International Journal of Zoology and Applied Biosciences **ISSN: 2455-9571**
Volume 2, Issue 5, pp: 232-240, 2017
https://doi.org/10.5281/zenodo.1312090
http://www.ijzab.com
Research Article

POTENTIAL EFFICACY OF *TRIBULUS TERRTRI* AGINST TOXIC IMPACT OF CHLORPYRIFOS ON HAEMATOLOGICAL ALTERATION IN THE FRESH WATER FISH *OREOCHROMMIS MOSSAMBICUS*

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Article History: Received 17th April 2017; Accepted 15th October 2017; Published 27th October 2017

ABSTRACT

Pesticides are stable compounds and they enter the aquatic ecosystem through the agriculture run off. The evaluation of nature and degree of harmful effects produced by the toxic substance in the aquatic organisms are evaluated by toxic tests. 96 hour LC_{50} values have generally been found to be satisfactory for the measurement of acute toxicity. Therefore, in this present study is an attempt to study the toxicity of the pesticide with respect to the hematology, of fish *Oreochrommis mossambicus.* The Chlorpyrifos affects not only fish but also organisms in the food chain through the process of consumption of one by the other. The pesticide enters the body tissues of the fish that affects physiological activities. The cytometric measurements of erythrocytes of sublethal exposure showed that there are not many differences from the control. In the control fish, the erythrocytes were oval in shape with elongated nucleus. Fish exposed to sublethal concentration of Chlorpyrifos showed abnormal size Reduction in the volume of the cytoplasm of cells and swelling of nuclei were observed in fish exposed to concentration. In the hematology, the total Red Blood Corpuscle and Haemoglobin content were decreased with the increasing hours of exposure of the chlorpyrifos 36% EC. The amount of the Mean Corpuscular Haemoglobin (MCH) also was increased. The constant increase in the differential count clearly indicates that the pesticide stress certainly stimulates the white blood cells to produce more at all times of exposure. It has been suggested that the enumeration of differential cell ratio counts provision of useful diagnostic procedure to assess the physiological stress in the fish.

Keywords: *Tribulus terrtri*, chlorpyrifos, Haematology, *Oreochrommis mossambicus.*

INTRODUCTION

Environment is the sum total of water, air and land interrelationships among themselves and also with the human being, other living organisms and other property. It includes all the physical and biological surroundings and their interactions. Environmental studies provide an approach towards understanding the environment of our planet and the impact of human life upon the environment (Overland, 2010). Environmental pollution occurs when the environmental degradation crosses the limit so that. It becomes lethal to living organisms. In India, pesticides constitute an important component in agriculture development and protection of public health since the tropical climate is very conducive to pest breeding. Kumar *et al.,* 2010). Contamination by pesticides in the aquatic ecosystem is a serious problem and fish are more frequently exposed to these pollutants and may be taken in through gills, skin and contaminated food (Li *et al.,* 2010). Fishes are widely used to evaluate the health of aquatic ecosystems because pollutants build up in the food chain and are responsible for adverse effects and death in the aquatic systems. Chlorpyrifos is a widely used organophosphatepesticide, second largest selling in India and used for more than a decade to control pests on cotton, paddy fields, pasture and vegetable crops (Rao *et al.,* 2006). Pesticides are one of the most potentially harmful

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toxic chemicals introduced into the environment. Though they have contributed considerably to human welfare, their adverse effects on non-target organisms are quite significant. Aquatic ecosystems that run through agricultural or industrial areas have a high probability of being contaminated by runoff and ground water leaching by a variety of toxic pesticides which pose a potential direct threat to freshwater organisms, particularly to sensitive animals, such as fish. Chlorpyrifos is an organophosphate insecticide and is highly toxic to freshwater fish. Fishes have an important role in the food chain; therefore, investigation of the effects of toxic pesticides such as chlorpyrifos on fish has a diagnostic significance in the evaluation of negative effects of pesticides on human health.

Fish immune system, important for defense against a variety of harmful pathogens is very sensitive to homeostatic adjustments via endocrine regulation and is influenced by the biochemical profile of the nervous system. Insecticides can alter the immune functions of the body and result in immune-depression, uncontrolled cell proliferation, and alterations of the host defense mechanism against pathogens (Srivasta *et al.,* 2004). Effects of chlorpyrifos on the immune factors of fish such as mRNA levels of IL-1β and IFN- γ2b in immune organs of common carp have been reported by Wang *et al.* (2004) and (De *et al.,* 2004), studied the immune response parameters of *Oreochromis niceties.* Results indicated that chlorpyrifos at 0.051 mg/L induced a diminishing in concentration of lgM in plasma. On the other hand, organisms exposed to high concentration of the pesticide showed an increase in the lysozyme activity. Saeed *et al., (*2005) reported that *O. Niceties* exposed to 0.422 and 0.211 mg/L of chlorpyrifos during 96 h caused a significant decrease in the phagocytic capacity and in the percentage of phagocytic cells present in blood.

Organophosphorus (OP) compounds are one of the most widely used pesticides in the world by replacing the organochlorine. OP pesticides have a common mechanism of action even though each of their chemical structure varies in their nature. They cause inhibition of the nervous tissue acetylcholinesterase (AChE) activity (Ahmad *et al*., 2012). AChE is an important enzyme that can be measured environmental bio-indicator in the animal body. Cholinesterase is involved in the signal transmission at neuromuscular junctions and is also intensely expressed in the organism nervous system. The main role of AChE is to catalyze the hydrolysis of acetylcholine into cholin and acetic acid at cholinergic synaptic sites (Varo *et al.,* 2007).

Haematology studies of cytometric measurement, red blood corpuscle estimation, total white blood corpuscle estimation, haemoglobin content and mean of corpuscle heamoglobin in the fresh water fish in *Labeo rohita* (Hamilton 1882) exposed in monocrotophos Tamizhazhagan (2015). Visvanathan *et al.* (2009) have reported decreasing in hematocrit, hemoglobin and red blood cell values of fish after their exposure to insecticide. Erythrocytes are the dominant cell type in the blood of fish; it is widely accepted that fish like most other vertebrates. The high erythrocyte number was associated with fast movement, predaceous nature, and high activity with streamlined body (Srinivasa *et al.,*1986) Leucocytes are involved in the regulation of immunological function in many organisms and the increase in WBC in stressed animals indicates a protective response to stress (Haniffa, 1990).

Similarly the significant increase of WBC count may be due to generalized immune response. In the present study is a haematology study of cytometric measurement, red blood corpuscle estimation, total white blood corpuscle estimation, haemoglobin content and mean of corpuscle haemoglobin in the *Tribulus terrtri* against toxic impact of chlorpyrifos on fresh water fish *Oriochrommis mossambicus*.

MATERIALS AND METHODS

Collection and maintenance of the experimental Animal

The freshwater fish *Oreochromis mossambicus* were collected from the VGM fish farm located in Kurinjipadi, Cuddalore district. The fishes were brought to the laboratory and transferred to the rectangular cement tanks 100 (175) of 500 liters capacity containing chlorine free aerated well water, fishes of the same size and weight were used irrespective of their sex for the experiments.

Tribulus terrestrial

It is proved that herbal medicine is effective in the treatment of many diseases. *Tribulus terrestrial* is an annual plant in the caltrop family (Zygophyllaceae) widely distributed around the world. That is adapted to grow in dry climate locations in which few other plants can survive. It is an invasive species in North America. Like many weedy species, this plant has many common names, including goat's-head, bindii, bullhead, burra gokharu, Bhakhdi, caltrop, small caltrops, cat's-head, devil's eyelashes, devil's-thorn, devil's-weed, puncture vine, puncturevine, and duckweed. It is native to warm temperate and tropical regions of the Old World in southern Europe, southern Asia, throughout Africa, and Australia. It can thrive even in desert climates and poor soil.

The potential protective role of *Tribulus terrestris* in acetaminophen-induced hepatotoxicity in *O. mossambicus* was investigated. Histopathological changes of liver, gill and muscle samples were compared with respective controls. The results of the present study specify the hepatoprotective and antioxidant properties of *T. terrestris* against acetaminophen-induced toxicity in freshwater fish, *O. mossambicus.*

Tribulus terrestris as a Supplementary Feed

Collection and preparation of *Tribulus terrestris:* The dried *T. terrestris* was collected from thiruvenkadu Village near to chidambaram. The *T. terrestris* powder was kept in carefully.

Preparation of *Tribulus terrestris:* Dried *T. terrestris* powder weight of 50g and rice brand powder was weighted at 50g for both the powders mixed well then adds sterilized water make titer paste. After this paste to make pellets (1gram) according to fish feed (artificial feed) sizes. Pellets were dried inside the room at normal temperature and avoid the direct sunlight. Pellets were packed in airtight bottle for experimental use.

Selection of Herbicide

In the present investigation, the herbicide chlorpyrifos has been selected. Chlorpyrifos is a broad leaf herbicide that has been used historically on agriculture and forestry. Herbicide is a chemical used to kill or otherwise manage certain species of plants considered to be pests.It is used most extensively on corn crops and to a lesser extent on other food crops such as asparagus, sugarcane and macadamia, pineapple as well as in conifer restoration plantings. Chlorpyrifos is a herbicide that does not occur naturally. Pure chlorpyrifos is an odorless, white powder that is not very volatile, reactive or flammable and that will dissolve in water.

Toxicity Studies

Acute toxicity tests were conducted to measure the impact of toxicant on aquatic animals within a short period of five hours. In the toxicity studies, the renewal technique of acute static test was adopted, in which fish were periodically exposed to the concentrations of the same composition, usually once in every 24 hours by transferring the animals from one test chamber to another. The LC_{50} is a statistical estimate of the concentration of toxic material in the water that kills 50 percent of the test animals under experimental conditions with specific time intervals (Sprague, 1973). This value is ideally suited for toxicity studies as it gives more acceptable and reproducible concentration required affecting 50 per cent of the organisms than any other value (Pickering and Henderson, 1966).

Hematological Studies

The blood was mixed well with the EDTA solution by using a needle and this sample was used for determining the Red Blood Corpuscle Count (RBC), Total Leucocyte Count (TLC) and Haemoglobin content (Hb). For RBC count, a method devised by Yokayama and later modified by Christensen *et al.*, was followed. For counting the total number of WBC, the pipette with white lead was used. The number of cells present in the four large corner squares marked by capital letter *'L'* was counted and multiplied by 10^3which give the total number of WBC per cubic millimeter of blood. Haemoglobin determination is the quickest means for detecting anaemia. However, many factors are known to influence the haemoglobin level. The Sahli Hellige method was followed for haemoglobin determination. Sahlis pipette was filled slightly above the 20mm mark. The pipette was wiped with a filter paper or cotton to remove excess blood and the volume was adjusted to exactly 20 $mm^3$by blotting the tip. The blood was expelled into a calibrated (transmission) test tube containing 2 ml of 0.1 N HCl. The pipette was rinsed several times in the acid solution. The sample was allowed to stand for 15 min. In the control fish, the erythrocytes were oval in shape with elongated nucleus fish, exposed to sublethal concentration of Monocrotophos showed abnormal size. Reduction in the volume of the cytoplasm of cells and swelling of nuclei were observed in fish exposed to concentration.

Statistical Analysis

The data obtained from the quantitative study were expressed as the mean S.E. The mean values were calculated from 6 individual observations. P-values were calculated by two tailed students't' test. The two mean values obtained were considered significant from each other. Since the calculator student's' ration was greater than 't' 0.05 or 0.01 value at the respective degree of freedom (df=n1+n2+-2). The't' values were calculated by using the formula (Trivedy and Goel, 1984).

$$T = \frac{X1 - X2}{\sqrt{SE_1^2 + SE_2^2}}$$

Where X_1 and X_2 represent the means of control and treated values, SE_1 and SE_2 were their respective standard errors. Standard error was calculated from the formula:

$SE = \frac{S.D}{\sqrt{n}}$ Where 'n' was total number of observations.

RESULTS

LC_{50} values for the fish

Median lethal concentration (LC_{50}) values for the fish *O. mossambicus* exposed to different period of chlorpyrifos are presented in the Table 1.

Haematological parameters

The quantitative changes of haematological parameters like RBC, WBC, Hb, PCV, MCV, MCH and MCHC of the blood cells in the freshwater fish *O. mossambicus* in all groups control (group 1), sublethal concentration of chlorpyrifos (group 2), chlorpyrifos along with Tribulus terrestris (group 3) and Tribulus terrestris alone (group 4) exposed to 24, 48, 72, 96 and 120 hrs are observed.

Red blood corpuscles (RBC)

In the present investigation chlorpyrifos treated fish (group 2) show the decrease in RBC (red blood cells) content when compared to control (group 1). The percentage changes are -22.71, -37.60, -14.61, -31.92 and -20.82 for 24, 48, 72, 96 and 120 hrs respectively. Whereas in the chlorpyrifos and Tribulus terrestris treated fish (group 3) record significant recovery from the effect of group 2. The

increased percentage recoveries are 53.39, 67.93, 75.93, 122.8 and 167.67 for 24, 48, 72, 96 and 120 hrs respectively. While in fish of *O. mossambicus* exposed to *T. terrestris* alone, when compared with control, a slight variation is noticed. Percentage changes in Tribulus terrestris (group 4) are -11.20, -2.91, -11.74, -4.23 and -5.47 for 24, 48, 72, 96 and 120 hrs respectively. The recorded RBC content in blood cell counts of four groups is statistically significant at 1% and 5% levels (Table 2).

White blood corpuscles (WBC)

The chlorpyrifos exposed fish (group 2) show a slight increase in the WBC count when compared to control fish (group 1). There are no noticeable changes in the control fish. The increased per cent changes are 24.39, 31.54, 37.08, 49.39 and 60.64 for 24, 48, 72, 96 and 120 hrs respectively. Whereas in the chlorpyrifos, along with *T. terrestris* treated fish (group 3) record significant recovery from the effect of chlorpyrifos (group 2). The percentage recoveries are 8.94, 26.89, 35.55, 38.88 and 43.41 for 120 hrs respectively. While in fish exposed to *Tribulus terrestris* alone (group 4), no changes are noticed and it is equal to normal. Percentage changes in *Tribulus terrestris* alone (group 4) are -8.85, -8.52, -3.33, -5.32 and -2.98 for 24, 48, 72, 96 and 120 hrs respectively. WBC content in blood cells counts for groups 2, 3 and 4 is statistically significant at 1% and 5% levels (Table 2).

Haemoglobin (Hb)

Hb levels of *O. mossambicus* fingerlings exhibit remarkable changes. In group 2, the values of Hb decrease than into control (group 1). The per cent changes are -7.46, -13.10, -11.22, -17.30 and -17.30 for 24, 48, 72, 96 and 120 hrs respectively, whereas the chlorpyrifos along with *Tribulus terrestris* treated fish (group 3) record significant recoveries from the effect of chlorpyrifos (group 2). Increased percentage recoveries are 4.76, 5.46, 14.97, 16.88 and 24.04 for 24, 48, 72, 96 and 120 hrs respectively. While in fish exposed to *T.ribulus terrestris* alone (group 4), slight variations are noticed compared with control (group 1). Percentage changes in *T. terrestris* alone (group 4) are -5.49, -5.15, -0.82, -3.66 and -2.91 for 24, 48, 72, 96 and 120 hrs respectively. Hb content in blood cell counts for groups 2, 3 and 4 is statistically significant at 1% and 5% levels (Table 2).

Packed cell volume (PCV)

In the present investigation, group 2 shows decrease in the level of PCV content when compared to group 1, group 3 and group 4. The per cent changes for group 2 are -17.44, -10.80, -15.86, -13.23 and -10.03 for 24, 48, 72, 96 and 120 hrs respectively. While in the fish exposed to group 3, PCV content decreases when compared to group 2. The per cent changes are -20.78, -13.17, -15.79, -18.64 and -12.93 for 24, 48, 72, 96 and 120 hrs respectively. Remarkable minimum value is noticed in group 4 and the percentage changes are -1.75, -2.09, -2.82, -0.03 and -0.46 for 24, 48, 72, 96 and 120 hrs respectively. The level of PCV content for the four groups is statistically significant at 1% and 5% levels (Table 2).

Mean corpuscular volume (MCV)

The chlorpyrifos exposed fish (group 2) show a slight decrease in the MCV content when compared to control fish. There are no noticeable changes in the control fish. The per cent changes are -13.30, -11.26, -6.57, -12.44 and -2.30 for 24, 48, 72, 96 and 120 hrs respectively. The chlorpyrifos along with *T. terrestris* treated fish (group 3) recorded significant recoveries from the effect of chlorpyrifos (group 2). The per cent recoveries are -5.04, -4.05, -3.94, -7.72 and -11.39 for 24, 48, 72, 96 and 120 hrs respectively. In fish exposed to *T. terrestris* alone (group 4), no changes occur and it is equal to normal. Percentage changes in *T. terrestris* (group 4) are -0.19, -1.35, -1.75, -1.71 and -0.84 for 24, 48, 72, 96 and 120 hrs respectively. MCV content in blood cell counts for groups 2, 3 and 4 is statistically significant at 1% and 5% levels (Table 3).

Mean corpuscular haemoglobin (MCH)

The chlorpyrifos exposed fish (group 2) show a slight decrease in the MCH content when compared to control fish (group 1). There are no noticeable changes in the control fish. The per cent changes are -15.71, -8.43, -4.06, -3.25 and -7.54 for 24, 48, 72, 96 and 120 hrs respectively. The chlorpyrifos along with *T. terrestris* treated fish (group 3) record significant recoveries from the effect of chlorpyrifos (group 2). The per cent recoveries are 29.0, 32.41, 20.37, 42.56 and 27.08 for 24, 48, 72, 96 and 120 hrs respectively. In group 4 fish, the MCH content is lower than in group 1. The percentage changes in group 4 are -3.68, -3.09, -4.84, -4.33 and -3.06 for 24, 48, 72, 96 and 120 hrs respectively. The recorded MCH content for the 4 groups is statistically significant at 1% and 5% levels (Table 3).

Mean corpuscular haemoglobin concentration (MCHC)

The level of MCHC content decreases when exposed to group 2 compared to control fish (group 1). The increased per cent changes for group 2 are -45.43, -43.32, -40.54, -39.19 and -37.91 for 24, 48, 72, 96 and 120 hrs respectively. In group 3, the increased percentage recoveries is -32.0, -29.87, -26.61, -23.73 and -21.89 for 24, 48, 72, 96 and 120 hrs in group 4, there are no markable changes. Slight variations are -16.49, -10.74, -7.22, -4.86 and -1.44 for 24, 48, 72, 96 and 120 hrs respectively. The recorded MCHC content of all 4 groups is statistically significant at 1% and 5% levels (Table 3).

Table 1. Median lethal concentration (lc_{50}) values for the fish *oreochromis mossambicus exposed* to different period of chlorpyrifos.

S. No.	Exposure period in hrs	LC_{50} value (mg/L)
1	3	26.27
2	6	24.36
3	12	22.76
4	24	22.24
5	48	21.85
6	72	21.38
7	96	21.99
8	120	20.00

Table 2. Changes in the level of RBC ($\times 10^6$/mm^3), WBC ($\times 10^3$/mm^3), Hb (g/L) and PCV (%) in *Oreochromis mossambicus* fingerlings exposed to 120 hrs sublethal concentration of chlorpyrifos and antidote *Tribulus terrtri*.

Groups		Hours of exposure				
		24	48	72	96	120
RBC (× 106/mm3)	Group 1 – Control	3.39 ± 0.02	3.43 ± 0.03	3.49 ± 0.03	3.54 ± 0.04	3.65 ± 0.04
	Group II - Chlorpyrifos	2.62** ± 0.01	2.14** ± 0.02	2.98* ± 0.04	2.41* ± 0.05	2.89* ± 0.05
	% COC	-22.71	-37.60	-14.61	-31.92	-20.82
	Group III - Chlorpyrifos + Tribulus terrtri	5.20* ± 0.03	5.76* ± 0.03	6.14* ± 0.04	7.89* ± 0.05	9.77* ± 0.06
	% COC	+53.39	+67.93	+75.93	+122.88	+167.67
	Group IV - Tribulus terrtri	3.01** ± 0.02	3.33** ± 0.02	3.08** ± 0.03	3.39** ± 0.04	3.45** ± 0.05
	% COC	-11.20	-2.91	-11.74	-4.23	-5.47
WBC (× 103/mm3)	Group 1 – Control	22.14 ± 0.08	22.16 ± 0.07	22.19 ± 0.08	23.12 ± 0.07	23.15 ± 0.09
	Group II - Chlorpyrifos	27.54** ± 0.05	29.15** ± 0.06	30.14** ± 0.07	34.54** ± 0.09	37.79** ± 0.08
	% COC	+24.39	+31.54	+37.08	+49.39	+60.64
	Group III - Chlorpyrifos + Tribulus terrtri	24.12** ± 0.07	28.12** ± 0.08	28.48** ± 0.06	29.11** ± 0.07	22.90* ± 0.06
	% COC	+8.94	+26.89	+28.34	+25.90	+29.15
	Group IV - Tribulus terrtri	20.18** ± 0.05	20.27** ± 0.06	21.45** ± 0.05	21.89** ± 0.04	22.46** ± 0.057
	% COC	-8.85	-8.52	-3.33	-5.32	-2.98
Hb (g/L)	Group 1 – Control	9.65 ± 0.12	9.69 ± 0.14	9.75 ± 0.18	9.83 ± 0.16	9.94 ± 0.16
	Group II - Chlorpyrifos	8.93* ± 0.23	8.42* ±0.21	8.65* ± 0.26	8.12* ± 0.19	8.22* ± 0.23
	% COC	-7.46	-13.10	-11.22	-17.39	-17.30
	Group III - Chlorpyrifos + Tribulus terrtri	10.11* ± 0.40	10.22* ± 0.19	11.21* ± 0.42	11.49* ± 0.21	12.33* ± 0.46
	% COC	+4.76	+5.46	+14.97	+16.88	+24.04

PCV (%)	Group IV - Tribulus terrtri	9.12* ± 0.13	9.19* ± 0.14	9.67* ± 0.18	9.47* ± 0.17	9.65* ± 0.19
	% COC	-5.49	-5.15	-0.82	-3.66	-2.91
	Group 1 – Control	48.5 ± 0.236	48.17 ± 0.437	59.12 ± 0.256	60.45 ± 0.237	62.16 ± 0.437
	Group II - Chlorpyrifos	24.41* ± 0.312	44.75* ± 0.239	49.15* ± 0.241	52.45* ± 0.245	56.12* ± 0.233
	% COC	-17.44	-10.80	-15.86	-13.23	-10.03
	Group III - Chlorpyrifos + Tribulus terrtri	20.49* ± 0.412	43.56* ± 0.317	49.78* ± 0.256	49.18* ± 0.255	54.12* ± 0.247
	% COC	-20.78	-13.17	-15.79	-18.64	-12.93
	Group IV - Tribulus terrtri	47.65* ± 0.242	49.18* ± 0.473	52.17* ± 0.484	60.43* ± 0.495	61.87* ± 0.455
	% COC	-1.75	-2.09	-2.82	-0.03	-0.46

Mean ± S.E. – Mean of six individual observations; (+/–) indicate the percentage change over control; * Significant at 5% level; **Significant at 1% level; %COC – % change over control.

Table 3. Changes in the level of MCV (fl), MCH (pg) and MCHC (%) in *Oreochromis mossambicus* fingerlings exposed to 120 hrs sublethal concentration of chlorpyrifos and antidote *Tribulus terrtri.*

Groups		Hours of exposure				
		24	48	72	96	120
MCV (fl)	Group 1 – Control	0.218 ± 0.94	0.222** ± 0.05	0.228** ± 0.04	0.233 ± 0.06	0.217 ± 0.07
	Group II - Chlorpyrifos	0.214** ± 0.06	0.197** ± 0.05	0.213** ± 0.06	0.204** ± 0.04	0.212** ± 0.05
	% COC	-13.30	-11.26	-6.57	-12.44	-2.30
	Group III - Chlorpyrifos + Tribulus terrtri	0.207** ± 0.02	0.213** ± 0.03	0.219** ± 0.06	0.215** ± 0.07	0.210** ± 0.08
	% COC	-5.04	-4.05	-3.94	-7.72	-11.39
	Group IV - Tribulus terrtri	0.216** ± 0.03	0.219** ± 0.04	0.224** ± 0.06	0.229** ± 0.05	0.235** ± 0.09
	% COC	-0.91	-1.35	-1.75	-1.71	-0.84
MCH (pg)	Group 1 – Control	27.43 ± 1.15	27.49 ± 1.42	27.45 ± 1.15	27.65 ± 1.18	27.69 ± 1.19
	Group II - Chlorpyrifos	23.12** ± 1.16	25.17** ± 1.17	26.42** ± 1.22	26.75** ± 1.27	25.45** ± 2.34
	% COC	-15.71	-8.43	-4.06	-3.25	-7.54
	Group III - Chlorpyrifos + Tribulus terrtri	35.39** ± 1.15	36.40** ± 1.65	33.15** ± 2.01	39.42** ± 0.69	35.19** ± 1.25
	% COC	+29.0	+32.41	+20.37	+42.56	+27.08
	Group IV - Tribulus terrtri	26.42 ± 2.55	26.64 ± 2.40	26.12 ± 2.05	26.45** ± 2.41	26.84** ± 1.35
	% COC	-3.68	-3.09	-4.84	-4.33	-3.06
MCHC (%)	Group 1 – Control	19.7 ± 0.08	19.18 ± 0.09	19.24 ± 0.06	19.34 ± 0.07	19.41 ± 0.05
	Group II - Chlorpyrifos	10.75** ± 0.02	10.87** ± 0.03	11.44** ± 0.04	11.7** ± 0.07	12.05** ± 0.06
	% COC	-45.43	-43.32	-40.54	-39.19	-37.91

Group III - Chlorpyrifos	13.39**	13.45*	14.12**	14.75**	15.16**
+ Tribulus terrtri	± 0.07	± 0.05	± 0.04	± 0.03	± 0.09
% COC	-32.0	-29.87	-26.61	-23.73	-21.89
Group IV - Tribulus	16.45**	17.12**	17.85**	18.40**	19.13**
terrtri	± 0.06	± 0.06	± 0.05	± 0.07	± 0.08
% COC	-16.49	-10.74	-7.22	-4.86	1.44

Mean ± S.E. – Mean of six individual observations; (+/–) indicate the percentage change over control; * Significant at 5% level; **Significant at 1% level; %COC – % change over control.

DISCUSSION

Fish blood is a patho-physiological reflector of the whole body and therefore blood parameters are important in diagnosing the structural and functional status of the fish exposed to toxicants (Rahman *et al.,* 2002). Pesticide toxicants are known to induce anaemia in fish. In the present study, it is found that a reduced RBC count and Hb contents in the *chlorpyrifos* exposed fish, may be due to inhibition of erythrocyte production or increase in the rate of erythrocyte destruction. Purves *et al.,* (2008) have reported that the anaemia is caused due to reduction in the erythrocyte production which supports the present findings. Houston (1997) have reported that the RBC count and haemoglobin content values significantly decrease resulting macrocytic anaemia in *Heteropneustes fossilis* exposed to zinc.In the present study, *chlorpyrifos* along with *T. terrestris* (group 3) exposure induces a noticeable concentration dependent on decreased Hb level may impair oxygen supply to various tissues, thus resulting in a slow metabolic rate and low energy production (Ahmad *et al.,* 1995). The significant decrease in the Hb concentration may also be due to either in increase in the rate at which Hb is destroyed or due to a decrease in the rate of Hb synthesis (Reddy and Bashamohiden, 1989).

Vasantharaja *et al.* (2012) have observed that the reduction in Hb content of toxicant exposed fish could be due to the inhibitory effect of the toxic substance on the enzyme system in the synthesis of Hb. Venkatesan et al., (2012) have reported decreasing haemoglobin, haematocrit level and erythrocyte count in *Channa punctuates* after exposure to either copper alone or copper and cadmium. Vettrivel *et al.,* (2013) have reported a decrease in RBC count with an increase in WBC and Hb level after chronic exposure of *Cyprinus carpio* to sublethal concentration of sodium selenite. According to Meenambal *et al.,* (2012), *O. niloticus* and *Chrysichthyes auratus* which exposed to *chlorpyrifos* result in a significant decrease in RBC, haemoglobin and haematocrit. It may be attributed to the lowering of the oxygen content of the water. Prabakaran *et al.,* (2014) has observed that high concentrations of cadmium decrease the per cent of haematocrit in the blood of rainbow trout.

Increase in the total leucocyte count has been attributed to several factors like increase in thrombocytes, lymphocyte or squeezing of WBC in peripheral blood in biochemical changes related from fish body. The industrial development and rapid urbanization has led to development of polluted zones discharging potentially toxic compounds in the environment. Especially, indiscriminate use of pesticides resulted in contamination of aquatic system has now become a global problem and is being extensively researched worldwide (Tamizhazhagan and pugazhendy 2016). In the present study, MCH is decreased from 24 to 120 hrs of sublethal treatment of chlorpyrifos. The decreased MCH and MCV levels may be a sign of hypochromic microcytic anemia (Tamizhazhagan *et al.,* 2017). MCH is significantly decreased in *C. idella* after 48 hrs exposure fenvalerate (Tamizhazhagan *et al.,* 2016; Srinivasa *et al.,* 1986) also report an increase in MCV and MCH of *Oreochromis mossambicus* after treatment with paper and pulp mill effluent, increase in MCHC together with MCV and MCH after exposure to zinc which is indicative of megaloblastic anaemia (Varo *et al.,* 2007). Total Hb concentration is reduced in the blood of triploids containing significantly fewer erythrocytes. The average volume of red blood cells (MCV) is greater in triploids, such that the mean cellular haemoglobin content (MCH) in erythrocytes of triploids is significantly greater than in diploids and the mean cellular haemoglobin concentrations (MCHC) are equivalent. Reported values for total blood haemoglobin (Hb) and MCHC concentrations in diploid and triploid fish are not consistent, whereas the mean cellular haemoglobin content (MCHC) is commonly reported to be higher in polyploids Rahman 2002: Houston 1997, report no differences in total Hb but higher MCH values in triploid shidrum (*Umbrina cirrosa*) compared to their diploid counterparts, since the mean cellular haemoglobin (MCHC) is significantly reduced at that time.

CONCLUSION

It is evident that chlorpyrifos presented in aquatic ecosystems can affect aquatic fauna in different ways. Alterations in physico-chemical properties of water, destruction of the delicate balance of the environment, entry into the food

chains and physiological damage to the vital tissues of aquatic fauna are the threatening issues of the modern day pesticides. Long term exposure to these products causes countless abnormalities and reduces the life span of organisms. Finally, we conclude that chlorpyrifos is highly toxic to fish, and impose life threatening effect on fish at both lethal and sublethal concentrations. Altered haematological responses can be used as tools in bioassessment to monitor ecotoxicological risks associated with pesticides such as chlorpyrifos to various fish.

ACKNOWLEDGMENTS

The authors are thankful to authorities of Bharathiar Universirty for the facilities provided to carry out this research work.

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