Journal of Entomology and Zoology Studies
Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
JEZS 2018; 6(2): 562-566

Received: 14-01-2018
Accepted: 17-02-2018

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Haematological changes in fresh water fish rosy barb *Pethia conchonius* exposed to an agrochemical calcium carbide

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Abstract
An attempt has been made in the present investigation to determine the acute toxicity of calcium carbide on the widely popular ornamental fish rosy barb *Pethia conchonius*. Short-term acute toxicity test was performed adopting renewal bioassay technique over a period of 96 h, using different concentrations (0, 0.1, 0.01, 1.0, 10, 50 and 100 $mg1^{-1}$) of calcium carbide to the fish. The 96 h LC_{50} value of calcium carbide to rosy barb *Pethia conchonius* for 96 h was found to be 57.5 $mg1^{-1}$. In sublethal concentrations ($1/10^{th}$ i.e. 5.75 $mg1^{-1}$ and $1/5^{th}$ i.e. 11.51 $mg1^{-1}$) fishes were exposed for 7^{th}, 14^{th}, 21^{th} and 28^{th} days and results showed significant changes ($p<0.05$) in haematological parameters as compared to the control. RBC counts significantly decreased from 1.540±0.001 on 1st day to 0.563±0.002 on 28th day; Hb significantly decreased from 2.211±0.015 on 1^{st} day to 0.871±0.020 on 28^{th} day and Hct significantly decreased from 21.587±0.026 on 1^{st} day to 10.846±0.019 on 28^{th} day. Similarly MCV, MCH and MCHC shows significantly decreasing trend. Whereas, WBC significantly increased from 50.030±0.033 on 1^{st} day to 82.131±0.171 on 28^{th} day of the study. All the estimated parameters showed significant alteration during exposure period indicated toxicity of calcium carbide to the rosy barb fish.

Keywords: Toxicity, rosy barb, calcium carbide, haematological parameters

1. Introduction
Calcium carbide apart from ripening of fruits and vegetables, it is being used in aquaculture ponds for eradicating the unwanted crabs and other burrowing animals [22]. Dumping of spent calcium carbide waste results to death of aquatic organisms [8]. The commonly held view was substantiated by the fact that the wastes from agrochemical, ripening and welding industries composed mainly of calcium hydroxide ($Ca(OH)_2$), which reacts over time with atmospheric carbon dioxide to form non-toxic calcium carbonate [20]. The chemicals calcium carbide reaches the aquatic environment through run-off which can affect the aquatic environment and organisms.
The fish, rosy barb, *Pethia conchonius* is a member of family Cyprinidae and It is native to rivers and fast flowing streams of Afghanistan, Pakistan, India, Nepal and Bangladesh [7]. Now it is very popular with aquarists throughout the world. It is, therefore, becoming a potential experimental model fish for biological and biotechnological research. In fact, it has been widely used in toxicological studies in recent years [2]. In addition, as evidenced by previous study [28], a rosy barb proved to have a high sensitivity to agrochemicals and therefore has been selected as a potential model fish for this study. This study investigated the acute and chronic effects of calcium carbide on various blood parameters of rosy barb, *Pethia conchonius*. This study also aimed to evaluate dose and duration dependent effects of calcium carbide in the experimental fish.

2. Material and methods
2.1 Experimental fish specimens and chemicals
Experimental research work was conducted in wet laboratory of college of fisheries science, Shirgaon, Ratnagiri, and Maharashtra during the month from June to august 2017. For conducting experiment, adult rosy barbs *Pethia conchonius* were collected and transported from local ornamental fish breeder of Ratnagiri, Maharashtra. The mean (±SD) wet weight and lengths of fishes were observed to be 2.55 ± 0.03 g and 5.6 ± 0.134 cm, respectively. To avoid any dermal infections, fishes were subjected to the prophylactic treatment by bathing them twice in 0.05% Potassium permanganate (KMnO4) for two minutes.

Then the fishes were acclimatised for two weeks under laboratory conditions in semi-static system. During the acclimatisation period, they were fed on commercial feed. Faecal matter and other waste materials were siphoned off daily to reduce ammonia content in water. For the present study, a commercial formulation of calcium carbide was purchased from a welding and fabrications workshop, Ratnagiri, Maharashtra was ground to powder in a dry morta.

2.2 Lethal toxicity (LC_{50}) study

Initially, a range finding test was conducted to ascertain the range of calcium carbide to be selected in the definitive test. During the trial, the test animals were exposed to a broad range of concentration on logarithmic scales (0, 0.001, 0.01, 0.1, 1.0, 10.0, and 100 $mg1^{-1}$). In narrow-range finding test of calcium carbide on rosy barb, the mortality percentage between 10% to 100% was considered.

Lethal toxicity (LC_{50}) study was carried out by following the standard guidelines [9]. To determine the LC_{50} of calcium carbide in rosy barb for 96 h by probit analysis method [10]. A total of 20 rosy barbs per group were exposed to the concentrations of calcium carbide in 40 litres of water in glass tanks for 96 h. Calcium carbide was always freshly dissolved in appropriate volume of water as a stock solution, and added immediately into the test solutions. After every 24 h, the dead animals were removed and the surviving ones were recorded.

2.3 Sublethal Experiment

During sublethal exposures for a period of 28 days, two concentrations were selected i.e. 1/10th and 1/5th of LC_{50}, such as 5.75 and 11.51 $mg1^{-1}$. At the end of every seven days of exposure periods i.e. 7th, 14th, 21st and 28th days, five fishes were randomly selected from each replicate for the haematological analysis.

2.4 Haematological Analysis

Blood was collected from control and calcium carbide treated groups by puncturing posterior caudal vein using 1.0 ml disposable insulin syringe having needle size of 0.30 x 8.0 mm. Syringe was rinsed with 1% of EDTA solution as anticoagulant and then needle was inserted into fish to take blood from posterior caudal region [19].

Red blood cells (RBC) and White blood cells (WBC) were counted by haemocytometer method [14]. Haemoglobin (Hb) concentrations were estimated by Cyanomethemoglobin method [27] and Hct was determined by the microhematocrit method [24]. Erythrocyte indices like mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were calculated according to standard formulas [5];

MCV (femtolitres) = Hct/RBC x 10

MCH (picograms) = Hb/RBC x 10

MCHC (g/dl) = (Hb in 100 mg blood / Hct) x 100.

3. Statistical Analysis

The LC_{50} value was estimated by probit analysis method [10]. In the sublethal testing, significance of differences was tested using one way ANOVA (Analysis of variance). Duncan multiple range test was used to check the significant differences. The differences were considered significant when $p<0.05$.

4. Results

4.1 Lethal toxicity (LC_{50}) experiment

The results of 96 h median lethal concentration value for rosy barb was calculated and presented in Fig 1. The LC_{50} value of calcium carbide for rosy barb was found to be 57.542 $mg1^{-1}$ (Probit log value 1.76 in Fig. 1) at 96 h.

4.2 Sublethal experiment

In Sublethal experiment, two concentrations such as 5.75 and 11.51 $mg1^{-1}$ were selected i.e. 1/10th and 1/5th of LC_{50} for a total period of 28 days. Sublethal effect of calcium carbide on haematological parameters of rosy barb, *Pethia conchonius* such as RBC, Hb, Hct, WBC, MCH, MCV and MCHC are shown in Fig. (2, 3, 4, 5, 6, 7 & 8) and the details are as follows;

RBC, Hb, Hct, MCH, MCV & MCHC levels in the experimental fish exposed to calcium carbide were significantly ($p<0.05$) decreased while WBC counts was significantly ($p<0.05$) increased as compared to those of the control group. RBC counts in calcium carbide exposed fishes showed decreasing trend. The control groups showed similar values throughout the exposure period with a value of 1.49±0.04 on 7th day and 1.49±0.05 on 28th day. In 5.75 $mg1^{-1}$ (1/10th concentration of LC_{50}), the RBC count decreased from 1.33±0.09 on 7th day to 0.66±0.08 on the 28th day. In higher concentration of 11.51 $mg1^{-1}$ (1/5th of LC_{50}), the count decreased from 1.12±0.05 on 7th day to 0.56±0.05 on 28th day. Fishes exposed to 11.51 $mg1^{-1}$ concentrations showed significantly lower RBC counts than that to 5.75 $mg1^{-1}$ concentrations (Fig. 2).

WBC counts in calcium carbide exposed fishes showed increasing trend. Lower values were observed in control group throughout the exposure period with a count of 50.03±0.09 and 49.03±0.12 on 7th and 28th day respectively. In 5.75 $mg1^{-1}$ (1/10th concentration of LC_{50}), the count increased from 52.55±1.49 on 7th day to 74.10±0.09 on the 28th day. In higher concentration of 11.51 $mg1^{-1}$ (1/5th of LC_{50}), the WBC count increased from 58.50±0.12 on 7th day to 82.13±2.83 on 28th day (Fig. 3).

Fishes exposed to sublethal concentrations of calcium carbide showed a significant decrease ($p<0.05$) in Hb as compared to control. Hb in calcium carbide experiment showed a decreasing trend in fishes. Control groups showed similar values throughout the exposure period i.e. 3.21±0.04 on 7th day to 3.22±0.04 on 28th day. In 5.75 $mg1^{-1}$ (1/10th concentration of LC_{50}), the values decreased from 2.59±0.06 on 7th day to 1.51±0.07 on the 28th day. In higher concentration of 11.51 $mg1^{-1}$ (1/5th of LC_{50}), the Hb decreased from 2.21±0.04 on 7th day to 0.87±0.05 on 28th day. Hb content was significantly decreased in the sublethal concentrations than that of control group (Fig. 4).

The trend of Haematocrit (Hct) value was same as that of RBC and Hb in sublethal concentrations of calcium carbide, which also showed decreasing trend. In control groups, there was no significant change throughout the exposure period with a value of 21.51±0.09 to 21.48±0.09 on 7th and 28th day respectively. In 5.75 $mg1^{-1}$ (1/10th concentration of LC_{50}), the Hct value decreased from 17.28±0.09 on 7th day to 12.43±0.09 on 28th day. In higher concentration of 11.51 $mg1^{-1}$ (1/5th of LC_{50}), the Hct decreased from 15.48±0.11 on 7th day to 10.85±0.05 on 28th day. Fishes exposed to 11.51 $mg1^{-1}$ concentrations showed significantly lower Hct value than fishes exposed to 5.75 $mg1^{-1}$ concentrations during the study period (Fig. 5).

MCV in calcium carbide exposed fishes showed decreasing trend. Control groups showed higher values throughout the exposure period with a value of 143.71±3.22 on 7th day and 143.93±5.46 on 28th day. In 5.75 $mg1^{-1}$ (1/10th concentration of LC_{50}), the values decreased from 138.81±6.00 on 7th day to 130.14±10.29 on the 28th day. In 11.51 mgl^{-1} concentration (1/5th of LC_{50}), the MCV decreased from 133.65±2.36 on 7th day to 122.81±1.91 on 28th day (Fig.6).

MCH value in calcium carbide exposed fishes showed decreasing trend. Higher values were observed in control groups with a value from 21.59±0.63 to 21.57±0.61 on 7th and 28th day respectively. In 5.75 mgl^{-1} (1/10th concentration of LC_{50}), the MCH value decreased from 19.84±1.07 on 7th day to 15.31±1.25 on the 28th day, whereas in 11.51 mgl^{-1} concentration (1/5th of LC_{50}), the value significantly decreased from 17.31±1.04 on 7th day to 8.46±0.48 on 28th day. Fishes in 11.51 mgl^{-1} concentration (1/5th of LC_{50}) showed significantly lower MCH value than fishes exposed to 5.75 mgl^{-1} concentration (1/10th of LC_{50}). However, MCH values were significantly higher in control than that of both treatments (Fig.7).

Fishes exposed to sublethal concentrations of calcium carbide showed a significant decrease in the MCHC as compared to control. Control groups showed higher values throughout the study period with a percentage of 14.903± 0.069 on 7th day to 14.989± 0.061on 28th day. In 5.75 mgl^{-1} (1/10th concentration of LC_{50}), the value decreased from 14.970± 0.075 on 7th day to 12.116± 0.175 on the 28th day. In higher concentration of 11.51 mgl^{-1} (1/5th of LC_{50}), the MCHC decreased from 14.287± 0.069 on 7th day to 8.034± 0.134 on 28th day. On 7th day there is no significant difference between 1/10th concentration and control group and on 14th and 21st day values shows increasing trend comparing to 1/10th with 1/5th concentration. While 1/5th concentration significantly decreased from 7th to 28th day of experiment (Fig.8).

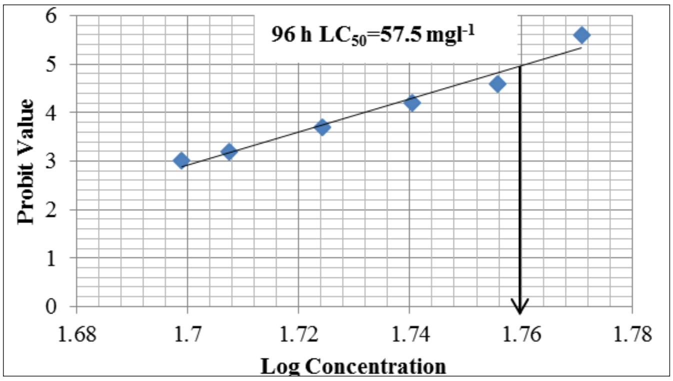

Fig 1: 96 h LC_{50} of calcium carbide concentration in rosy barb

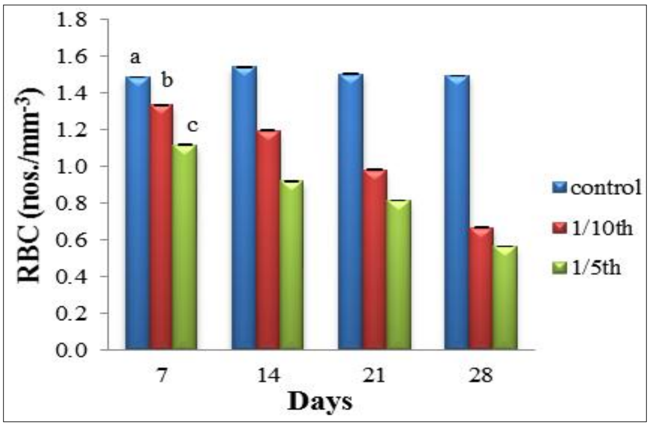

Fig 2: RBC changes in rosy barb exposed to sublethal concentrations of calcium carbide. Mean values with different alphabetic and numeric superscripts are significantly different ($p<0.05$).

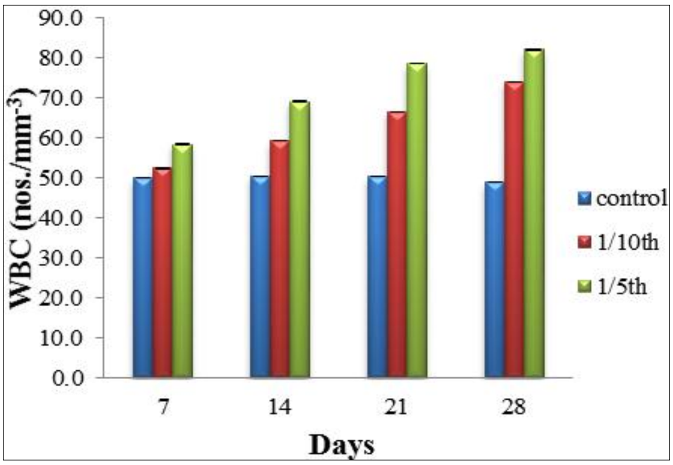

Fig 3: WBC changes in rosy barb exposed to sublethal concentrations of calcium carbide. Mean values with different alphabetic and numeric superscripts are significantly different ($p<0.05$).

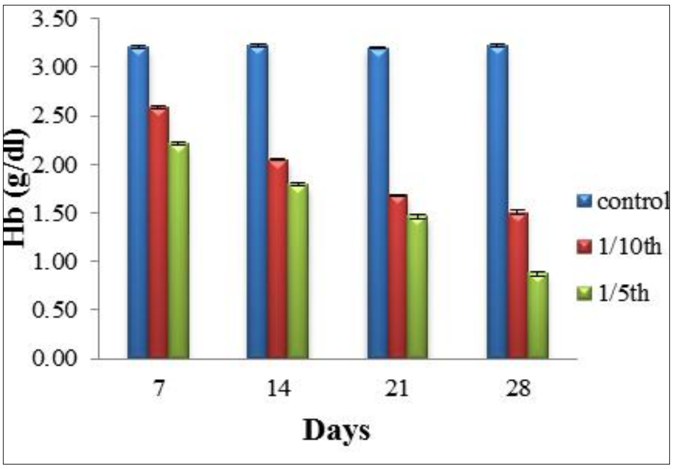

Fig 4: Hb changes in rosy barb exposed to sublethal concentrations of calcium carbide. Mean values with different alphabetic and numeric superscripts are significantly different ($p<0.05$).

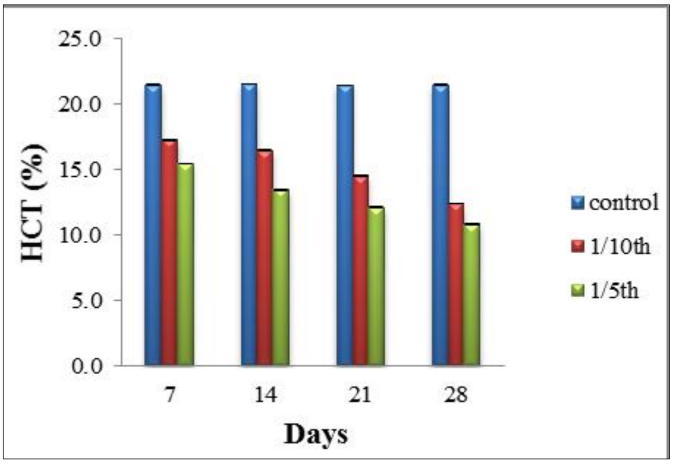

Fig 5: Hct changes in rosy barb exposed to sublethal concentrations of calcium carbide. Mean values with different alphabetic and numeric superscripts are significantly different ($p<0.05$).

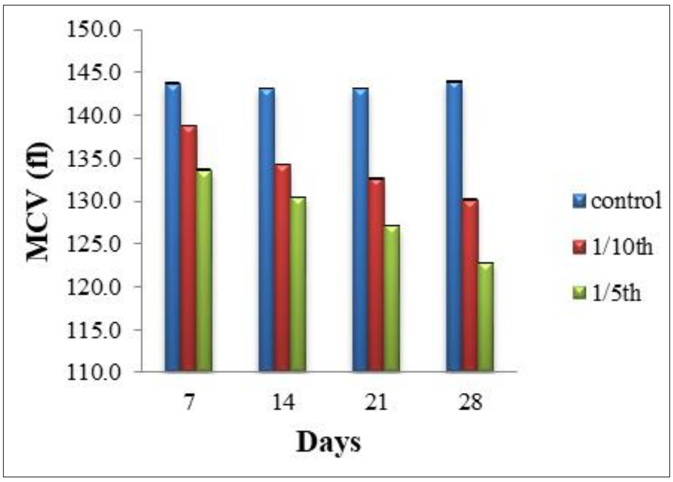

Fig. 6: MCV changes in rosy barb exposed to sublethal concentrations of calcium carbide. Mean values with different alphabetic and numeric superscripts are significantly different ($p<0.05$).

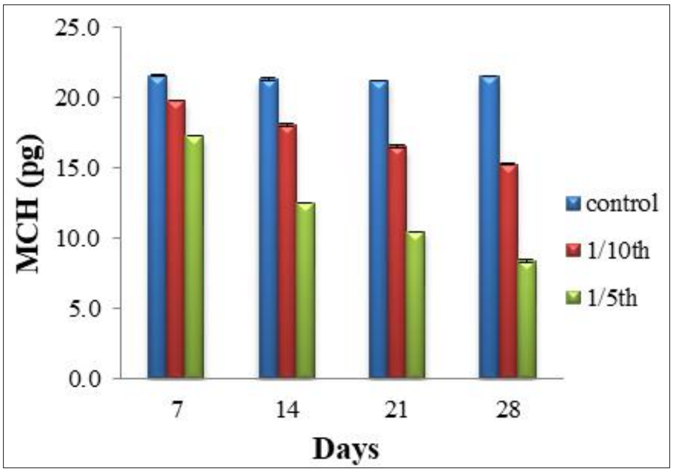

Fig. 7: MCH changes in rosy barb exposed to sublethal concentrations of calcium carbide. Mean values with different alphabetic and numeric superscripts are significantly different ($p<0.05$).

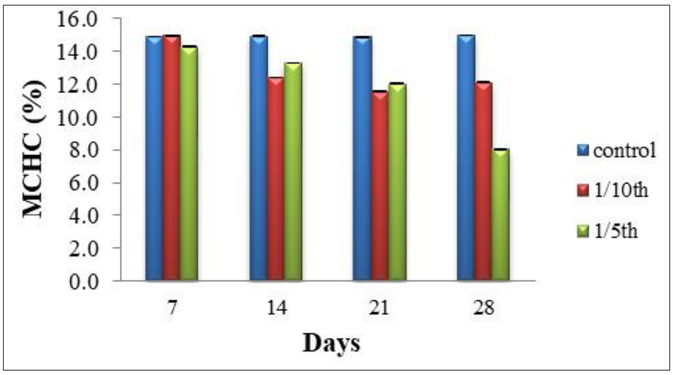

Fig 8: MCHC changes in rosy barb exposed to sublethal concentrations of calcium carbide. Mean values with different alphabetic and numeric superscripts are significantly different ($p<0.05$).

5. Discussion

The effect of calcium carbide on rosy barb *Pethia conchonius* indicated that toxicity of the agrochemical is both time and concentration dependent. However, in the present study, LC_{50} value for rosy barb exposed to calcium carbide was estimated to be 57.5 mg1 $^{-1}$ at 96 h but the LC_{50} value for rosy barb exposed to nonylphenol was 1.72 mg1^{-1} and for copper and zinc were 0.5 mg1^{-1} and 9 mg1^{-1} to *Puntius parrah* respectively, these values were lower compared with the present study [4].Calcium carbide treatments caused different alterations in haematological constituents of rosy barb *Pethia conchonius.* The results indicated, time and dose dependent decrease in Hct and Hb throughout the duration of exposure. There was also a significant reduction in RBC counts on 7, 14, 21 and 28 days may be due to inhibition of erythropoiesis, haemosynthesis and osmoregulatory dysfunction or due to increase in the rate of erythrocyte destruction in haematopoietic organs [21, 1, 16]. They have reported significantly decrease in RBC count in high concentration of cypermethrin in *Prochilous lineatus*.

The WBCs in fish respond to various stressors including infection and chemical irritants [26]. Thus, altered number of WBCs is a normal reaction on the exposure of the toxicant [12]. In the present investigation, the significant increase in WBCs count might have resulted from the excitation of defence mechanism of the fish to counter the effect of pesticide[11]. Similar kinds of results have previously recorded by other researchers for some pesticides such as, diazinon [25] and curzate [6].

Calcium carbide exposure to rosy barb led to a significant decrease in Hb thereby suggesting that the fishes were under stress of anaemia. Similar results with the low level of Hb indicating anaemic conditions in fish caused hemolysis [15]. The reduction in Hb content in fish exposed to toxicant could also be due to the inhibitory effect of toxic substance on the enzyme system responsible for synthesis of Hb [22].

In present study, Hct values were significantly decreased compare to control group when rosy barb exposed to calcium carbide. Similar results were also observed by Li *et al.* (2011) [13]. They have observed significant decrease in Hct levels in rainbow trout when exposed to carbamazepine chemical. Similarly results significant reductions were reported in Hct values existed in *Cyprinus carpio* and *Labeo rohita* that were exposed to cypermethrin and carbofuran respectively [3, 1]. The decrease in the PVC in *C. carpio* treated with sublethal concentration of chlorpyrifos due to either rapid oxidation of hemoglobin to methemoglobin or release of oxygen radical brought about by toxic stress of insecticide [17].

In the present study, significant decrease in MCV, MCH and MCHC in rosy barb fishes exposed to calcium carbide could be probably due to stress induced by acetylene gas and confirm the occurrence of haemolytic anaemia in experimental fish which exaggerates further disturbances in haemopoitic activity. Howeverer, the decrease in MCV and MCH might be due to high percentage of immature red blood cell in the circulation [18]. Similar findings have also been reported for *Labeo rohita* due to cypermethrin and carbofuran [1]. MCHC value was significantly decreased in sublethal concentration of calcium carbide than the control fish. Similar findings of significant decrease in MCHC level in *Prochilous lineatus* exposed to sublethal concentration of cypermethrin [16].

6. Conclusion

From the forgoing account, it is concluded that fishes exposed to calcium carbide exhibited dose and duration dependent alterations in many haematological parameters such as RBC, Hb, Hct, MCV, MCH, MCHC (Significantly decreases) and WBCs (significantly increases) over a period of 7th day. The results verifies that the vicissitudes in haematological indices may be used as sensitive biomarkers for animal health evaluation, especially in regions that are naturally affected by agrochemicals, causing stress in fish on exposure to elevated levels in the water. Based on the results presented, it is concluded that the use of the agrochemical, calcium carbide for ripening of fruits and vegetables in farms and fields near fish-inhabiting resources has to be judicious.

7. Acknowledgements

The authors would like to acknowledge college of fisheries, Shirgaon, Ratnagiri for providing the necessary facilities during experimental research work.

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