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ORIGINAL RESEARCH ARTICLE

Protective Role of *Spirulina* on the Variation of Haematological Parameter Induced by Herbicide Atrazine in the Fresh water Fish *Cyprinus carpio* (Linn)

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ABSTRACT

The protective effect of *Spirulina* against atrazine toxicity stress and haematological parameters was evaluated in fresh water fish *Cyprinus carpio* (Linn). Exposure to atrazine along with *Spirulina* for 120 hours by an acute toxic dose 0.5 mg/L showed variation in the haematological parameters. *Spirulina* treatment shows marked elevation in the blood haemoglobin, RBC and haematocrit and the recovery was noticed. Atrazine induced haematological change were also minimized with the treated atrazine along with *Spirulina*. The observed level of WBC is increased (group II), when compared with control. These results suggest the *Spirulina* algae might play a role in reducing the toxic effect of atrazine and Sits haematological effects seem to mediate such a protective effect.

Key word: *Spirulina*, Atrazine, Haematological, RBC, Hb and *Cyprinus carpio*.

1. INTRODUCTION

Environmental pollution caused by pesticides especially in aquatic ecosystem, has become a serious problem. The widespread use of pesticides to increase crop production, which resulted in a series of toxicological and environmental problem, in particular the appearance of resistant species of pest and toxic effects in human and animals. Worldwide herbicide usage has increased dramatically during the past two decades, coinciding with changes in farming practices and increasingly intensive agricultural concern for the possible threat to human health posed by exposure to these chemicals [1, 2]. The use of haematological parameters as fish health indicators have been proposed by [3]. Haematological parameters are used as in index of fish health status in a number of species to detect physiological changes following different stress condition like exposure to pollutants, disease, metals hypoxia, etc., [4].

Blood is the most important and abundant body fluid. Its composition often reflects the total physiological condition. The main route for any pesticides through the gills. From the gills, it is transported to various part of the body via the blood stream. Blood provides an ideal medium for toxicity studies. The haematological parameters have been considered as diagnostic indices of pathological condition in animal. Fish blood can serve as a valuable in detecting changes taking place in animal. Some authors [5,6,7,8] have reported a decrease in haematocrit haemoglobin and red blood cells values of some fish after their exposure to insecticides. Saxena *et al*. (2002)[9] showed significant changes in the haematology of the common freshwater fish *Cyprinus carpio* on exposure. The information suggests that haematological parameters could be used as potential biomarkers of herbicides. *Spirulina* is a group of Blue green algae, and it's grown by fresh water medium. *Spirulina* which is grown be eco-friendly alkaline pH levels of the *Spirulina* culture. *Spirulina* is sun-dried, which gives it a special energy and wonderful taste. In its World Food Conference of 1974, the United Nation launched *Spirulina* as being possibly "the best food for the future". The aim of present investigation was to study the changes induced by sublethal concentration of atrazine exposure on some haematological parameters of *Cyprinus carpio* and chelating properties of *Spirulina* against toxicity.

2. MATERIALS AND METHODS

Specimens of *Cyprinus carpio* were obtained from Navarathna fish farm from Pinnalur village and introduced into large cement tiles tank (4×4) disinfected with potassium permanganate and washed thoroughly prior to introduction of fish (to prevent the fungal infection). Fish were acclimatized for about 15 days before the

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commencement of the experiment. They were fed on commercial fish feed which given daily at morning hours. LC_{50} of atrazine was calculated by the log – dose / profit regression line was recorded. The test fishes were grouped in four groups (1,2,3 and 4) having Group I control, Group II atrazine, Group III Atrazine + *Spirulina*, Group IV *Spirulina*) were dissolved 0.5 mg/L in each groups for sublethal concentration of atrazine for a period of 120 hours. Simultaneously a control was maintained to compare to toxicant values.

The experimental sublethal concentration was 0.5 mg/L of atrazine, and the exposure time was 120 hours. Experimental were performed under semi static condition and aquaria water was changed every 24 hours with the appropriate herbicide amount. A Group of 8 fishes were exposed to each groups and exposure period. At the same time, 8 specimens which are used as a control were kept in clean trough. Experimental chemical atrazine was purchased from (TATA Atrataf 50% WP) mft by Rallis India Limited, Mumbai. The supplemented diet *Spirulina* were collected from Aurospriul commercial farm, from Aurovile village, Pondicherry.

At the end of the each exposure time blood was collected by dissection of caudal peduncle [10,11] with a haparinized micro syringe and transferred to special tubes containing potassium EDTA as an anticoagulant agent. The following haematological assays were performed: Erythrocyte Count (RBC) in **1 µ** of blood: the red blood cells were counted in the Bukar chamber following 200 x dilution in the Hyam fluid. Leukocyte count (WBC) in **1µ** of blood: The white blood cells were counted in the Burker chamber, following dilution in the Truki fluid [13]. Haemoglobin counted (Hb): Photometrically, using the cyanomethaemoglobin techniques, with Drabkin reagent, at 540nm [13]. Haematocrit (Hct): In heparin – covered microhaematocrit tubes, using haematocrit centrifuge and standards reading devices. The assay listed above was performed with standards ANALAB reagent. The following erythrocyte – related indices were calculated: Mean haemoglobin content (MCH), Mean Cell Haemoglobin Count (MCHC), Mean erythrocyte volume (MCV). Blood samples of each fish were used to prepare by – Grunwald and Giemsa stained smears to read to leukocyte pattern percentage contribution of individual's leukocyte from were calculated based on 200 cells examined [12,13].

3. RESULTS AND DISCUSSION

In the present Investigation, the fishes were exposed to atrazine (Group 2) when compared to controls (Group 1) the RBC content was decreased gradually, the percentage decrease was 5.5, 1.49, 1.71, 23.94 and 20.44, for the period of 24, 48, 72, 96, and 12 hours respectively. The observed value of Group III atrazine along with *Spirulina* exposed fish shows gradually recovered. The percent changes over control were, 4.16, 0.89, 1.14, 0.15, 0.18 for 24, 48, 72, 96 and 120 hours respectively. The observed value of Group IV *Spirulina* supplemented fish, when compared to controls, the RBC content decreased gradually. The percent changes over control were 26.94, 37.81, 32.85, 28.16, 24.30 for 24, 48, 72, 96 and 120 hours respectively. The results of this study showed that, the RBC, haemoglobin and haemotocrit values significantly decreased in atrazine treated fish when compared to control. The decreases in RBC might be due to effect of pesticides on blood forming organ (Bone marrow and liver) and inhibition of many steps of biosynthesis of fish, as the results of pesticide exposure. But the exposure of *Spirulina* supplements diet, which is having highly rich nutrients. So, gradually increased in RBC from Group IV when compared with control.

The decreased value of content indicates acute anemia. The anemia could be due to destruction of RBC [14,15]. The observed values of WBC (Group II) fishes exposed to atrazine, when compared to controls, the WBC content was increased gradually. The percent change over control 35.18, 35.31, 35.60, 36.05 and 35.77 for 24, 48, 72, 96 and 120 hours respectively and also increased in Group III exposure to atrazine along with *Spirulina* the percent change over control were 20.37, 19.88, 20.84, 22.67, 17.43 for 24, 48, 72, 96 and 120 hours respectively. The recorded value of Group IV *Spirulina* exposure shows slightly decreased when compared to Group II and III.

The increase in WBCs content recorded in this work when compared with control and other parameters could be due to the attempt of the fish to fight against the antigens (pollutant) and this augmented the production of more WBC to improve the health status of the fishes which agreed with the reports of [16,17]. The increased in WBC count can be correlated with an increased in antibody production which helps in survival and recovery of fish exposed to sub-lethal concentration of pesticides [18]. [19] reported an increased in TLC in

Channa punctatus exposed fenvalerate. The level of haemoglobin content Group II atrazine treated fish, when compared to control, the haemoglobin content was decreased. The observed values of Group III exposure to atrazine along with *Spirulina* values were decreased. The recorded values of Significant reduction in haemoglobin in experimental animals might be destruction (decrease in haemoglobin) has been reported by [20]. Exposure to heavy metals or pesticides leads to reduced haemoglobin content and haematocrit *via* disorders in haemopoietic and accelerated disintegration of erythrocyte cell membrane [21]. The decrease in blood haemoglobin and red blood cells is not unconnected with the presence of stressor which caused haemodilution to occur die to the impaired osmoregulation [22] when trichlorefon (on organophosphate pesticide commonly used for pest control) was exposed to fish for 120 hours. The increase Hb content could be explained as a process where the body tires to replace the oxidized denatured Hb [23]. The experimental values of PCV (Packed Cell Volume) in the fishes exposed to atrazine, when compared to control PCV content were decreased. The recorded values of Group III Atrazine and *Spirulina* exposed to fish, values were decreased. Group IV *Spirulina* alone exposed to fish shows increases in the PCV content.

The haematocrit values of *Cyprinus carpio,* which was exposed to different cadmium concentration, showed differences [24]. The fishes exposed to atrazine were decreases in the packed cell volume levels and fall in the number of red blood cells followed by PCV confirms anemia in *Clarias batrachus.* [25] reported no effect on the hematological profiles of common carp exposed to 45 µg simazine. However, significant decrease in PCV has been reported by [26] in common carp exposed to atrazine. [27] also reported lower values of RBC and PCV after metribuzin exposure.

The value of Group II Atrazine treated fish, when compared to control, the MCV value was significantly lower in the Group I control and also observed value of Group III atrazine along with *Spirulina* exposed to fish. The present study MCH values also reduced after exposure of atrazine to various concentrations. Because of the exposure herbicide a slight increased in MCV while all the erythrocyte - based parameters. Another type of haematological response to the effect of organophosphorus compounds was significant increased of mean corpuscular volume (MVC) associated with increment of haematocrit value and Group IV *Spirulina* alone exposure fish, slightly increases in Hb values compared with other groups. Changes in hematological parameters might have been brought about by cypermethrin as an anemic condition due to decreased synthesis of Hb and RBC number in bone marrow cells. drop of MCHC. This response was registered in common carp after acute exposure of phenitrothion, imidan and dichloros [28].

In the present study the observed values of atrazine treated fish, when compared to control the MCH values were decreased. Where as in Group III atrazine along with *Spirulina* exposed *and* Group IV *Spirulina* exposed for shows slightly increase. The result shows Erythrocytes and inhibition of erythropoiesis, which is confirmed by increased in MCH values. The atrazine exposed fish showed a reduction in the mean corpuscular haemoglobin. The reduction in the erythrocyte count and the haemoglobin content accompanied by increased MCH and MCV were revalued by [5] in carp exposed for 24 hours to cypermethrin (0.02 mg kg^{-1})

The observed values MCHC in Group II atrazine treated fish, when compared to control. The MCHC content was increased. Above these values (Group II and IV) slightly increase, when compared to control. Erythrocyte and inhibition of erthropoiesis, which is confirmed by increased MCHC values. The MCHC levels increased in tilapia *Oreochromis mosambicus* when it was exposed cadmium [29], our results agree with those obtained in previous studies. Some authors reported by cadmium exposure resulted in increased in MCHC. The increased iron level after intoxication was (*Cyprinus carpio* L) after exposure to diazinon has also been reported by [30].

The present results also revealed that, the haematological parameters were compared in atrazine exposed in *Cyprinus carpio* fed *Spirulina* supplemented diets as against atrazine exposed fish fed *Spirulina* – free diet. It suggests the protective effect of against atrazine toxicity in *Cyprinus carpio. Spirulina* has 14% phycocyanin and it stimulates the erythropoetin (EPO) hormone production for haematopoesis [31]. Phycocyanin also regulates the production of white blood cells even when bone marrow stem cells are damaged by toxic chemicals or radiation. [32] reported that *Spirulina* added feed improved the tolerance of *Poecilia reticulata* when exposed to an azo dye methyl red by considerable reduction in the cytotoxic effects on RBC's count at higher concentration of the dye. The result of this study

showed that the haematological parameters of RBC and HB were significantly decreased in atrazine treated with supplement exposure of *Spirulina* when the erythrocytes sedimentation rate was significant by increased as compared with control.

Table 1: Haemotological parameters of the *Cyprinus carpio (*Linn.) exposed to atrazine and supplement feed *Spirulina*

	Duration of exposure				
Parameters	**24**	**48**	**72**	**96**	**120**
(**RBC** Total count (Million / cu mm**)** Group -1: Control	3.60 ± 0.18	3.35 ± 0.13	3.50 ± 0.17	3.55 ± 0.14	3.62 ± 0.21
Group -2:Atrazine	3.40 ± 0.14 -5.5	3.30 ± 0.16 -1.49	3.44 ± 0.02 -1.71	2.70 ± 0.13 -23.94	2.88 ± 0.1 -20.44
Group -3:Atrazine + Spirulina	3.45 ± 0.17 -4.16	3.32 ± 0.19 -0.89	3.46 ± 0.17 -1.14	3.04 ± 0.13 -0.15	3.15 ± 0.11 -0.18
Group -4 :*Spirulina*	4.57 ± 0.18 +26.94	4.60 ± 0.23 +37.81	4.65 ± 0.18 +32.85	4.55 ± 0.27 +28.16	4.50 ± 0.22 +24.30
WBC Total Count (Million / cu mm) Group -1: Control	540 ± 27.0	538 ± 26.9	542 ± 27.1	538 ± 26.9	545 ± 32.7
Group –2:Atrazine	730 ± 29.2 +35.18	728 ± 29.12 +35.31	735 ± 29.4 +35.60	732 ± 36.6 +36.05	740 ± 37.0 +35.77
Group –3: Atrazine + *Spirulina*	650 ± 26.0 ±20.37	645 ± 32.85 +19.88	655 ± 32.75 +20.84	660 ± 39.6 +22.67	640 ± 32.0 +17.33
Group -4:*Spirulina*	580 ± 29.0 +7.40	575 ± 28.75 +6.87	852 ± 23.82 +7.38	577 ± 28.85 +7.22	585 ± 35.1 +7.33
Hb count (g / 100 ml) Group -1: Control	13.8 ± 0. 69	13.0 ±0. 65	13.5 ± 0. 67	13.2 ± 0. 66	13.6 ± 0.68
Group -2 :Atrazine	11.8 ± 0.70 -14.49	11.4 ± 0. 57 -12.30	11.6 ± 0 .69 -14.07	11.2 ± 0. 56 -15.15	10.9 ± 0. 54 -19.85
Group -3:Atrazine + *Spirulina*	12.8 ± 0.64 -7.24	12 .5 ±0. 62 -3.84	12.8 ± 0.60 -0.64	12. 0 ± 0.72 -0.72	12.9 ± 0.71 -0.77
Group –4: *Spirulina*	14.8 ± 0.88 +7.24	14.4 ± 0.86 +10.76	14.1 ± 0.84 +4.44	14.0 ± 0.56 +6.06	14.5 ± 0.72 +6.61
PCV Group-1: Control	34.18 ±1.74	34.40 ±1.72	34.02 ±1.70	34.5 ± 2.07	34.7 ± 2.08
Group -2 :Atrazine	32.7± 1.96 -6.03	32.4 ±1.62 -5.81	31.9 ±1.27 -6.23	31.6 ±1.89 -9.17	31.02 ±1.86 -10.6
Group -3:Atrazine + *Spirulina*	32.7±1.96 -2.87	33.6 ±1.68 -2.32	33.0 ±1.65 -2.9	32.9 ±1.31 -4.63	32.6 ±1.63 -6.05
Group -4 :*Spirulina*	40.06 ± 2.40 +15.02	37.4 ±1.87 +8.72	39.6 ±1.98 +16.40	40.5 ± 2.02 +17.39	38.0 ±1.52 +9.51
MCV Group -1: Control	130.61 ± 6.53	131.89 ±1.49	132.77 ± 7.6	132.91 ± 6.64	132.22 ± 7.93
Group –2: Atrazine	113.82 ± 5.69 -12.85	112.61 ± 5.63 -14.61	113.10 ± 4.52 -14.81	117.03 ± 7.02 -11.94	110.00 ± 6.6 -16.80
Group –3: Atrazine + *Spirulina*	120.60 ± 6.03 -7.66	120.90 ± 6.04 -8.33	121.81± 7.30 -8.25	119.93 ± 7.19 -9.76	118.85 ±5.94 -10.11
Group –4:*Spirulina*	142.38 ± 7.11 +9.01	137.56 ± 6.87 + 4.29	140.46 ± 5.61 + 5.79	139.37 ± 5.57 + 4.86	141.61 ± 5.66 +7.10
MCH Group I :Control	30.25 ±1.51	30.45 ±1.52	31.28 ±1.56	31.11±1.55	31.78 ±1.91
Group –2: Atrazine	28.91± 1.44 -4.42	27.87 ±1.39 -8.47	27.52 ±1.37 -12.02	28.56 ±1.42 -8.19	28.31 ± 1.69 -10.91
Group -3:Atrazine + *Spirulina*	29.97±1.19 -0.92	29.88 ±1.19 -1.87	28.8 ±1.15 -7.89	29.50 ±1.47 -5.17	29.81 ± 1.19 -6.19
Group -4: *Spirulina*	32.31±1.61 + 6.80	31.70 ±1.58 +4.10	32.56 ±1.95 +4.09	32.16 ±1.28 +3.37	32.89 ±1.31 +3.49
MCHC Group –1: Control	28.56 ±1.14	28.44 ±1.70	29.94 ±1.49	29.92 ±1.49	28.87±1.73
Group -2 :Atrazine	33.60 ± 1.34 + 17.64	33.97 ± 1.35 +17.44	34.22 ± 1.35 +19.44	34.22 ± 1.36 +14.29	35.18 ± 1.75 +22.06
Group -3:Atrazine + *Spirulina*	31.09 ± 1.86 + 8.85	31.81 ± 1.59 +11.84	31.81 ±1.59 + 11.84	32.17 ±1.93 +7.44	33.61± 2.01 +16.41
Group -4 :*Spirulina*	29.87± 1.49 +4.58	29.83 ±1.78 +4.88	29.83 ±1.78 +4.88	31.16 ±1.58 +4.07	30.29 ±1.21 +4.91

Mean +SE (Mean of six individual observations)
**Significant at 5% level of "t" test.*

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