</td>
<td>83.33±4.16</td>
<td>0.121</td>
<td>76.00±4.00</td>
<td>83.33±4.16</td>
<td>0.375</td>
</tr>
<tr>
<td>Neutrophil (%)</td>
<td>10.00±3.605</td>
<td>7.00±1.73</td>
<td>0.375</td>
<td>10.00±3.605</td>
<td>7.00±1.73</td>
<td>0.076</td>
</tr>
<tr>
<td>Eosinophil (%)</td>
<td>0.667±0.577</td>
<td>0.667±0.577</td>
<td>0.792</td>
<td>0.667±0.577</td>
<td>0.667±0.577</td>
<td>0.157</td>
</tr>
<tr>
<td>Basophil (%)</td>
<td>0.33±0.577</td>
<td>1.00±1.00</td>
<td>0.479</td>
<td>0.33±0.577</td>
<td>1.00±1.00</td>
<td>0.157</td>
</tr>
</tbody>
</table>

Fig 4: Regression lines of range-finding (R₁) of chlorpyrifos.

Fig 5: Regression lines of range-finding (R₂) of chlorpyrifos.

Fig 6: Regression lines of range-finding (R₃) of chlorpyrifos.
Differential leukocyte count: The increase in the number of leukocytes (leucocytosis) is attributed to the increase in the defence activity of fishes under stress of pesticide exposure. Das and Mukherjee (2001) related the increase in the TLCs in *Labeo rohita* due to stimulated lymphopoiesis and/or enhanced release of lymphocytes from lympho-myeloid tissues. Such a lymphocyte response might be due to the presence of toxic substances or may be associated with the pollutant induced tissue damage as opined by Haniffa (1990). Some workers reported the initial increase in leukocyte count of fish species exposed to pesticides followed by the a drastic decrease which is generally associated to the fact that initially the TLC count is raised to fight the infection caused in fish species due to pesticide exposure. Increase in time of exposure of pesticide, generally in acute levels is associated with the complete destruction of hematopoietic system as an indicative of hematotoxicity in fishes (Ali and Rani, 2009). Ahamad (2011) reported decrease in TLC of *Cyprinus carpio* to malfunctioning of the haematopoietic system caused by exposure to organophosphates. He suggested that the changes in leukocyte system manifest in the form of leucocytosis with heterophilia and lymphopenia, which are characteristics of leukocytic response in animals exhibiting stress (Table 4).

**CONCLUSION**

The study suggests that organophosphorous compounds are dangerous for fish health. Therefore natural waterbodies must be checked for their entry at all point sources of pollution. Moreover, non judicious use of pesticides and their application around the catchment areas must be stopped so as to prevent their entry to natural water bodies. Otherwise they will not only affect fishes, but also humans who directly consume them.

**REFERENCES**


