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Research Article
Impact of Glyphosate Herbicide on Haematological Parameters of Koi Carp, *Cyprinus carpio* (Linnaeus, 1758) Fingerlings

[1]Felix Sanudi, [1]Sudhakar Indulkar and [2,3]Kumbukani Mzengereza

[1]College of Fisheries Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth, 415629 Ratnagiri, India
[2]Department of Fisheries and Aquatic Science, Mzuzu University, Private Bag 201, Mzuzu, Malawi
[3]United Graduate School of Agricultural Sciences, Kagoshima University, 1-21-24 Korimoto, 890-0065 Kagoshima, Japan

Abstract

Background and Objective: Herbicides are chemicals which are employed to kill or control vegetation. Herbicides being used in agriculture industry have agents with the ability to cause death to plants and animals. Sub-lethal toxicity bioassay experiment was conducted to determine the toxicity of glyphosate herbicide on Koi carp, *Cyprinus carpio* fingerlings. **Materials and Methods:** Koi carp fingerlings with mean length 8.06±0.99 cm exposed to sub-lethal concentrations i.e., 1/10th (3.6 mg L^{-1}) and 1/5th (6.6 mg L^{-1}) of glyphosate. Blood samples were collected from both control and experimental group fishes by puncturing posterior caudal vein using a disposable 1.0 mL tuberculin syringe having needle size 0.45×13 mm for analysis at 7B day interval for a period of 28 days. Total white blood and red blood cells counts were carried out using Neubauer Haemocytometer. Haemoglobin percentage was determined following the Cyanmethemoglobin method. The micro-haematocrit method was used to determine the haematocrit. The derived haematological indices of mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were calculated using the following standard formulae. **Results:** Findings revealed significant decrease ($p<0.05$) in red blood cells (1.06±0.008 to 0.60±0.029), haemoglobin (11.2±0.116 to 7.45±0.029), haematocrit (18.2±0.664 to 14.7±0.404), mean corpuscular haemoglobin concentration (62.7±1.628 to 45.8±1.199) and increase in white blood cells count (204.0±3 to 216.5±0.404), mean corpuscular volume (170.4±7.707 to 255.2±18.65), mean corpuscular haemoglobin (106.2±7.707 to 116.8±6.500). **Conclusion:** This indicated that glyphosate has impact on haematological parameters of exposed fishes. Thus, it is harmful to target, non-targeted species and the environment.

Key words: Lethal toxicity, glyphosate, herbicide, haematological parameters, Koi carp, *Cyprinus carpio*

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Corresponding Author: Kumbukani Mzengereza, Department of Fisheries and Aquatic Science, Mzuzu University, Private Bag 201, Mzuzu, Malawi

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Herbicides represent the largest proportion of pesticides used in agriculture. Worldwide use of pesticides was estimated to be 2.4 billion kg during 2007, of which the herbicides contribute 40% i.e., 950 million kg[1]. The active components in herbicides possess toxicity potential to cause harm in plants, aquatic biota[2]. Enormous amounts of herbicides are found in the environment such as in the soil and water[3].

Most herbicides used in agriculture, except biocides such as disinfectant chlorine and the wood preservative, pentachlorophenol are specific to a group of pests because of their specificity of mode of action. This is particularly the case for the herbicides that are designed to control plants. For example, photosynthetic inhibitors such as urea and triazine herbicides specifically target components of the photosynthetic apparatus[4], which are not found in animals. Therefore, animals are usually less sensitive to herbicides than plants. However, at significantly large concentrations, herbicides can be toxic to animals through narcosis[5].

Glyphosate is widely used for controlling weeds such as grasses, broad leafed weeds and shrubs[6]. Its worldwide use has resulted in pollution of surface water, rivers and streams. Glyphosate gained its importance because it has wide range of applications in agriculture, including its use in genetically modified seed fields which are resistant to the herbicide and its low persistence in the environment[7]. However, the herbicide contains surfactants which are lethal to aquatic organisms, thereby, making the formulations not appropriate for use in aquatic environments[8].

Koi carp is an important ornamental fish species of Asian origin which is sold at a good price in the market[9]. Koi carp was chosen as a representative of the Cyprinidae family, which is one of the major groups of cultured species worldwide.

Change in haematological parameters is a widely used measure of stress in organisms. Thus in the present study, changes in haematological parameters were analyzed as a measure of toxicity to evaluate the impact of glyphosate in Koi carp fingerlings.

MATERIAL AND METHODS

Sublethal toxicity bioassay experiments were conducted in the College of Fisheries, Ratnagiri wet laboratory for a period of 56 days from December, 2018 to January, 2019.

Test animals: Koi carp (*Cyprinus carpio*) fingerlings with mean length 8.06±0.99 cm were obtained from a freshwater fish seed hatchery for use in the present study. The fishes were acclimatized for a period of 2 weeks prior to the start of the bioassay experiments. Water temperature and pH were recorded daily and ranged from 28-29°C and 6.5-7.0, respectively. Feeding was done twice a day during the acclimatization period using commercial pelleted feed.

Test solution: Glyphosate 41% SL herbicide (Trade name: CEDAAR) was procured from a local agro-dealer in Ratnagiri market. Its chemical name is (N-(phosphonomethyl)-glycine). The test liquid was measured using a volumetric pipette for making stock solution. The test solution was prepared immediately prior to commencement of each experiment.

Collection of blood: Blood samples were collected from both control and experimental group fishes by puncturing posterior caudal vein using a disposable 1.0 mL tuberculin syringe having needle size 0.45×13 mm. Ethylenediaminetetra-acetic acid (EDTA) was used as anticoagulant[10] and the blood sample was immediately transferred into EDTA tubes for analysis.

Red blood cells (RBC) count: Total RBC counts were carried out using Neubauer Haemocytometer[11]. Blood was diluted at a ratio of 1:200 with Hayem's fluid[12]. Erythrocytes were counted in the loaded Haemocytometer chamber and total numbers were recorded[13] as 10^6 mm^{-3}.

White blood cells (WBC) count: Total WBC counts were determined by Neubauer Haemocytometer[11]. Blood was diluted at a ratio of 1:20 with Turk's diluting fluid and placed in a Haemocytometer. Four large (1.0 sq.mm) corner squares of the Haemocytometer were counted under the microscope. Cells touching the boundary lines were not counted. The total number of WBC was expressed[13] as $x10^3$ mm^{-3}.

Haemoglobin (%): Haemoglobin (%) was determined following the Cyanmethemoglobin method[14]. A quantity of 20 µL of blood was added to 5 mL of Drabkin's reagent (Potassium Ferricyanide, Potassium, Cyanide and Potassium Dihydrogen phosphate) and was allowed to stand for 4 min and read against blank at 546 nm. The absorbance of Cyanmethemoglobin standard was read in a similar manner. The Haemoglobin content was expressed as a percentage.

Haematocrit (HCT) value (%): The micro-haematocrit method was used to determine the haematocrit (PCV)[15]. The derived

haematological indices of mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were calculated using the following standard formulae[16]:

$$\text{MCV (fL)} = \frac{\text{PCV}}{\text{RBC}} \times 10$$

$$\text{MCH (pg)} = \frac{\text{HGB}}{\text{RBC}} \times 10$$

$$\text{MCHC} = \frac{\text{HGB in 100 mg blood}}{\text{HCT}} \times 100$$

Statistical analysis: Haematological parameters of Koi carp, *Cyprinus carpio* (Linnaeus, 1758) were analyzed statistically using one way analysis of variance (ANOVA) followed by Duncan's Multi Range Test using SPSS software (Version 14) to determine the significant differences and homogeneity of variances in measured variables among experimental and control groups.

RESULTS AND DISCUSSION

Haematological parameters such as white blood cells (WBCs), red blood cells (RBCs), haemoglobin (HBG), haematocrit (HCT) value, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were analyzed as a measure of stress in Koi carp fingerlings exposed to glyphosate herbicide. The fishes were subjected to 2 sublethal dose s, which are 1/10th (3.3 mg L^{-1}) and 1/5th (6.6 mg L^{-1}) of the LC_{50} value. Controls i.e., without herbicide were also set for comparison with fingerlings exposed to the said herbicide.

White blood cells (WBCs)(x 10^3 mm^{-3}): There was gradual increase in WBCs in fishes exposed to glyphosate for a period of 28 days. WBCs ranged from 204.0±3.1754 to 205.0±0.4619, 211.5±0.8083 and 216.5±0.4041 in control, 1/10th and 1/5th concentrations, respectively. In 1/10th concentration, WBCs increased as the exposure period increased i.e., from 204.0±3.1754 to 211.5±0.8083. One-way analysis of variance (ANOVA) showed significant difference ($p<0.05$) in WBCs on 21st and 28th day but there was no significant difference in WBCs from day 0 up to day 14. Duncan multiple range test revealed no significant difference ($p>0.05$) in WBCs among sampling days in control and 1/10th concentrations. Significant difference ($p<0.05$) was observed between 21st and day 28th 1/5th concentration (Fig. 1a).

The decrease may be attributed to disruption of WBCs development and discharge from tissue reservoirs[17-19].

Red blood cells (RBCs) count (10^6 mm^{-3}): Red blood cells count for Koi carp fingerlings exposed to glyphosate ranged from 1.06±0.0087 to 1.16±0.0837, 0.72±0.0231 and 0.60±0.0289 in control, 1/10th and 1/5th concentrations of the LC_{50} respectively during exposure period of 28 days. ANOVA indicated no significant difference ($p>0.05$) in RBCs count up to day 28 in control. In the 1/10th and 1/5th concentrations of the LC_{50}, values significantly decreased ($p<0.05$) from 1.06±0.0087 to 0.72±0.0231 and 0.60±0.0289, respectively during the exposure period of 28 days (Fig. 1b).

Duncan multiple range test between days showed significant difference between some days in 1/10th and 1/5th concentrations. The decline in RBC count could be due to anemia after exposure, which might be attributed to malfunction of red blood cells and hemoglobin synthesis and escalation of red blood cells damage in blood forming sites[20,21]. Red blood cells formation inhibition and damage were also reported by Saravanan *et al.*[21] in *Cyprinus carpio* exposed to Roundup.

Haemoglobin (HGB)(%): Haemoglobin of Koi carp fingerlings exposed to glyphosate ranged from 11.2±0.1155 to 10.75±0.3753, 8.1±0.0577 and 7.45±0.0289 in control, 1/10th and 1/5th concentrations of the LC_{50}, respectively during 28 days. Haemoglobin showed no significant decrease ($p>0.05$) from 11.2±0.1155 to 10.75±0.3753 in control. In 1/10th and 1/5th concentrations of the LC_{50} of glyphosate, HGB significantly decreased ($p<0.05$), respectively from 11.2±0.1155 to 8.1±0.0577 and 7.45±0.0289. One way ANOVA indicated significant difference ($p<0.05$) in HGB among treatments of Koi carp fingerlings exposed to glyphosate. Significant difference in HGB was also found between days in 1/10th and 1/5th concentrations during the exposure period of 28 days (Fig. 1c).

The reduction in HGB value may be due to herbicide interference of chemosynthesis and elevated red blood cells destruction in blood forming systems[22]. Further, lower levels of hemoglobin of treated fish might also be due to the disruption in synthesizing of iron[23].

Haematocrit (HCT) value: Haematocrit values of Koi carp fingerlings exposed to glyphosate ranged from 18.2±0.6640 to 17.8±0.4330, 15.5±0.2890 and 14.7±0.4040 in control, 1/10th and 1/5th concentrations, respectively during the 28 days of exposure period. In control, there was no significant

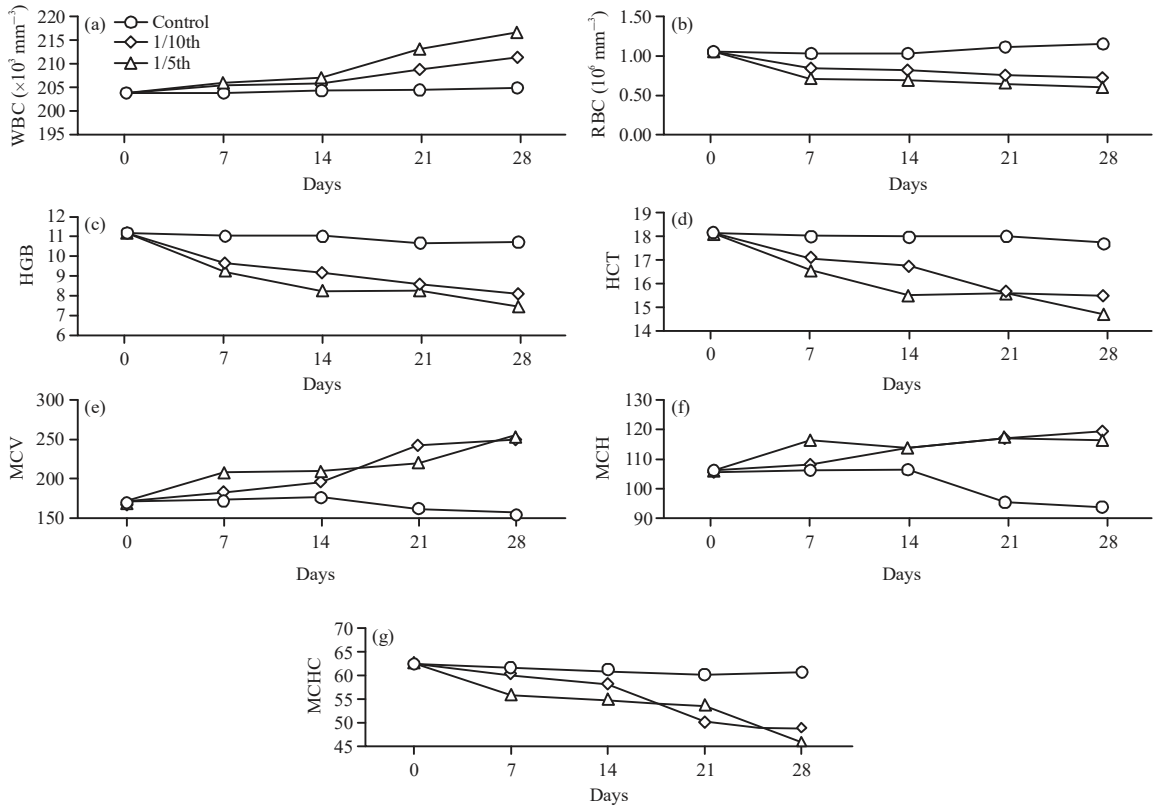

Fig. 1(a-g): (a) White blood cells count, (b) Red blood cells count, (c) Haemoglobin, (d) Haematocrit, (e) Mean corpuscular volume, (f) Mean corpuscular haemoglobin and (g) Mean corpuscular haemoglobin concentration for Koi carp fingerlings exposed to different concentrations of glyphosate

difference ($p>0.05$) in HCT value which remained almost same and decreased from 18.2±0.6640 to 17.8±0.4330 during the 28 days exposure period. In 1/10th and 1/5th concentrations of the LC_{50}, HCT values significantly decreased ($p<0.05$) during the exposure period of 28 days (Fig. 1d).

Reduction in haematocrit value was also reported after C*yprinus carpio* was exposed to a glyphosate-based herbicide[24]. The reduction in HCT after exposure to glyphosate may be due to impairment of haemopoietic procedures increased breakdown of membranes of RBCs[22].

Mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC): Values for MCV and MCH significantly increased ($p<0.05$) (MCV: 170.4±7.7072 to 251.5±10.9482 and 255.2±18.6529, MCH: 106.2±7.7072 to 119.5±2.8153 and 116.8±6.5002 in 1/10th and 1/5th, respectively) while MCHC decreased significantly (62.7±1.6277 in control to 48.0±1.3472 and 45.8±1.1997 in 1/10th and 1/5th, respectively) during the accumulation period of 28 days (Fig. 1e-g).

The observation suggests haematological effects of glyphosate on Koi carp fingerlings. Exposure of *Cyprinus carpio* to sublethal levels of Roundups caused increase in MCV and MCH values and resulted into reduction in MCHC value[24]. The increase in MCV and MCH and reduction MCHC may be due to anemia. The change in MCV and MCH values may be due to more release of undeveloped cells[21].

CONCLUSION

Glyphosate has been found to cause negative impacts on Koi carp fish. Fingerlings exposed to glyphosate exhibited decrease red blood cells, haemoglobin, haematocrit and mean corpuscular haemoglobin concentration and increment in mean corpuscular volume and mean corpuscular haemoglobin. The results indicated that glyphosate induces stress in fish and leads to haematological damage even at sub-lethal concentrations. Therefore, use of glyphosate for control of weeds in agricultural fields should be regulated to ensure appropriate use.

SIGNIFICANCE STATEMENT

The study revealed that sublethal concentrations of glyphosate are toxic to fishes. The findings of this study will help policy makers to make informed decisions concerning glyphosate use. The knowledge derived from this study will also add to the scientific construct with regard to glyphosate toxicity.

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