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Effect of organophosphate pesticide, ethephon on serum biochemical parameters of fresh water ornamental fish rosy barb, *Pethia conchonius* (Hamilton, 1822)

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Abstract
In the present study, effects of various concentrations of ethephon exposures on days 7, 14, 21 and 28 for many serum biochemical parameters in the freshwater teleost fish, *Pethia conchonius* were investigated. The 96h LC_{50} value of ethephon was estimated by using log-dose probit regression line method. On the basis of LC_{50} value, the sub-lethal concentrations were determined as 6.40 and 12.81 mgl^{-1} which is $1/10^{th}$ and $1/15^{th}$ of LC_{50} respectively. Well acclimated fishes from both control and treated group were sacrificed after 7, 14, 21 and 28 days and blood samples were collected. Various biochemical parameters such as serum total protein, serum albumin, serum globulin, and serum glucose has been studied as diagnostic tools. In general significant effects ($p<0.05$) from different concentrations and time of exposure were observed in exposed fishes. It was found that significant alterations in all the biochemical parameters were dose dependant as well as duration dependent. Results indicated that serum albumin, serum globulin and serum total protein decreased significantly where as serum glucose increased with increase in ethephon concentration and time of exposure when compared with control groups.

Keywords: Ethephon, Rosy barb, serum biochemical parameter, toxicity

Introduction
Ethephon [(2-chloroethyl) phosphonic acid] (ETF) is a major plant growth regulator (PGR) that promotes fruit ripening, abscission, flower induction, and other responses by releasing ethylene gas, a natural plant hormone [24, 7, 11, 28] which spontaneously decomposes at physiological pH [33]. In India, it is being extensively used to accelerate the post-harvest ripening of bananas, mangoes, pineapples etc. Unlike, other four classes of plant hormones, ethylene diffuses easily through the air from one plant to another and its residual effect in soils remains and come to the aquatic environment during rainy period [27]. The rosy barb, *Pethia conchonius*, a member of family cyprinidae has a short life span and produces great numbers of big, transparent eggs that are fertilized externally. It is native to rivers and fast flowing streams of Afghanistan, Pakistan, India, Nepal and Bangladesh and is now very popular with aquarists throughout the world [1]. It is, therefore, becoming a potential experimental model fish for biological and biotechnological research. In fact, this fish has been used as a model for clinical toxicology/phycology trials [9], genetics [29] developmental biology and fish behaviour [15, 1].

In addition, as evidenced by our previous study [32], rosy barbs proved to have a high sensitivity to agrochemicals and therefore selected as a potential model fish for this study. However, little information is available about the serum biochemical effects of ethephon to fish. The aim of this study was to investigate the serum biochemical parameters of toxicity of ethephon to evaluate the extent of tissue damage in rosy barbs.

2. Materials and Methods
2.1 Chemicals
Ethephon used in the experiments was purchased from local agriculture fertilizer shop in Ratnagiri. All other chemicals were analytical reagents.

2.2 Animals and treatments
Adult rosy barbs *Pethia conchonius* (Average body weight 2.55 ± 0.03 g) obtained from a local fish dealer were maintained in dechlorinated water at 25±1ºC. The fishes were fed twice in every 24 h with commercial feed and acclimatized for two weeks before exposure to the test pesticide.
A stock solution of the chemical was prepared having the strength of 1000 mgl^{-1} by adding the known quantity of pesticide in 1000 ml distilled water. Calculated amount of stock solution was added to the water and mixed thoroughly to arrive at a required level of working concentrations.

2.3 Lethal toxicity (LC50) study
Lethal toxicity (LC_{50}) study was carried out by following the standard guidelines of EPA/ROC (1998). To determine the LC_{50} of ethephon in rosy barb for 96 h by probit analysis method [8]. A total of 20 rosy barbs per group were exposed to the concentrations of ethephon in 40 litre glass tanks having dechlorinated water for 96 h. Ethephon 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.

2.4 Sublethal Experiment
During sublethal exposures for a period of 28 days two concentrations were selected i.e. $1/10^{th}$ and $1/5^{th}$ of LC_{50}, such as 6.40 and 12.81 mgl^{-1}. At the end of every seven days exposure periods i.e. 7^{th}, 14^{th}, 21^{st} and 28^{th} days, ten fingerlings were sacrificed for serum biochemical analysis such as serum albumin, serum globulin, serum glucose and serum protein.

2.5 Blood collection and serum separation
The blood was collected from their cut caudal vein in to a plain sterilized glass centrifuge tubes. For the separation of serum blood was allowed to clot in the tubes and then centrifuged at 2500 rpm for 30 minutes. The supernatant (serum) was separated and used for estimation of various biochemical parameters such as serum albumin, serum globulin, serum glucose and serum protein.

2.6 Methods of biochemical estimations
Serum protein was estimated by Biuret method [23], albumin was estimated by Bromocresol green binding method [5] and serum glucose was estimated by the method as per Nelson (1944).

2.7 Statistical Analysis:
The LC_{50} value was estimated by probit analysis method [8]. 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 ($P<0.05$) among the control and treatments.

3. Results
3.1 Experiment 1: Lethal toxicity (LC_{50}) experiment
Initially, a range finding test was conducted to ascertain the range of ethephon pesticides 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 mgl^{-1}). In narrow-range finding test of ethephon on rosy barb, the mortality percentage between 10% to 100% was considered. The results of 96 h median lethal concentration value for rosy barb was calculated and presented in Fig. 1. The LC_{50} value of ethephon for rosy barb was found to be 64.034 mgl^{-1} at 96 h.

3.2 Experiment 2: Sublethal experiment
In Sublethal experiment, two concentrations such as 6.40 and 12.81 mgl^{-1} was selected i.e. $1/10^{th}$ and $1/5^{th}$ of LC_{50} for a total period of 28 days. Sublethal effect of ethephon pesticide on serum parameters of rosy barb, *Pethia conchonius* is given in Fig. 2, 3, 4, 5 and the details are as follows;

3.2.1 Serum Albumin
The data of serum albumin (gdl^{-1}) in rosy barb fishes on exposure to sublethal concentrations of ethephon are presented in Fig. 2. In control group serum albumin values were 2.08±0.03, 2.08±0.03, 2.08±0.03 and 2.08±0.03 on 7^{th}, 14^{th}, 21^{st} and 28^{th} day respectively. In 6.40 mgl^{-1} ($1/10^{th}$ concentration of LC_{50}), the serum albumin values were from 1.59±0.04 on 7^{th} day to 1.56±0.03 on 14^{th} day, 1.55±0.04 on 21^{st} day and 1.56±0.02 on the 28^{th} day. In higher concentration of 12.81 mgl^{-1} ($1/5^{th}$ concentration of LC_{50}), the serum albumin values were 1.39±0.04, 1.33±0.03, 1.22±0.02 and 1.17±0.03 on 7^{th}, 14^{th}, 21^{st} and 28^{th} day respectively. ANOVA showed a significant decrease in serum albumin values on 7^{th}, 14^{th}, 21^{st} and 28^{th} day when exposed to sublethal concentrations of ethephon as compared to control ($p<0.05$). Compared to the fishes exposed to 12.81 mgl^{-1} concentration ($1/5^{th}$ of LC_{50}), the serum albumin values were significantly higher in fishes exposed to 6.40 mgl^{-1} ($1/10^{th}$ of LC_{50}) ($p<0.05$).

3.2.2 Serum Globulin
The data of serum globulin (gdl^{-1}) in rosy barb fishes on exposure to sublethal concentrations of ethephon are presented in Fig. 3. The serum globulin values in control groups were 5.34±0.07, 5.33±0.02, 5.32±0.04 and 5.31±0.01 on 7^{th}, 14^{th}, 21^{st} and 28^{th} day respectively. In 6.40 mgl^{-1} ($1/10^{th}$ concentration of LC_{50}), the serum globulin values were from 4.46±0.03 on 7^{th} day to 4.16±0.03 on 14^{th} day, 4.06±0.06 on 21^{st} day and 3.70±0.07 on the 28^{th} day. In higher concentration of 12.81 mgl^{-1} ($1/5^{th}$ concentration of LC_{50}), the serum globulin values were 4.17±0.03, 3.77±0.08, 3.15±0.05 and 2.76±0.07 on 7^{th}, 14^{th}, 21^{st} and 28^{th} day. ANOVA showed a significant decrease in serum globulin values on 7^{th}, 14^{th}, 21^{st} and 28^{th} day when exposed to sublethal concentrations of ethephon as compared to control ($p<0.05$). Compared to the fishes exposed to 12.81 mgl^{-1} concentration ($1/5^{th}$ of LC_{50}), the serum globulin values were significantly higher in fishes exposed to 6.40 mgl^{-1} ($1/10^{th}$ of LC_{50}) ($p<0.05$).

3.2.3 Serum Glucose
The data of serum glucose (gdl^{-1}) in rosy barb fishes on exposure to sublethal concentrations of ethephon are presented in Fig. 4. The serum glucose values recorded in control groups were 44.18±0.50, 44.25±0.48, 44.08±0.41and 43.87±0.37 on 7^{th}, 14^{th}, 21^{st} and 28^{th} day respectively. In 6.40 mgl^{-1} ($1/10^{th}$ concentration of LC_{50}), the serum glucose values were from 47.85±0.39 on 7^{th} day to 51.83±1.66 on 14^{th} day, 57.64±1.80 on 21^{st} day and 63.37±1.10 on 28^{th} day. In concentration of 12.81 mgl^{-1} ($1/5^{th}$ of LC_{50}), the serum glucose values were 54.13±0.54, 68.74±0.38, 82.73±2.07 and 98.33±0.95 respectively on 7^{th}, 14^{th}, 21^{st} and 28^{th} day. ANOVA showed a significant increase in serum glucose

values were on 7th, 14th, 21st and 28th day when exposed to sublethal concentrations of ethephon as compared to control ($p<0.05$). Compared to the fishes exposed to 12.81 mgl^{-1} concentration (1/5th of LC_{50}), the serum glucose values were significantly lower in fishes exposed to 6.40 mgl^{-1} (1/10th of LC_{50}) ($p<0.05$).

3.2.4 Total blood or Serum protein

The data of serum protein (gdl^{-1}) in rosy barb on exposure to sublethal concentrations of ethephon are presented in Fig. 5. The serum protein values recorded in control groups were 2.83±0.03, 2.84±0.04, 2.88±0.04 and 2.86±0.02 on 7th, 14th, 21st and 28th day respectively. In 6.40 mgl^{-1} (1/10th concentration of LC_{50}), the serum protein values were 2.73±0.03, 2.63±0.03, 2.53±0.03 and 2.35±0.03 on 7th, 14th, 21st and 28th day respectively. In higher concentration of 12.81 mgl^{-1} (1/5th concentration of LC_{50}), the serum protein values were 2.56±0.03, 2.46±0.03, 2.24±0.04and 2.08±0.04 respectively, on 7th, 14th, 21st and 28th day respectively. ANOVA showed a significant decrease in serum protein values on 7th, 14th, 21st and 28th day when exposed to sublethal concentrations of ethephon as compared to control ($p<0.05$). Compared to the fishes exposed to 12.81 mgl^{-1} concentration (1/5th of LC_{50}), the serum protein values were significantly higher in fishes exposed to 6.40 mgl^{-1} (1/10th of LC_{50}) ($p<0.05$).

4. Discussion

In the present study, LC_{50} value for rosy barb exposed to ethephon was 64.034 at 96 h, while the 96 h LC_{50} value for rosy barb exposed to nonylphenol was 1.72 mgl^{-1} [2] and for copper and zinc were 0.5mgl^{-1} and 9 mgl^{-1} to *Puntius parrah* respectively [3].

Analysis of serum biochemical constituents used in detection and diagnosis of metabolic disturbances and diseases in fishes [13]. Incline in the serum protein, albumin and globulin levels considered with a stronger innate response in fishes [31]. In the present study, the lowest plasma protein, albumin and globulin content were reported in 1/5th sublethal concentration of ethephon on 28th day. Abating in serum total protein, albumin, globulin and albumin to globulin ratios are strong indicators of an immunosuppressive effect of the pesticides [18]. Also, decreased protein, albumin and cholesterol levels in fish are associated with stress-mediated mobilization to cope with enhanced demand for energy imposed by the pesticides [14]. Presently observed decrease in total protein, albumin and globulin level are indicative of similar effects. Similar results were reported by Padma *et al*. (2012) when *Channa Punctatus* were exposed to the sublethal concentration of imidacloprid insecticide.

Depletion of serum albumin was found in Nile tilapia, *Oreochromis mossambicus* exposed to benomyl [17]. Also similar results of decreasing serum albumin were observed in *Channa punctatus* under ondofil toxicity [26]. It was also witnessed in *Channa punctatus* exposed to sub-lethal concentration of Nuvan [22]. Similar findings were also observed in *Clarias gariepinus* [20] and in *Heteropneustes fossilis* when exposed to Nuvan [25].

Glucose measurements show many inconsistencies and should be a complement of stress tests rather than a main indicator. Glucose is one of the most important sources of energy for the animals. It has been reported as an indicator of stress caused by physical factors [4, 16]. In many fish species, the blood glucose level has the tendency to increase due to experimental stress. In the present study, the significant increase in glucose which was time dependent may be considered to be manifestation of stress induced by the both agrochemicals. Wedemeyer *et al*. (1984) stated that high levels of blood glucose caused by disorders in carbohydrate metabolism due to physical and chemical stresses. A variety of stressors stimulate the adrenal tissue resulting in increased level of circulating glucocorticoids and catecholamines [12]. Both the groups of hormones produce hyperglycemia. It is generally thought that under conditions of stress, hyperglycemia may provide additional energy during times of high metabolic need such as “fight and fight: response [10].

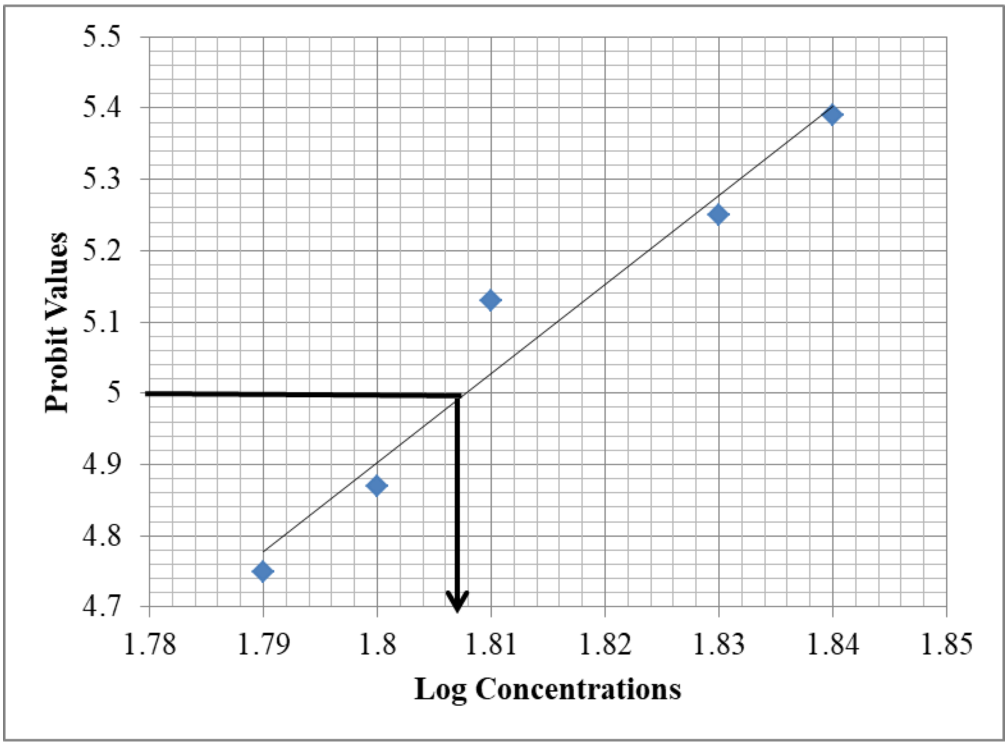

Fig 1: Probit values against log concentrations of ethephon

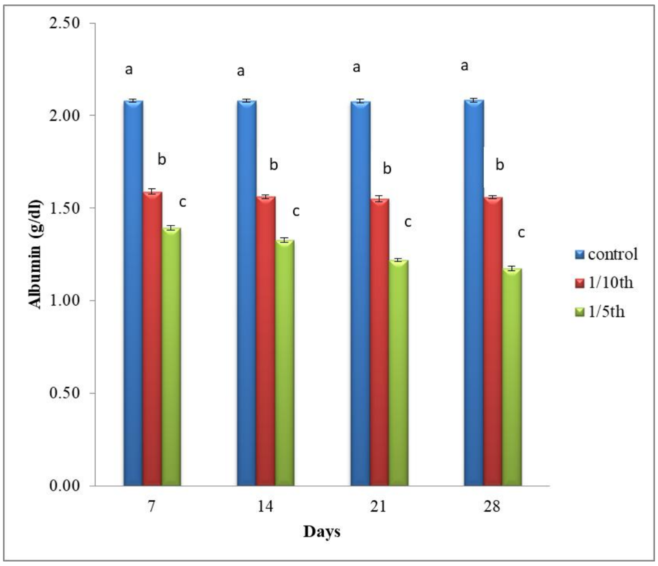

Fig 2: Variation in serum albumin of rosy barb exposed to sublethal concentrations of ethephon.

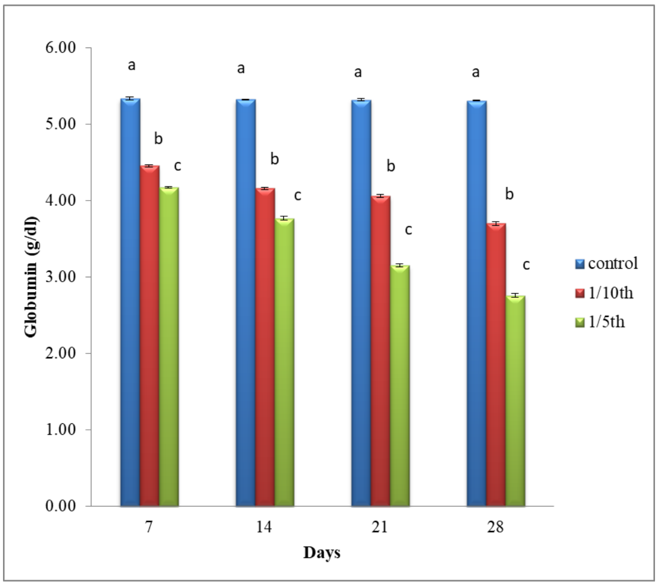

Fig 3: Variation in serum globulin of rosy barb exposed to sublethal concentrations of ethephon

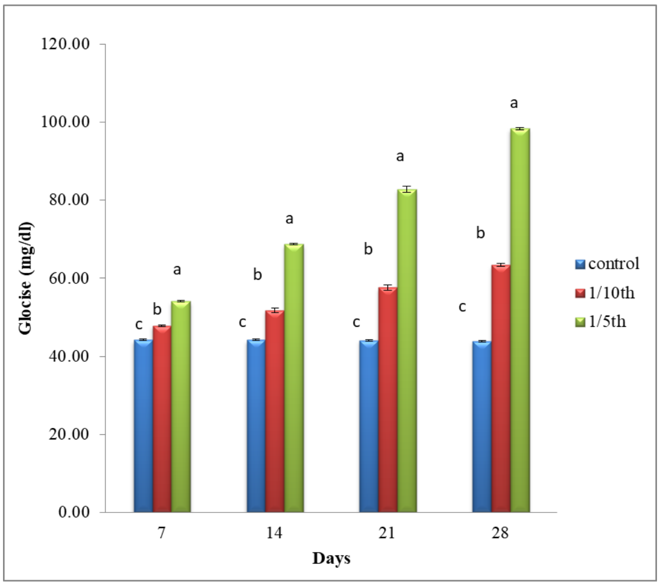

Fig 4: Variation in serum glucose of rosy barb exposed to sublethal concentrations of ethephon

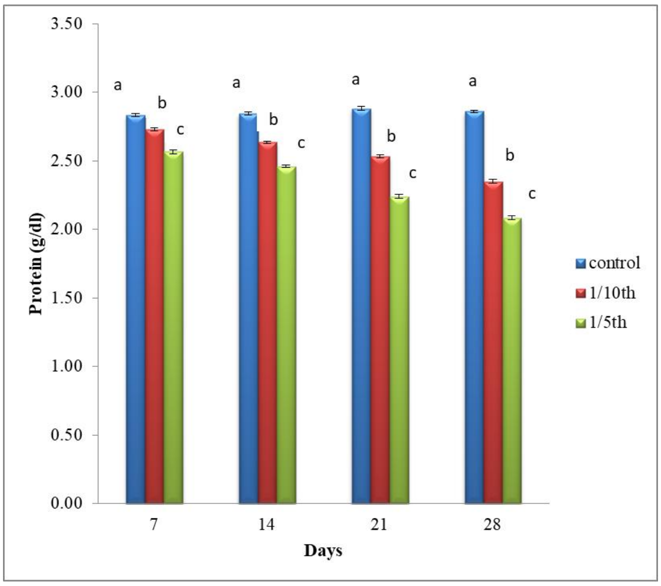

Fig 5: Variation in serum protein of rosy barb exposed to sublethal concentrations of ethephon

5. Conclusion

The results of the present investigation revealed that fishes exposed to ethephon exhibited dose and duration dependent alterations in many biochemical components such as serum total protein, serum albumin, serum globulin and serum glucose of the rosy barb (*Pethia conchonius*). Ethephon affects the target as well as non-target organisms.

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7. References

1. Bhattacharya H, Lun L, Gomez RGD. Biochemical effects to toxicity of CCl_4 on rosy barbs (*Puntius conchonius*). Our Nat. 2005; 3:10-25.
2. Bhattacharya H, Zhang S, Xiao Q. Comparison of histopathological alterations due to sublethal CCl_4 on rosy barb (*Puntius conchonius*) and Amphioxus (*Branchiostoma belcheri*) with Implications of liver ontogeny. Toxicology Mechanisms and Methods. 2008; 18:627-633.
3. Ciji PP, Nandan SB. Toxicity of copper and zinc to *Puntius parrah* (Day, 1865). Marine Environmental Research. 2013; 93:38-46.
4. Daniel CS, Agarwal S. Human red blood cell membrane damage by endosulfan. Toxicol. Lett. 1986; 32:113-118.
5. Doumas BT, Biggs HG. Determination of serum albumin. Stand. Method of Clinical Chemistry, Acad. Press, New York, 1971; 7:175.
6. EPA. Reregistration Eligibility Decision (RED): Ethephon. List A Case 0382. U.S. Environmental Protection Agency. Office of Prevention, Pesticides and Toxic Substances. Washington, D.C. 20460, 1995. Available from: http://www.epa. gov/REDs/0382.pdf.
7. EPA/ROC, Standard guide for conducting acute tests with fishes: static renewal test for common carp: NIEA B904.10B. Environmental Protection Administration of the Republic of China, Taipei, Taiwan, 1998, 81-96.

8. Finney DJ. Probit analysis. 3rd edn. Cambridge University Press, London, 1971, 508-510.
9. Gill TS, Tewari H, Pande J. Use of the fish enzyme system in monitoring water quality: effects of mercury on tissue enzymes. Comp. Biochem. Physiol. 1990; 97:287-292.
10. Gross GG, Wood CM. The effects of acid and acid/aluminium exposure on circulating plasma cortisol levels and other blood parameters in the rainbow trout *Salmogardineri*. Journal of Fish Biology. 1988; 32:63-76.
11. Haux JE, Quistad GB, Casida JE. Phosphobutyrylcholinesterase: Phosphorylation of the Esteratic Site of Butyrylcholinesterase by Ethephon [(2-Chloroethyl) phosphonic Acid] Dianion. Chem Res Toxicol. 2000; 13:646-651.
12. Hontela A, Daniel C. Effects of acute and sublethal exposure to cadmium on the interregnal and thyroid function in rainbow trout *Onchorhynchus mykiss*. Aquat. Taxicol. 1996; 35:171-182.
13. Jamalzadesh HR, Keyvan MR. Comparison of blood indices in healthy and fungal infected Caspian salmon (*Salmo trutta caspius*). African. J Biotechnology. 2009; 8:319-322.
14. Jee JH, Masroor E, Kang JC. Response of cypermethrin induced stress in haematological parameters of Korean rockfish, *Sebastes schlegeli* (Hilgendorf). Aquaculture research. 2007; 36:898-905.
15. Kirankumar S, Anathy V, Pandian TJ. Hormonal induction of supermale golden rosy barb and isolation of Y-chromosome specific makers. Gen. Comp. Endocrinol. 2003; 134:62-71.
16. Manush SM, Pal AK, Das T, Mukherjee SC. Dietary high protein & vitamin C influences in mitigating stress due to chelate claw ablation in *Macrobrachium rosenbergii* males. Comparative Biochemistry & Physiology. 2005; 142A:10-18.
17. Min EY, Kang C. Effect of water born benomyl on the haematological and antioxidant parameters of Nile tilapia, *Oreochromis niloticus*. Pest. Bioch. and physiology. 2008; 2:138-143.
18. Nayak AK, Das BK, Kohli MPS, Mukherjee SC. The immunosuppressive effect of α-permethrin on Indian major carp, rohu (*Labeo rohita* Ham.). Fish Shellfish Immun. 2004; 16(1):41-50.
19. Nelson N. A photometric adaptation of the Somogyi method for the determination of glucose. Journal of biological Chemistry. 1944; 153(2):375-380.
20. Ogamba EN, Inyng IR, Azuma IK. Effect of Paraquat dichloride on some metabolic and enzyme parameters of *Clarias gariepinus*. Current Research. Journal of biological Sciences. 2011; 31:186-190.
21. Padma PB, Rachel V, Maruthi YA. Acute toxicity effect of imidacloprid insecticide on serum biochemical parameters of fresh water teleost *Channa punctatus*. Int. J. Int sci. Inn. Tech. Sec. A. 2012; 1(2):18-22.
22. Rani R, Gautam RK. Biochemical studies on blood of *Channa punctatus* (Bloch.) after Nuvan toxicity. Ph.D. thesis Department of Zoology, Dr. Bhim. Rao Ambedkar University, Agra. 2009, 288.
23. Reinhold JG. Total protein, albumin and globulin. Stand. Methods. Clin. Chem, 1953; 1:88.
24. Segall Y, Grendell RL, Toia RF, Casida JE. Composition of Technical Ethephon [(2-Chloroethyl) phosphonic Acid] and Some Analogues Relative to Their Reactivity and Biological Activity. Journal of Agricultural and Food Chemistry. 1991; 39:380-385.
25. Shaikh IA, Gautam RK. Effect of organophosphate pesticide Nuvan on serum biochemical parameters of fresh water cat fish *Heteropneustes fossilis* (Bloch). 2014; 3(10):1-6.
26. Sharma G, Malla FA, Singh S. An assay on the effect of indofil toxicity on the total serum protein content of *Channa punctatus* (Bloch), biology and Medicine. 2009; 1:25-27.
27. Silpi U, Chantuma P, Kosaisawe J, Thanisawanyangkura S, Gohet E. In: Sainte-Beuve Jerome (ed). Biotechnology and Rubber Tree: Proceedings of IRRDB Symposium, Montpellier, France, 2001, 25-28.
28. Tomlin CDS. The Pesticide Manual: A World Compendium. 11th Ed. The British Crop Protection Council, 1997.
29. Varadi L, Hidas A, Varkonyi E, Horvath L. Interesting phenomena in hybridization of carp (*Cyprinus carpio*) and rosy barb (*Barbus conchonius*). Aquaculture. 1995; 129:211-214.
30. Wedemeyer G, Mcleay. Assessing the tolerance of fish and fish population to environmental stress. The problems and methods of monitoring in: contaminate effects on fisheries on fisheries cairnus W.V. Hodson PV and Nriagu J.O (Eds.). John Wiley and Son Inc New York. 1984, 164-195.
31. Wiegertjes GF, Stet RJM, Parmeatier HK, Van-Muiswinkel WB. Immunogenetics of disease resistance in fish; a comparable approach developmental and comparative Immunology. J exp. Boil. 1996; 20:365-381.
32. Xiao Q, Zhang SC, Zhao B. Toxic effects of nonylphenol on the gonad of adult rosy barb. Chin. J. Environ. Sci. 2007; 28(11):2580-2585.
33. Zhang N, Casida JE. Novel Irreversible Butyrylcholinesterase Inhibitors: 2-Chloro-1-(substituted-phenyl) ethylphosphonic Acids. Bioorganic & medicinal chemistry. 2002; 10:1281-1290.