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RESEARCH ARTICLE

IMPACT OF TRIAZOPHOS ON *CHANNA PUNCTATUS*: HEMATOLOGICAL AND HISTOPATHOLOGICAL STUDIES

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ABSTRACT

The indiscriminate use of pesticides in agriculture can affect the production of fish by creating different physiological anomaly which can also affect the human health by biomagnifications. Therefore, the present study was aimed to evaluate the Sublethal toxicity of triazophos (o,o-Diethyl-o-(1-phenyl-1H-1,2,4,- triazol-3yl) thiophosphate), an organophosphate (OP) pesticide, on histopathological and haematological parameters of a freshwater fish, *Channa punctatus.* Fishes were exposed to 5% and 10% of LC_{50} concentration, 0.068 mg/l of triazophos for 96 h maintaining ten fishes in each group, along with the control group. For histopathological studies, tissues under study were dissected and fixed appropriately and for haematological studies, blood sample were collected. Histopathological changes in liver include vacuolization and increase in number of kupffer cells; in gills, changes in the architecture of primary and secondary gill lamella; and in kidney, shrinkage of glomerular and kidney tubules were observed. In hematological parameters significant increase in total WBC, MCV and significant decrease in RBC, Hb, MCH, MCHC were observed with respect to control. This preliminary study indicates the physiological effect of triazophos on *C. punctatus*. However, further molecular and biochemical studies may supplement the mechanism of action of this pesticide.

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INTRODUCTION

Wide use of pesticides in agriculture to control the pests causes pollution of aquatic system thus indirectly posing pollution problem (Ganeshwade, 2012). As organophosphates are used extensively due to their rapid biodegradability and non-persistent nature, but as the remnants of these compounds are present in nature for longer can cause countless abnormalities to the different aquatic biota including fish (Naveed *et al.,* 2010; Chishti *et al.,* 2013). Although these exhibit relatively low level of toxicity in mammals (WHO, 1992), may bioaccumulate in humans and can cause toxic consequences (Go *et al.,* 1999). High toxicity of synthetic pesticides has been found in some aquatic arthropod (Bradbury and Coats 1989) as well as zooplankton communities. Synthetic pesticides are one of the important groups of aquatic pollutants affecting fish health (Ram, 2014) by inducing histopathological changes in fish (Barlas, 1999; Cengiz *et al.,* 2001). Haematological indices also act as indicators of changes in the internal and/or external environment of fish (Kori-Siakper and Oboh, 2011). Thus, studies on these parameters may be used as indicator of physiological changes due to different stress conditions affecting fish health (Blaxhall *et al.,* 1972; Duthie *et al.,* 1985).

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The pesticide namely an organophosphate, triazophos (o,o-Diethyl-o-(1-phenyl-1H-1,2,4,- triazol-3yl) thiophosphate) which is widely used as insecticide to control the pests on the paddy and on cotton fields due to its low toxicity to mammals (particularly in human) (Naveed and Janaiah, 2011) but reports delineating the effect of Triazophos on histo-pathology in *Channa punctatus* (Bloch) are scanty. Thus, present study was aimed to assess the impact of acute exposure of triazophos in terms of Histopathological changes to the gill, liver and kidney tissues as well as haematological parameters of fish, *Channa punctatus*.

MATERIALS AND METHODS

Animals and maintenance

Healthy fishes (average length 9-11 cm, average weight 21±2 gm.) were obtained from local fish market of Allahabad, Uttar-Pradesh, India (25.43° N, 81.84° E). Immediately after procurement, the fishes were treated with potassium permanganate solution ($KMnO_4$, 0.5% w/v) for 1 min to remove any kind of sub-cutaneous contamination/adherents(s). The fishes were acclimatized under laboratory conditions for two weeks in glass aquarium containing de-chlorinated aerated tap water at room temperature (25 ± 2ºC) with commercially available food pelette (Tokyu, India) *ad libitum*.

Procurement of chemicals

The pesticide (Triazophos; commercially known as Sutathion, Pune, Maharashtra, India) was procured from local supplier for chemicals, Allahabad, Uttar-Pradesh, India. The chemicals for histo-pathology and haematology were also procured from local supplier of Allahabad, Uttar-Pradesh, India.

Assessment of water quality

Before exposure, the quality of water was evaluated as per APHA guideline (APHA, 1985). The parameters were noted as adequate for fishes to be exposed for different concentrations of LC_{50} values of pesticide which are as follows: pH 6.8 ± 0.2, DO 6.8 ± 0.5 mg/l and total hardness 111.4 ± 4 mg/l.

Experimental design

The LC_{50} for triazophos exposure was determined as 0.068 mg/l (Singh *et al.,* 2016). To study the effect of triazophos, fishes were divided into three different groups (control, 5% of LC_{50} and 10% of LC_{50}). Ten fishes (n=10) were placed in each three glass aquaria containing 12l water. The stock solution of triazophos used in this study was always freshly prepared when needed. Equal volume of acetone was mixed in the control aquarium, as the pesticide (triazophos) was solubilized in acetone. The exposure of fishes to the pesticide was continued for 96 h.

Sample collection

Immediately, after 96h of exposure, blood was collected in heparinised vial and was processed for haematological parameters. After sacrifice, the desired tissues (gill, liver, and kidney) were collected, washed in chilled PBS and further processed for histopathological studies.

Preparations for histopathology

The histo-pathological preparations were done following the protocol published elsewhere (Ghosh and Haldar, 2015). In brief, the tissues (gill, liver and kidney) were fixed in 10% Neutral Formaline for overnight. After fixation, the tissues were washed under running tap water and were dehydrated using graded alcohols (from 30% to absolute). After that the tissues were transferred into absolute alcohol and xylene (50:50), pure xylene. Further, they were transferred to xylene + wax (50:50) and three grades of waxes. Then the block was prepared and 6µm thin sections were cut using a microtome (Leica Microtome, Leica, Leitz, Germany). Then, the sections were spread on 0.1% gelatin coated slides and the slides were processed for Hematoxylene-Eosin counter staining. The slides were dipped in xylene (for de-waxing), passed through down-grade alcohols (from absolute to 30%) and water and was stained in Ehlrich's hematoxylin. After that, the slides were counter-stained by eosin, dehydrated by different grades of alcohol (from 30% to absolute), cleared in xylene and was mounted in DPX. The next day, the slides were snapped by Nikon, 80i, Japan with a camera setup attached to the microscope and computer.

Hematological preparations

Total RBC and WBC (by Neubaur's Hemocytometer), gram percentage (g%) hemoglobin (by Shali's hemoglobinometer),

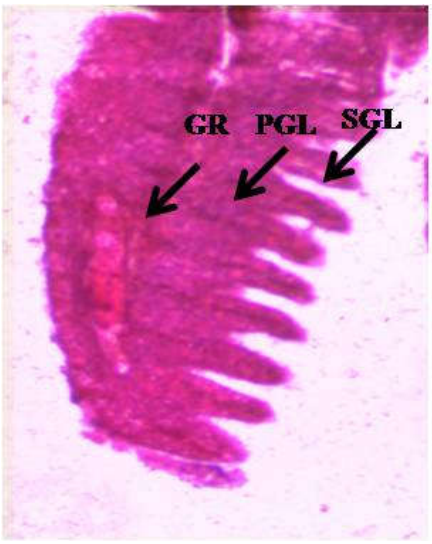

Fig. 1A. showing histo-architecture of control gill. GR Gill Raker, PGL: Primary Gill Lamellae, SGL: Secondary Gill Lamellae

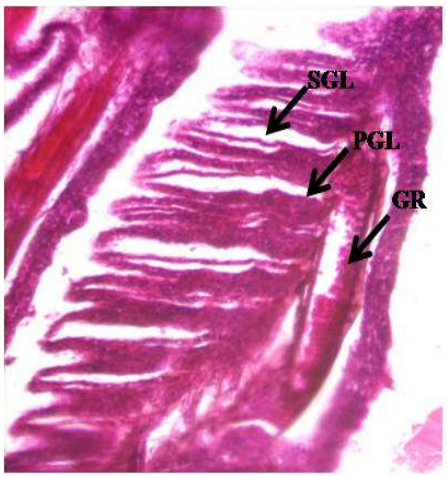

Fig. 1B. Histo-architecture of 5% Triazophos treated gill. GR Gill Raker, PGL: Primary Gill Lamellae, SGL: Secondary Gill Lamellae.

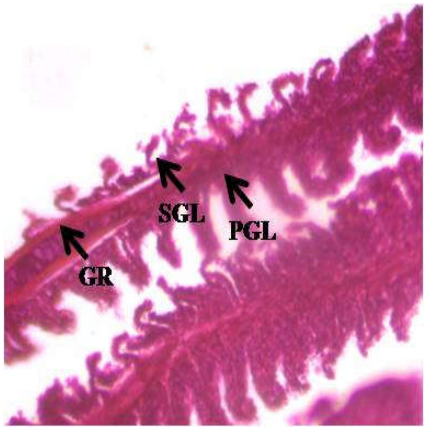

Fig. 1C. Histo-architecture of 10% Triazophos treated gill. GR Gill Raker, PGL: Primary Gill Lamellae, SGL: Secondary Gill Lamellae.

Differential Leucocyte Count (DLC, by Giemsa stain) and hematocrit were estimated by using standardized protocols. From the results of %Hb, hematocrit and total RBC counts the Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH) and Mean Corpuscular Hemoglobin Concentration (MCHC) were calculated using proper formula.

Statistical analyses

The data of total RBC and WBC counts, g% hemoglobin, DLC, hematocrit value, MCV, MCH and MCHC are represented as mean ± SEM and were analyzed by One Way Analysis of Variance (One Way ANOVA) on Microsoft Office Excel work-sheet. To note the variations between control *vs* experimental groups and among the experimental groups (5% and 10% groups), Duncan's Multiple Range Post-hoc Test was applied. The results were considered to be significant at $P \leq 0.05$ and $P \leq 0.01$ levels (confidence levels 95% and 99% respectively). All of the statistical analyses were done in accordance to Brunning and Knitz, 1977.

RESULTS

Histo-pathological observations

A. *Gill:* The general histo-architecture of gills was noted in control groups with intact primary and secondary gill lamellae along with gill filament attached to gill raker (Fig. 1A). In case of 5% group, the primary and secondary gill lamellae lost their basic structure (due to degeneration in gill filament) as found in control (Fig. 1B). But, in case of 10% group, the entire structure of both primary and secondary gill lamellae along with gill filament was not only degenerated but also they are dispersed from their attachment to gill raker (Fig. 1C).

B. *Liver:* The normal histo-architecture of liver was noted in control groups where intact Glisson's capsules (with assembled hepatocytes) and hepatic artery were observed (Fig. 2A). But, in the 5% group, the integrity of Glisson's capsules was partially lost (which was identified by increased intercellular spaces) and hepatocytes and kupffer cells were found to be present in the dilated hepatic artery (Fig.2B) in comparison to control. But, in 10% group, the integrity of Glisson's capsules was severely affected along with high disposition of hepatocytes and kupffer cells in more dilated hepatic arterial area in comparison to control (Fig. 2C).

C. *Kidney:* The basic histo-architecture of kidney is having glomerulus which is found to be intact in control fishes (Fig. 3A). But, the histo-anatomy of kidney is lost in 5% Triazophos treatment group as marked by swelling of diameter of glomerulus and Bowman's capsule as compared to control (Fig. 3B). In case of 10% Triazophos treatment group, the degeneration of kidney is more marked by swelling of Bowman's capsule and increase in inter cellular spaces.

Hematological parameters

A. *Total RBC Count*

We noted significantly low level of total RBC count in 5% and 10% Triazophos treatment groups ($p < 0.01$) in comparison to control. But, among the experimental groups the variation was not significant ($p > 0.05$, Table-1).

B. *G% of Hb*

We noted significantly low level of G% of Hb in 5% and 10% Triazophos treatment groups ($p < 0.01$) in comparison to control. Among the experimental groups (between 5% and 10%) the variation was significantly low in 10% than 5% ($p < 0.05$, Table-1).

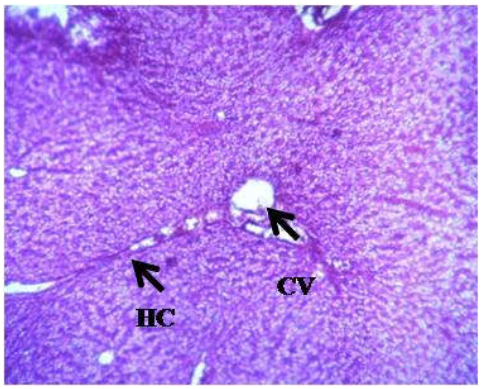

Fig. 2A. Histo-architecture of Control liver. HC: Hepatic cord (Glisson's Capsule), CV: Central Vein

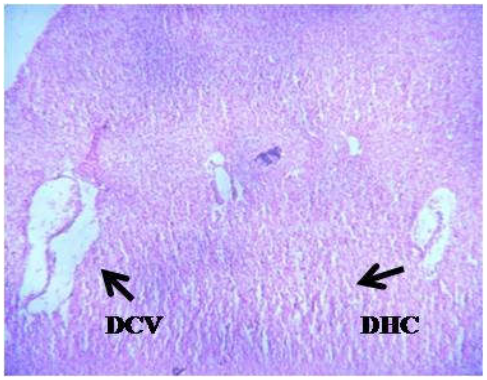

Fig. 2B. Histo-architecture of 5% Triazophos treated liver. DHC: Degenerated Hepatic cord (Glisson's Capsule), DCV: Degenerated Central Vein

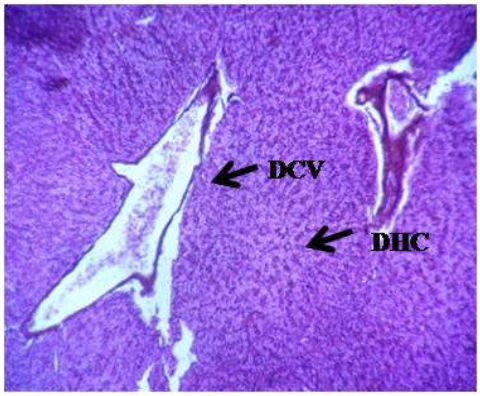

Fig. 2C. Histo-architecture of 10% Triazophos treated liver. DHC: Degenerated Hepatic cord (Glisson's Capsule), DCV: Degenerated Central Vein

C. *% Hematocrit*

We noted significantly low level of % hematocrit in 5% and 10% Triazophos treatment groups ($p < 0.01$) in comparison to control. Among the experimental groups (between 5% and 10%) the variation was significantly low in 10% than 5% ($p < 0.05$, Table-1).

D. *MCH level*

We noted significantly low level of MCH in 5% and 10% Triazophos treatment groups ($p < 0.01$) in comparison to control. But, among the experimental groups the variation was not significant ($p > 0.05$, Table-1).

Table 1. Impact of Triazophos on hematological parameters of *Channa punctatus*. Data represented as Mean ± SEM (n=10/group). (+ indicates % increase values; - indicates % decrease values). (*p < 0.05 control *vs* experimental groups; ** p < 0.01 control *vs* experimental groups; [a] p < 0.05 5% of LC_{50} vs 10% of LC_{50}; [b] p < 0.01 5% of LC_{50} vs 10% of LC_{50})

Sl. No	Conc. of Triazophos (mg/l)	Total RBC Count ($x10^6/mm^3$)	Hematocrit (Percentage, %)	MCH (pg)	MCHC (g/dl)	%Hb	MCV	Total WBC Count ($x10^3/mm^3$)	Differential Leukocyte Count (%)				
									Neutrophil	Basophil	Eosinophil	Lymphocyte	Monocyte
1	Control (0)	3.81 ± 0.12	78.75 ± 2.34	22.2 ± 0.23	0.07±0.002	5.8±0.15	205±25.2	5.9±0.08	45.71±0.71	1.46±0.69	10.11±0.35	35.66±1.1	7.06±0.25
2	5% of LC_{50}	1.71 ± 0.15** (-55.11)	73.5 ±2 .39* (-6.67)	15.2 ± 0.31** (-31.5)	0.05±0.001** (-28.6)	3.8±0.22** (-34.4)	409±30.1** (+99.5)	6.8±0.12* (-15.2)	58.87±0.55* (+28.8)	1.88±0.61 (+28.7)	9.21±0.39 (-8.9)	28.12±1.23 (-21.1)	1.92±0.06** (-72.8)
3	10% of LC_{50}	1.51 ± 0.17** (-60.36)	68.5 ± 4.5**a (-13.01)	14.6 ± 0.15** (-34.2)	0.03±0.001** (-57.1)	2.2±0.12**a (-62.1)	417±32.5** (+103.4)	7.6±0.19**a (-28.8)	59.61±0.34*a (+30.4)	1.11±0.31 (-23.9)	9.11±0.42 (-9.9)	29.20±1.12 (-17.9)	0.97±0.04**a (-86.3)

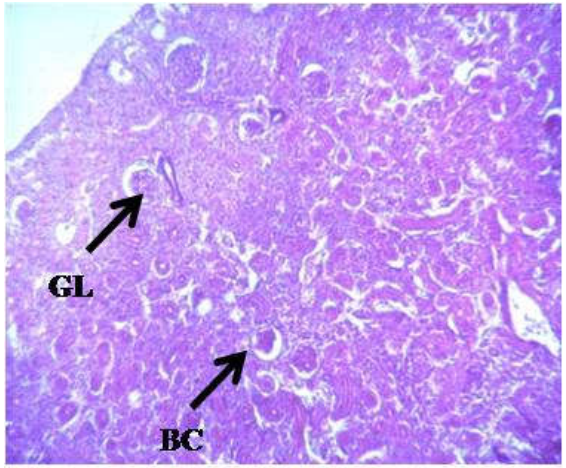

Fig. 3A. Histo-architecture of Control kidney. BC: Bowman's Capsule, GL: Glomerulus

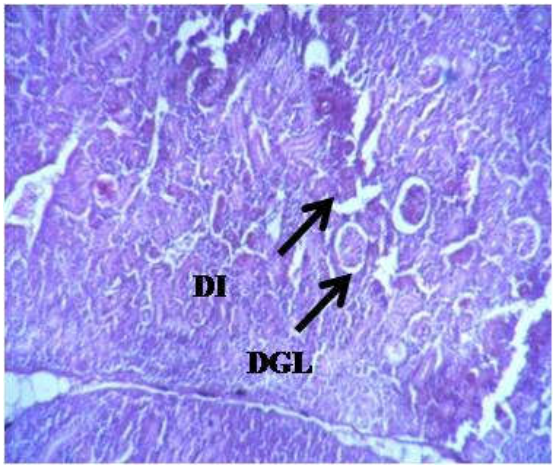

Fig. 3B. Histo-architecture of 5% Triazophos treated kidney. DI: Degenerated Interstitum, DGL: Degenerated Glomerulus

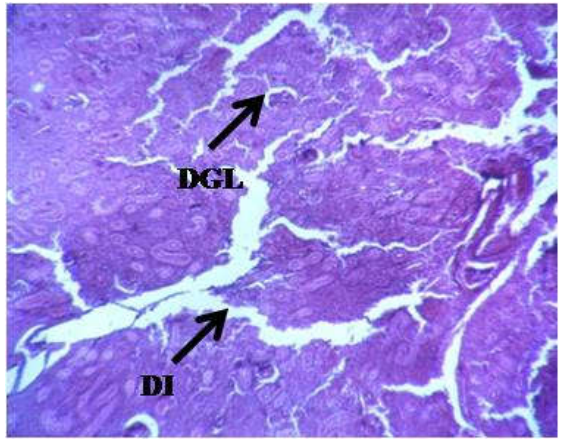

Fig. 3C. Histo-architecture of 10% Triazophos treated kidney. DI: Degenerated Intestitium DGL: Degenerated Glomerulus

E. *MCHC level*

We noted significantly low level of MCHC in 5% and 10% Triazophos treatment groups ($p < 0.01$) in comparison to control. But, among the experimental groups the variation was not significant ($p > 0.05$, Table-1).

F. *MCV level*

We noted significantly high level of MCV in 5% and 10% Triazophos treatment groups ($p < 0.01$) in comparison to control. But, among the experimental groups the variation was not significant ($p > 0.05$, Table-1).

G. *Total WBC count*

We noted significantly high levels of total WBC count in 5% ($p < 0.05$) and 10% ($p < 0.01$) Triazophos treatment groups when compared to control. The total WBC count was more significantly high in 10% Triazophos treated group than 5% ($p < 0.05$, Table-1).

H. *Differential Leukocyte Count*

In fishes, two major classes of WBCs were identified which are granulocytes and agranulocytes. Among the granulocytes, % neutrophil level was found to be significantly high in 5% ($p < 0.05$) and 10% ($p < 0.05$) Triazophos treated groups tan control. The level of increase was more significantly high in 10% group than 5% ($p < 0.05$). Other granulocytes (eosinophil and basophil) did not show significant variations among all of the groups and between the experimental groups ($p > 0.05$, Table-1). In agranulocytes, % monocyte level was noted to be significantly high in 5% and 10% Triazophos treatment groups ($p < 0.01$), and the 10% exposure group showed more variation than 5% exposure ($p < 0.05$). However the variation in % lymphocyte level was not significant ($p > 0.05$, Table-1).

DISCUSSION

Studies on histopathological and hematological changes assist to identify the environmental impacts of pesticides (Kumari and Kumar, 1997; Parry, 1998) as the pesticide exposure significantly damages number of physiologically important organs which in turn brings about different behavioral changes like loss of equilibrium, irregular movements, increase in opercular movements, imbalance and finally leading to death. In the present study, histological alterations were found in different tissues (gill, kidney and liver) of Triazophos exposed fish (*Channa punctatus*). It is evident from the above findings that, even the sub-lethal doses of Triazophos (i.e. 5% and 10% LC_{50}) are highly toxic to fish. The histological changes in the organs of experimental fish increased with increasing dose exposure. Previous report considering several histopathological changes in kidney, liver and gills, due to impact of industrial effluents in the fish, *Channa punctatus* and *Hetropneustes fossils* is available (Kumari and Kumar, 1997). Kidney is one of the very important organs which are affected by contaminants in water as it acts as a filter in animal body. Elsan treatment in *Channa punctatus* also revealed significant decrease in the dimension of Bowman's capsule and glomerulus, and the tubules lost their regular shape due to precipitation of cytoplasm and karyolysis (Banerjee and Bhattacharya, 1995). Hypertrophy of renal cells, changes in the nuclear structure, formation of vacuoles, necrosis and degeneration of renal components were noticed on the renal cells of *Cyprinus carpio* exposed to malathion and sevin (Dhanapakiam and Sampoorni, 1998; Cengiz *et al.,* 2001) demonstrated lesions in the kidney tissues of fish exposed to deltamethrin, tubular degeneration observed in catfish, *Ictalurus punctatus* upon exposure to methyl mercury (Kendall, 1975). Sublethal concentration of phenolic compounds exhibited degeneration and dissolution of epithelial cells of renal tubules, hypertrophy and necrosis in *Notopterus notopterus* (Gupta and Dalela, 1986).

In another vital organ liver, abnormal changes were also found. Fish liver can be regarded as the body's detoxification center and hence a target organ for various xenobiotic substances. Necrosis, which is a passive and unregulated mode of cell death, shows that the capacity to maintain homeostasis was affected. Thus occurrence of necrosis may be one of the important reasons for decreased lysosomal membrane stability leading to the leakage of lysosomal marker enzyme acid phosphatase to the soluble fraction. Pycnotic nuclei observed indicate that the cells became hypofunctional. Pycnosis results in irreversible condensation of chromatin in the nucleus of a cell. Acute toxic injury usually includes cloudy swelling or hydropic degenerations and pycnosis, karyorrhexis and karyolysis of nuclei (Hawkes, 1980; Hinton *et al.,* 1988). Cloudy swelling, bile stagnation, focal necrosis, atrophy and vacuolization have been reported in the *Corydora spaleatus* exposed to methyl parathion by Cengiz *et al.,* 2001 who also reported hypertrophy of hepatocytes, increase of kupffer cells, circulatory disturbance, narrowing of sinusoids, pycnotic nuclei, fatty degeneration and focal necrosis in the liver of *Gambusia affinis* exposed to deltamethrin. The cellular degeneration in the liver may be also due to oxygen deficiency as a result of gill degeneration and/or to the vascular dilation and intravascular haemolysis with subsequent stasis of blood (Mohamed, 2001). In teleost gills are critical organs which perform respiratory, osmoregulatory and excretory functions. Due to their lipophilicity, pyrethroids have a high rate of gill absorption, (Polat *et al.,* 2002). The main feature observed in gills exposed to sublethal concentration of pesticide was partial degeneration of epithelium of secondary gill lamellae. In some place adjacent secondary gill lamellae appeared to adhere each other. Fusion of secondary gill lamellae resulting in reduction of respiratory surface and vacuolization was also observed. Our results are inconsistent with the earlier reports (Rao *et al.,* 2003; Butchiram *et al.,* 2009; Choudhan and Pandey, 1987; Srivastava and Shrivastva, 1983), studied effect of sublethal concentration of malathion chloride on the histopathology of the gills of *Channa gachua* and observed hyperplasia, hypertrophy vacillation in primary gill lamellae, pycnotic nuclei and increase in volume of pillar cells. Our findings are in agreement with Tilak *et al.,* 2007) who reported that the effect of butachlor technical and machete 50% EC has induced marked pathological changes in fish gills. The changes included secondary filaments lost their original shape and cutting of secondary gill filaments, pillar cell nucleus showed necrosis and developed vacuoles in the secondary gill epithelium. The degeneration in gill is due to intimate contact of gills with toxicant may lead to desertion of normal respiratory area that is damage of gill tissue which in turn may reduce the diffusion capacity of the gill.

The diffusion capacity of gill is directly associated with the oxygen carrying capacity of hemoglobin residing in RBC. The total RBC count, % Hb content, % Hematocrit value, Mean

Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH) and Mean Corpuscular Hemoglobin Concentration (MCHC) were found to be significantly low in a dose dependent manner upon pesticide exposure in fish. Similar reports of RBC count and other hematological parameters are available in other fish models like *Cyprinus carpio* and *Puntius ticto* (Satyanarayan *et al.,* 2004). Thus, lower levels of these parameters indicate that utilization of oxygen is differential and might have channelized to modulate different physiological processes including immunity. In the cell-mediated immune response parameters, the TLC and DLC were found to be significantly high in exposed fishes especially the percent neutrophil level. However, in earthworms the count of coelomocyte which act as immunogenic cell was significantly high in pesticide exposed groups. This may be due to the effect of pesticides on the primary line of defense such as mucus and skin of fishes, and body wall of earthworms which got injured and to maintain the homeostasis the Cell–mediated immune parameters were high. These findings are in agreement with other reports (Yonar, 2010; Kathya *et al.,* 2010).

Conclusion

Depending upon our preliminary results, we may suggest that the detrimental impact of organophosphate on both target and non-target organisms are almost similar in terms of hematological and histopathological aspects. However, further biochemical and molecular aspects are yet to be explored to identify the exact mechanism of action of organophospahates and other pesticides in fishes.

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Conflict of interest

None of the authors has any conflict of interest in submitting this manuscript (including financial).

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