Voyager: Vol. VII, 2016
ISSN: (P): 0976-7436, (e): 2455-054X
ICRJIFR
Impact Factor
3.8919

Study of Nitrite Toxicity on RBC Count of Fish *Heteropneustes Fossilis*

Renu Verma
Associate Professor
Dept. of Zoology, C.S.S.S. (P.G.) College,
Machhara (Meerut)
Email - renu20081970@gmail.com

Dr. Neelam Panwar
Associate Professor
Dept. of Zoology, Meerut College,
Meerut
Email – panwarneelam15@gmail.com

ABSTRACT

Nitrite is an intermediate product in the oxidation of ammonium to nitrate. An elevated ambient nitrite concentration is a potential problem for fresh water fish since nitrite is actively taken up across gills in competition with chloride. Nitrite is a well-known toxicant for fish as well as disrupter of multiple physiological functions.

So, the present study aims at the effect of different nitrite concentrations of sodium nitrite on total erythrocyte count in fresh water fish Heteropneustes fossilis. During the present investigation, a decrease in RBC count was recorded. Haemopoitic tissue hypoxia results on the long term nitrite toxicity which results into decrease of red blood cell production and hence to reduce the blood haemoglobin level.

Key words: *Nitrite, fishes, toxicity, Heteropneustes fossilis, Total erythrocyte count, haemopoitic tissue, hypoxia.*

ISSN: (P): 0976-7436, (e): 2455-054X
ICRJIFR
Impact Factor
3.8919

INTRODUCTION

Nitrite is a natural component of the nitrogen cycle in ecosystems and its presence in the environment is a potential problem due to its well documented toxicity to animals (e.g. Lewis and Morris 1986; Jensen 2003).

Aquatic animals are at higher risk of nitrite intoxication than terrestrial animals. Since nitrite in the ambient water can be actively taken up across the gill epithelium and can accumulate to very high concentrations in the body fluids. Studies on fish and crustaceans revealed that nitrite induced a large variety of physiological disturbances, many of which contribute to toxicity (Jensen 1996, 2003).

Nitrite is an intermediate and important product in bacterial nitrification and dentrification process in the nitrogen cycle. Elevated concentrations of nitrite can be found in water receiving nitrogenous effluents, in various hypoxic environments or in effluents from industries producing metals, dyes and celluloids (Pitter 1999).

Elevated Nitrite concentrations cause great problems in intensive culture of commercial fish species and ornamental fish (Dvorak 2004; Svobodova, et al. 2005a).

Present work shows the effect of nitrite exposure on R.B.C. count of species *Heteropneustes fossilis*.

MATERIALS AND METHODOLOGY

Fishes were collected from local fish market. In the laboratory, the animals were acclimated over a 20 days period before the beginning of the experiment in order to recover from stress of handling and transportation. Inorganic salt sodium nitrite ($NaNo_2$) was selected as a toxicant for the study.

The fishes were divided into ten sets of eight fishes each. Out of these ten sets, one set is treated as control and, others were experimental groups, in which stock solution of sodium nitrite was added ranged from 50 to 250 mg/L with increments of 25 mg/L. The experiment was determined in triplicate.

To avoid the effect of seasonal environmental changes on the fish. The physico-chemical characteristics of the water during the experiment were:

- Temperature 23ºC – 28ºC
- pH 7.0 – 7.5
- Hardness 106mg/L as calcium carbonate
- Dissolved oxygen > 6.0 mg/L
- $CL^{—}$ 38.6 mg/L
- NO^{-}_{2} and ammonia < 0.01 mg/L

Survival of *Heteropneustes fossilis* during exposure to NO^{-}_{2} was determined in triplicate in a series of nine treatments. Blood samples were taken out from caudal vein.

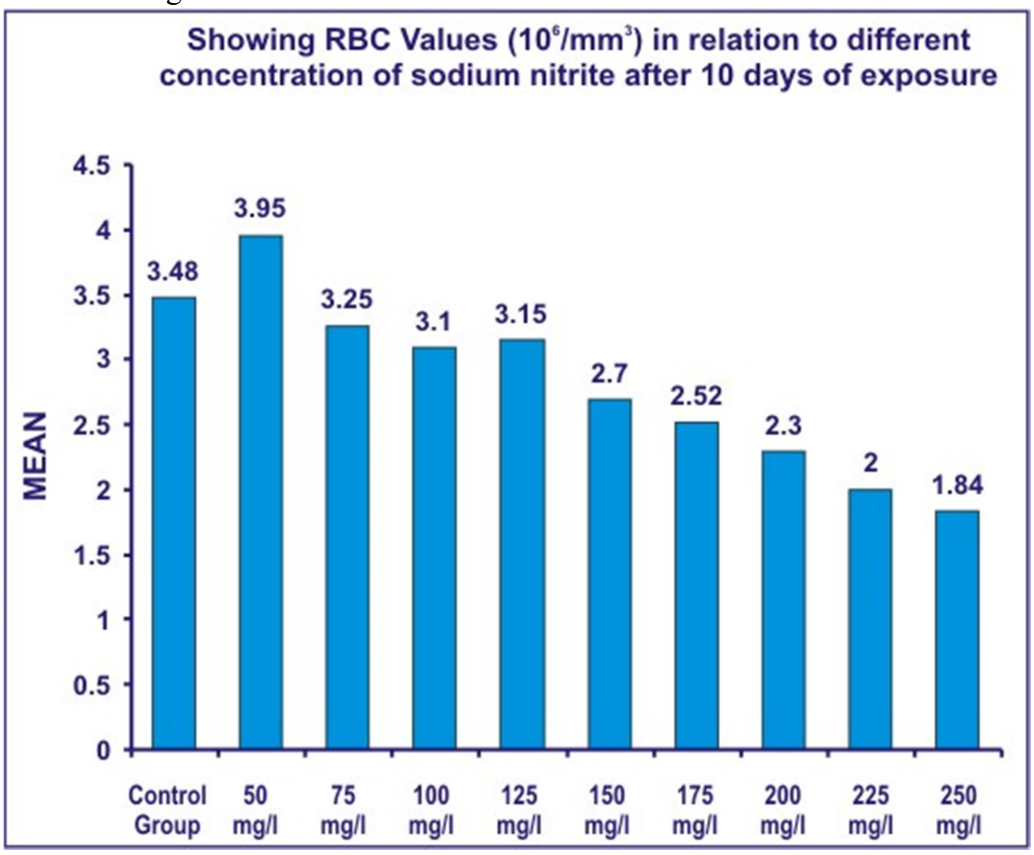
Determination of R.B.C. count was done by Neubauer haemotocytometer. Student 't' test was applied to find out if there is any significance difference between the control and experimental group.

ISSN: (P): 0976-7436, (e): 2455-054X
ICRJIFR
Impact Factor
3.8919

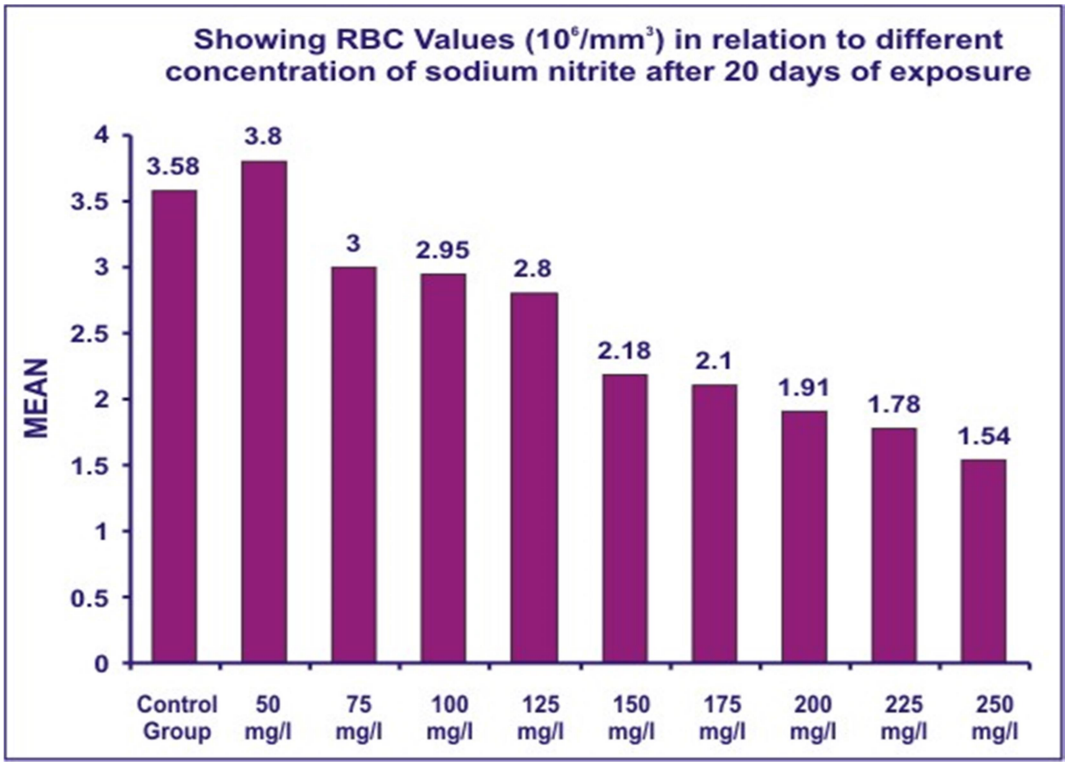
Showing RBC (10^6/mm^3) values in relation to different concentration of sodium nitrite and time; values are mean ± S.D.

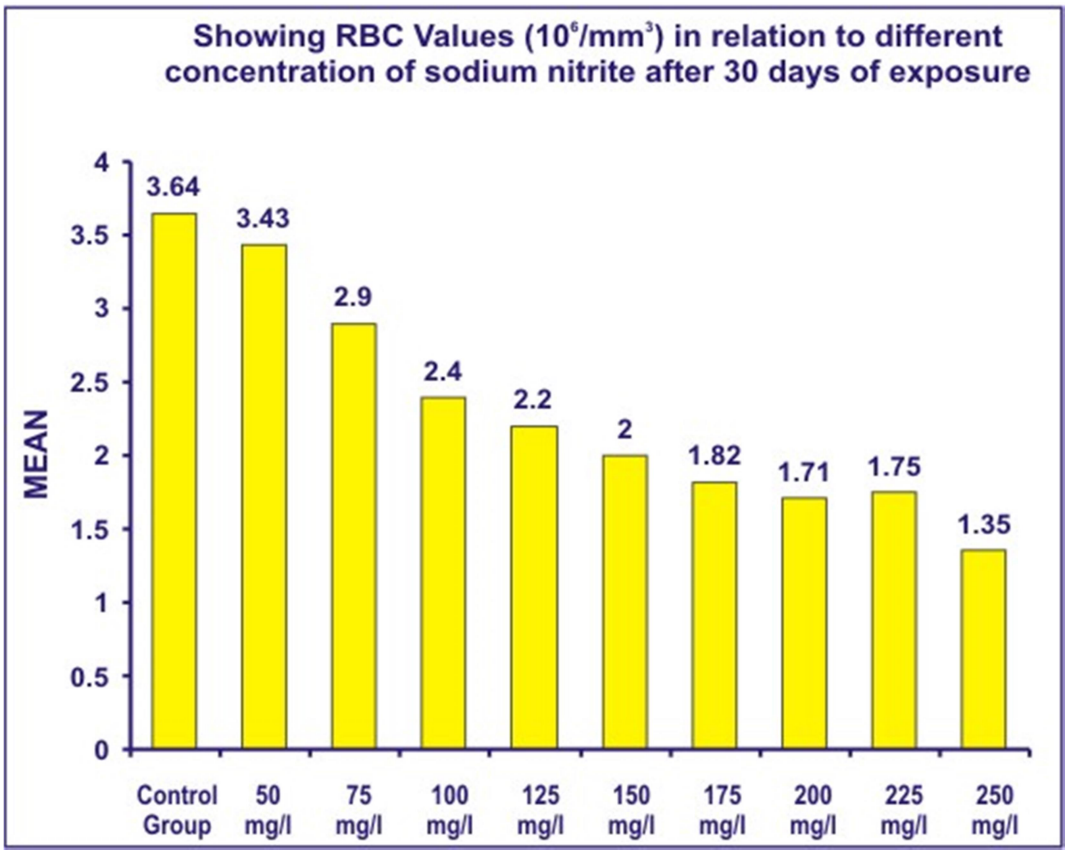
Time in days	Control Group	Experimental group treated with different concentration of Sodium nitrite								
		50 mg/l	75 mg/l	100 mg/l	125 mg/l	150 mg/l	175 mg/l	200 mg/l	225 mg/l	250 mg/l
10 days	3.48± 0.64	3.95± 0.58^{NS}	3.25± 0.25*	3.10± 0.20*	3.15± 0.35*	2.70± 0.35*	2.52± 0.25*	2.30± 0.35*	2.00± 0.50*	1.84± 0.84*
20 days	3.58± 0.67	3.80± 0.34^{NS}	3.00± 0.85^{NS}	2.95± 0.67*	2.80± 0.84*	2.18± 0.53*	2.10± 0.74*	1.91± 0.44*	1.78± 0.20*	1.54± 0.35*
30 days	3.64± 0.24	3.43± 0.52^{NS}	2.90± 0.64*	2.40± 0.25*	2.20± 0.50*	2.00± 0.40*	1.82± 0.84*	1.71± 0.25*	1.75± 0.34*	1.35± 0.25*
60 days	4.02± 0.85	3.00± 0.25*	2.75± 0.24*	2.15± 0.75*	2.15± 0.35*	1.85± 0.35*	1.70± 0.65*	1.50± 0.34*	1.51± 0.84*	1.15± 0.35*

* Significant at 0.05 level
NS – Non Significant

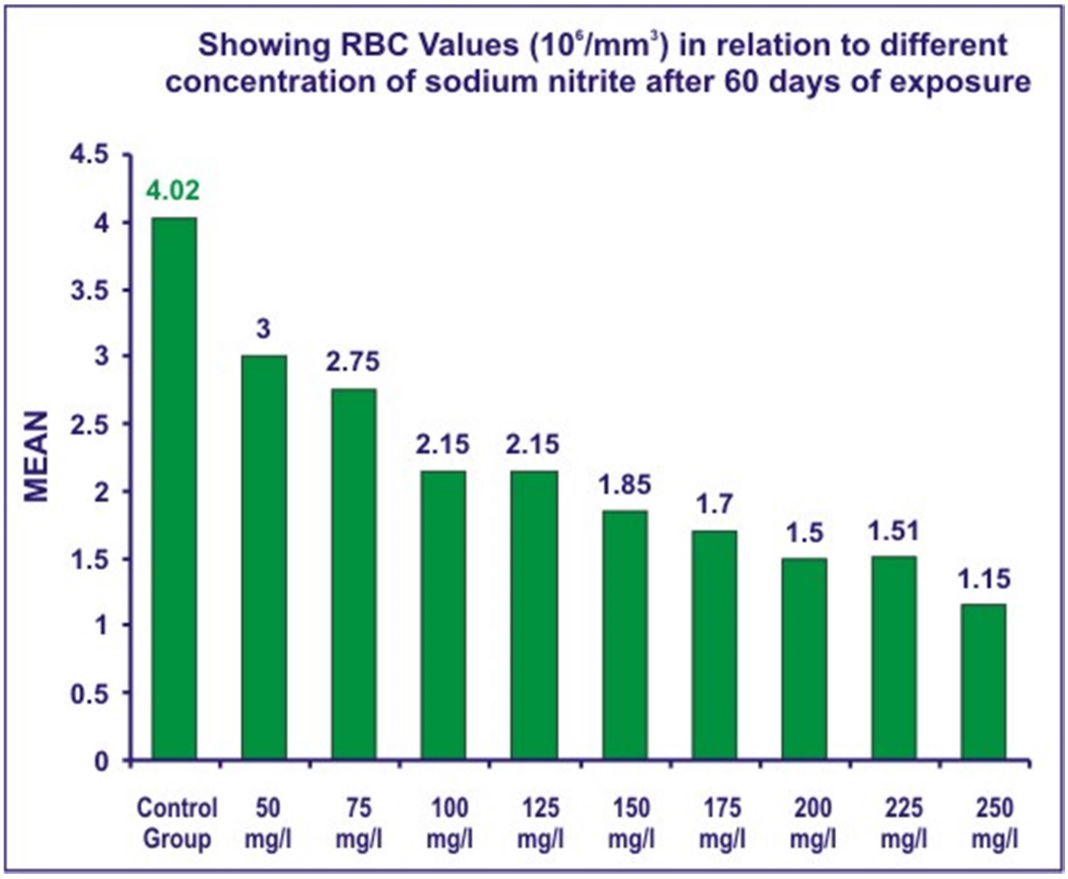


ISSN: (P): 0976-7436, (e): 2455-054X
ICRJIFR
Impact Factor
3.8919





ISSN: (P): 0976-7436, (e): 2455-054X
ICRJIFR
Impact Factor
3.8919



RESULT AND DISCUSSION

RBC values for control fish was 3.48 (10^6/mm^3) after 10 days exposure. Whereas, a significant decrease ($p<0.05$) in RBC values was observed in all treatments except the first one, which was found insignificant. The same pattern was observed for 20 days exposure, During this exposure, except first and second treatments all the treatments were found significant with continuous decrease in values as compared to the control group, which was containing RBC values 3.56 (10^6/mm^3). Similar pattern was observed during 30 days and 60 days exposure. However, it was interpreted that:

- Fish exposed to nitrite concentration showed a constant significant decrease in RBC values as the exposure period increases.
- There was a constant significant decrease in RBC values as the concentration of nitrite increases.

During the present investigation a decrease in RBC count was recorded. In my opinion this decrease of RBC may be due to haematopoietic tissue hypoxia, resulting on the long term nitrite toxicity which results into decrease of red blood cell production and hence, to reduce the blood haemoglobin level. Decrease in RBC count has been reported using different animals including rats (S.K. Rawat et al., 2013) dog (Guillermo A. Zebbalos, 1995), Pig (Jensen F.B., 2005). Further, nitrites have been reported to induce a reduction in RBC level in human (Harely and Robin, 1962).

The present result is in part, comparable to those obtained by Svobodova et al. (2005a) (1983a and b), where they found changes in haematological values, the total erythrocyte count had

decreased. The present investigation is also supported by Costa L.D.F., Miranda – Filho K.C. and Sampiro L.A. (2008), who investigated the decreased RBC count in juvenile Pompano *Tranchinotus marginatus,* when exposed to acute ammonia and nitrite exposure at different salinity.

It is likely that the decrease in RBC count might be due to the disruptive action of sodium nitrite on the peripheral red cells. Similar decrease in RBC count was also observed in the fish, *Puntius ticto* (Chauhan et al. 1983). A similar suggestion was made by Verma, G.P. and Panigrahi, P., McConnell R. (1985), who reported toxicity to the fat head minnow exposed to nitrite. Because fish are quite resistant to death from methemoglobinemia or from CO inactivation of haemoglobin, it has been suspected that nitrite toxicity involves mechanisms other than methemoglobinemia. Alternative mechanism of nitrite induced mortality has yet not been well demonstrated. John Davidson et al. (2014) also supported decrease of R.B.C. count with increased nitrite toxicity in juvenile Rainbow trout.

REFERENCES

1. Christine C. Helms, Mark T., Gladwin and Daniel B., Kim Shapiro (2018): Erythrocytes and Vascular function: Oxygen and Nitric Oxide, https://doi.org/10.3389/fph 45:2018.00125.

2. Costa, L.D.F., Miranda – Filho K.C. Severo, M.P., Sampiro, L.A. (2008): Tolerance of juvenile pompano *Tranchinotus marginatus* to acute ammonia and nitrite exposure at different salinity levels. Aquanture 285: 270-272.

3. Dvorak P. (2004): Selected specificity of aquarium fish disease (in Czech). Bulletin VURH Vodnany, 40, 101–108.

4. Guillermo, A. Zeballos, Robert D. Bermstein, Carl I. Thompson, Paul R. Forfia, Nahid Seyedi, Weigun Sen, Pawel M. Kamiski (1995): https://doi.org/10,1161/01.CER91.122982/

5. Harley, J.D. and Robin, H. (1962): The effect of nitrate ions on intact human erythrocytes, Blood 20, 710.

6. Jensen, F.B. (2005): Nitrite transport into pig erythrocytes ad its potential biological role. Acta Physiol Scand 184: 243-251.

7. Jensen, F.B. (1996): Uptake, Elimination and Effects of Nitrite and Nitrate in Freshwater Crayfish (*Astacus astacus*). *Aquatic Toxicology* 34:95–104

8. Jensen, F.B. (2003): Nitrite Disrupts Multiple Physiological Functions in Aquatic Animals. *Comp. Biochemistry and Physiology* Part A, (135):9–24.

9. John Davidson, Christopher Good, Carla Welsh, Steven, T. Summer felt (2014). Comparing the effect of high Vs low nitrite on health of juvenile rainbow trout. (*Concorhynchus myskiss*). Aquacultural Engineering, 59, 30-40.

10. Lewis W.M., Morris D.P. (1986): Toxicity of nitrite to fish - a review.

11. Mc Connell R. (1985): Toxicity of nitrate to the fat head minnow, Colorado Department of Health File Report, Denver.

12. Pitter P. (1999): Hydrochemistry (in Czech). Editor VSCHT, Prague. p. 568

13. Rawat, R.K. Singh, FW Bansode, Poonam Singh, Rana P. Singh (2013) Nitrate induced toxicity on some haematological parameters of Charles Foster rats, J. Recent Av. Appl. Sci. 28, 35-38.

14. Smith, G.R., K.G. Temple, D.A. Vaala, H.A. Dingfelder (2005): Effects of Nitrate on the tadpoles of two Ranids (*Rana catesbeiana* and *R. clamitans*). *Archives of Environmental Contamination and Toxicology* 49:559–62.

15. Svobodova et al. (2005a): Nitrite poisoning of fish in aquaculture facilities with water-recirculating systems: Acta Veterinaria Brno, 74, 129–137.

16. Verma, G.P. and Pangirahi, P. (1998): Effect of agrofen on blood parameters of *Oreochromes mossambicules*, Proc. Natl. Acad. Sci. India B. Biol. Sci., 68 (1) 29-36