Vol 4, Issue 6, 2017 ISSN 2349-7041

Research Article

ACUTE TOXICITY AND RESPIRATORY RESPONSES IN FRESHWATER FISH, LABEO ROHITA EXPOSED TO AN AGROCHEMICAL INDOXACARB

NAGARAJU BANTU*[1], RAVI KUMAR K[2] AND VENKATARATHNAMMA VAKITA[2]

[1]Department of Chemistry, Aksum University, Ethiopia, Eastern Africa ,[2]Department of Zoology, Acharya Nagarjuna University, Guntur, India

Email: nagaraju.bantu301@gmail.com

ABSTRACT

Experimental fish were exposed to different concentrations of Indoxacarb for different hours, percent mortality was recorded. The 96 h LC50value of toxicant to the fish were found to be 0.0521 mg/L. Throughout the experimental period, the fish showed severe respiratory distress and rapid opercular movements leading to the higher amount of toxicant uptake, increased secretion of mucus higher ventilation volume, decrease in oxygen uptake efficiency, labored breathing and engulfing of air through the mouth Behavioral patterns were observed in during exposure period, test organism showed normal behavior in control group but jerky movements, hyper secretion of mucus, opening and closing of mouth for gasping, losing scales, hyperactivity were observed experimental group.

Keywords: Mortality, value, exposure, oxygen, behavior and scales.

INTRODUCTION

The undue persistence, high mammalian toxicity and developing resistance of the organochlorine, organophosphate and carbamate insecticides led to a ban or restriction on their use in many developed and developing countries. To increase productivity, modern methods are practiced in agriculture. One integral part of this is to resort to increased use of pesticides to curb agricultural losses due to pests [1]. Unfortunately, after use pesticides do not stay in their place of application but move to the other parts of the environment and ultimately to the aquatic environment. Since pesticides are poisons and are meant to kill, during their sojourn through the different compartments of the globe, they kill a host of other non-target organisms [2].The work of [3, 4] and several such other workers has clearly established that the pesticide residues are transported to the aquatic environment either through surface runoff or through precipitation into which they get in by evaporation from cropland. The increasing awareness of the environmental hazards of pesticides necessitated the testing of toxicity of different pesticides to different aquatic organisms. Different types of toxicity tests serve different purposes. The 96 h toxicity test or the short term or acute toxicity test is one of the most commonly employed tests in the evaluation of toxicity. The modern aquatic toxicity protocols in use are the results of a series of attempts at the standardization of the test methodology. The earliest and one of the most useful of these test methods is that of [5]. This forms the basis for all other attempts. In the standard methods of the American Public Health Association [6] bioassay and toxicity test procedures are described in detail. Indoxacarb is an insecticide with a new class of chemistry and with a new mode of action. It is a reduced risk pesticide with a low mammalian toxicity and a benign profile for avian and aquatic toxicity. Indoxacarb is a broad spectrum insecticide with activity on codling moth, white apple leafhopper, Panda leaf roller, and Lacanobia fruit worm. Hence, in the current study, acute toxicity and the effect of sub lethal and lethal concentrations of indoxacarb on the oxygen consumption of fish.

MATERIALS AND METHODS

The freshwater fish, *Labeo rohita* is an edible and commercially valuable fish. Live fish of size 6-7 cm and 6-8 g weight were brought from a local fish farm Nandivelugu, India and acclimatized at 28 ± 2^0C in the laboratory for 15 days. During acclimatization period, if 5% mortality is observed the total batch was discarded. Indoxacarb (14.5% S.C) was supplied by Rallies India Ltd. Hyderabad. The water used for acclimatization and conducting experiments was clear unchlorinated ground water and the hydrographical conditions of the water were shown in the table 1

Duration of the test

The concentration of pesticide, which may normally be sub-lethal during short-term exposures (24 or 48 h), may prove to be lethal, if the exposure time is extended (up to 96 to 120 h). Since, the toxicity of the poison is a function of time; it is customary to expose the test organisms over a fixed period of time to the toxicant usually for 24, 48, 72 and 96 h. The containers of the test media are of 10 liters capacity; wherein for each test five containers were used and in each container 10 fishes were introduced. Experiments were conducted to determine the toxicity of indoxacarb in various concentrations in static system. The data on the mortality rate of the fish was recorded. The dead fish were removed. The toxicity tests were conducted to choose the mortality range from 10% to 90% for 24, 48, 72 and 96 h in static system. Finney's probit analysis [7] was followed to calculate the LC50 values. The fish were exposed to lethal (48 h LC50 of 0.05649 mg/L) and sub lethal were (1/10 of 48 h LC50 of 0.005649 mg/L).

Description of respiratory chamber

The apparatus used for the measurement of whole animal oxygen consumption is a wide mouthed bottle, which is called a respiratory chamber (RC). Its mouth was fitted with a four-holed stopper (S) and through one of the holes a thermometer (T) was passed to know the temperature of the medium in the respiratory chamber. From the remaining three holes three glass tubes were passed whose outer ends were fitted with rubber tubes. These three tubes served as delivery tubes and designated as T1, T2 and T3 respectively. They were fitted with pinch locks P1, P2and P3. T1 was connected with the reservoir (R) and though this water could be drawn (inlet) into the respiratory chamber. T2 was atmospheric tube; useful for testing the air tightness of the respiratory chamber. Through the T3 tube (outlet) water samples from the respiratory chamber were collected for estimation of dissolved oxygen. The respiratory chamber was

coated black to avoid photochemical reactions and to keep the animal activity at normal during the experiment.

Only one fish was introduced into each respiratory chamber and was filled with water drawn through T1 from the reservoir. After checking the air tightness pinch lock P2 was closed and pinch lock P3 was opened slightly so that a very gentle and even flow of water was maintained through the respiratory chamber. This was continued for 15 minutes to facilitate the animal in returning to a spate of normalcy from the state of excitement, if any, due to the handling and also to allow the animal to adjust to the darkness in the chamber (acclimatization).

Collection of the initial and final samples

After allowing the animal to settle in the chamber, the initial sample was collected from the respiratory chamber through T3. After the collection of initial sample, the respiratory chamber was closed by closing P3 first and then P1 after one hour. The next sample was collected from the respiratory chamber. Likewise, other samples were also collected at the end of each hour for total 22 hours period of the experiment.

Along with three experimental fish chambers, one respiratory chamber without fish (control) was maintained. The control serves to estimate the initial amount of oxygen.

The amount of oxygen consumed was calculated per gram body weight per hour.

$$\text{Gram body weight/hour} = \frac{\text{O2 consumed by fish / } \alpha\text{-}\beta \text{ x N of hypo x 8 x 1000}}{\text{Vol. of the sample taken x Correction factor}}$$

Wt. of the fish x Time interval for each sample; α = hypo rundown before exposure; β = hypo rundown after exposure; N= Normality of Hypo

Statistical analysis

Student's t-test was employed to calculate the significance of the differences between control and experimental means. P values of 0.05 or less were considered statistically significant.

RESULTS AND DISCUSSION

The results of the present work with reference to observed percent mortality for Avaunt (Indoxacarb 14.5% S.C) for 24, 48, 72 and 96 h to the fish *Labeo rohita* in static system are given in tables 2,3,4&5. The results of the present work with reference to the observed percent mortality for indoxacarb were compound with the other animals and different pesticides LC_{50} values with the indoxacarb. The percent mortality and probit mortality increased with the increasing concentration of indoxacarb. The percent mortality plotted against log concentration of indoxacarb gave sigmoid curves. The 24, 48, 72 and 96 h. LC_{50} of indoxacarb was obtained by taking the mean LC50 derived from the percent and probit mortality Curves.

Table 1: Chemical analysis of water used for experiments (mg/L).

Variable	Results
Turbidity	8 silica units
pH at 28^0 C	8.1
I Phenolphthalein	Nil
II Methyl orange (as $CaCo_3$)	452
Total hardness	232
Calcium hardness	52
Magnesium hardness	32
Carbonate hardness	150
Nitrite nitrogen (as N)	Nil
Sulphate (as SO_4)	trace
Chloride (as Cl)	40
Fluoride (as F)	1.8
Iron (as Fe)	Nil
Dissolved oxygen	8-10
Temperature	28^0±2^0 C

Table 2: Determination of static LC_{50} – 24 h

S.no.	Concentration mg/L	Percent Mortality	Probit value	LOG (100*conc.) =X	X*X	Y*Y	XY
1	0.054	20	4.1584	0.740362	0.548136	17.29229	3.078724
2	0.056	30	4.4756	0.755874	0.571346	20.03099	3.382993
3	0.058	50	5	0.770852	0.594212	25	3.854260
4	0.060	70	5.5244	0.785329	0.61674	30.51899	4.338476
5	0.062	80	5.8416	0.799340	0.638945	34.12429	4.669427

X 0.770352 ; Y 5 ; SXX 0.0021739 ; SYY 1.9665718 ; SXY 0.065082 ; Slope B 29.938321 ; Variance B 46.000953 ; Variance A 27.398906 ; M 0.770352; LC_{50} = 0.0569 mg/L

Table 3: Determination of static LC_{50} – 48 h

S.no.	Concentration mg/L	Percent Mortality	Probit value	LOG (100*conc.) =X	X*X	Y*Y	XY
1	0.052	20	4.1584	0.72427	0.524575	17.29229	3.011828
2	0.054	30	4.4756	0.740362	0.548136	20.03099	3.313567
3	0.056	50	5	0.755874	0.571346	25	3.779374
4	0.058	70	5.5244	0.770852	0.594212	30.51899	4.258494
5	0.060	80	5.8416	0.785329	0.61674	34.12429	4.587582

X 0.7553391 ; Y 5 ; SXX 0.0023296 ; SYY 1.9665718 ; SXY 0.0673716 ; Slope B 28.919837 ; Variance B 42.925846 ; Variance A 24.590787 ; M 0.7553391 ; LC50 = 0.05649 mg/L

Table 4: Determination of static LC_{50} – 72h

S.no.	Concentration mg/L	Percent Mortality	Probit value	LOG (100*conc.) =X	X*X	Y*Y	XY
1	0.050	20	4.1584	0.707570	0.500655	17.29229	2.94235
2	0.052	30	4.4756	0.72427	0.524575	20.03099	3.241569
3	0.054	60	5.2533	0.740362	0.548136	27.59716	3.889347
4	0.056	70	5.5244	0.755874	0.571346	30.51899	4.175755
5	0.058	90	6.2816	0.770852	0.594212	39.45849	4.842183

X 0.7397871 ; Y 5.13866 ; SXX 0.0025027 ; SYY 2.8688078 ; SXY 0.0836428 ; Slope B 33.420985 ; Variance B 39.956777 ; Variance A 21.967744 ; M 0.7432173; LC50 = 0.05422 mg/L

Table 5: Determination of static LC_{50} – 96 h

S.no.	Concentration mg/L	Percent Mortality	Probit value	LOG (100*conc.) =X	X*X	Y*Y	XY
1	0.048	20	4.1584	0.69019	0.476370	17.29229	2.870111
2	0.050	40	4.7467	0.707570	0.500655	22.53116	3.358623
3	0.052	60	5.2533	0.72427	0.524575	27.59716	3.804838
4	0.054	70	5.5244	0.740362	0.548136	30.51899	4.090059
5	0.056	90	6.2816	0.755874	0.571346	39.45849	4.748103

X 0.7236559 ; Y 5.19288 ; SXX 0.0026959 ; SYY 2.5680928 ; SXY 0.0824442 ; Slope B 30.581625 ; Variance B 37.093744 ; Variance A 19.525175 ; M 0.7256316; LC_{50} = 0.0521 mg/

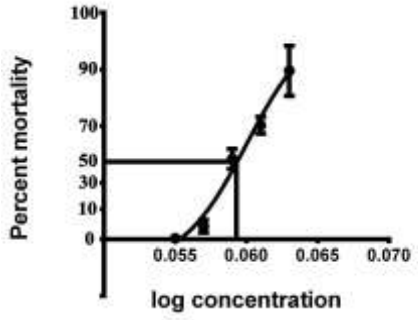

Fig.1: The graph showing sigmoid curve between percent mortality of fish against log concentration in fish, *Labeo rohita* on exposure to indoxacarb

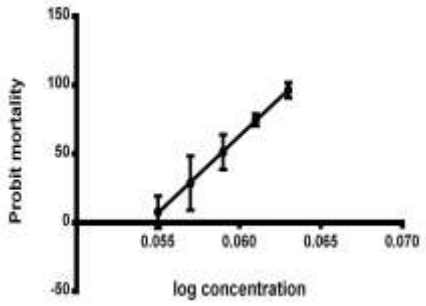

Fig.2: The graph showing linear curve between percent mortality of fish against log concentration in fish, *Labeo rohita* on exposure to indoxacarb

When the present LC_{50} values of indoxacarb to the test organisms are compared to the degree of harmfulness shown in the above table, it was observed that the pesticide indoxacarb is extremely toxic to the fish, *Labeo rohita*.

The toxicity of indoxacarb, the DPX-KN 127 Isomer and associated degradates are moderately to very highly toxic to freshwater and estuarine/marine fish on an acute basis with LC_{50}'s ranging from 0.024 to >1.3 mg/L [8]. Chronic toxicities range from 0.0006 to 0.0184 ml/L for estuarine fish and invertebrates and from 0.004 to 0.15 mg/L for freshwater fish and invertebrates. They are also moderate to very highly toxic to freshwater and estuarine/marine invertebrates on an acute basis with EC 50 ranging from 0.029 to 2.9 mg/L

Brugger and Kannuch (1997) reported the LC_{50} values of DPX-MP 062 to blue gill as 900 ppb, for rainbow trout as 650 ppb and for sheep head minnow 96 h-LC_{50} as 374 ppb. These authors also reported the LD_{50} values for rat and bobwhite quails as 1730 ppm and 808ppm respectively. In the present study also the LC_{50} values for 24, 48, 72 and 96 h for indoxacarb to the fish *Labeo rohita* are in agreement with the earlier reports.

The reported EC_{50} value for *Daphnia magna* is 600ppb, for mallard duck is >5620 ppm and the 96 h LC_{50} for Mysid shrimp is 54.2ppb to DPX-MP 062. These values are less than the LC_{50} values of indoxacarb to freshwater fish *Labeo rohita* when compared to other fish species of marine /estuarine and freshwater fish.

Carbamates are moderately toxic to fish. Carbaryl has a 96 h LC_{50} of 2 to 39 mg/L and carbofuran 150 to 87 µg/L for many freshwater American fish [9] organophosphates has negligible chronic toxicity, but some of them have moderate to high acute toxic. [10] Reported that median lethal concentrations (LC_{50}) of monocrotophos for 24, 48, 72 and 96 h were 0.0041, 0.0039, 0.0037 and 0.0036 ppm respectively, whereas the LC_{50} values of synthetic pyrethroid lambda cyhalothrin for 24, 48, 72 and 96 h were 0.0026, 0.0024, 0.0022 and 0.0021 ppm, to fish *Labeo rohita*. The LC_{50} value of malathion an organophosphate pesticide to freshwater fish was found to be 9.0 µl/l, reported by [11]. [12] Reported that the 96 h LC_{50} values of botanical pesticide, Kethrin and an organophosphate pesticide Dichlorvos was found to be 21.68ppm and 16.71ppm to freshwater fish *Labeo rohita*, respectively.[13]reported that acute toxicity of dimethoate to freshwater fish *Colisa fasciatus* (Bl. & Schn.) for 24, 48, 72 and 96 h were found to be 22.15 mg l^{-1}, 21.99 mg l^{-1}, 21.74 mg l^{-1} and 21.65 mg l^{-1}, respectively. Temperature, hardness, pH, alkalinity and biological factors such as sex, age, weight and physiological status are reported to have profound effects on the acute toxicity of dimethoate. The estimated LC_{50} values of dimethoate to freshwater fish *Labeo rohita* were found to be 17.532 mg l^{-1}, 17.321 mg l^{-1}, 16.721 mg l^{-1}, and 16.350 mg l^{-1} for 24, 48, 72, and 96 h, respectively [14]. Variation in lethal concentration (LC) values of dimethoate in different species occurs probably due to differences in susceptibility and tolerance related to differences in rates of bio-accumulation, biotransformation and excretion of toxicant. It is evident form the above stated LC_{50} values that organochlorines, organophosphates, carbamates and synthetic pyrethroids are more toxic. Due to their persistence and high toxicity on fish and mammals, a restriction on their use is imposed.

The morphological and behavioral changes exhibited by the test fish can be taken as a useful parameter in assessing the toxicity caused by pesticides to some extent [15]. Thus studies on symptomology need much emphasis in understanding the changes in animals [16]. In the present study, the behavioural changes observed in the test fish were, swimming near the water surface, hyper-excitability, muscular incoordination, hyperactivity, erratic movement, loss of buoyancy, increased gill mucus secretion and restlessness before death. These activities may be due to the increased metabolic rate and interference of the pesticide with neural transmission.

Oxygen consumption

The comparative data on the whole animal oxygen consumption of control and experimental fish, calculated per gram body weight/h in sublethal and lethal concentrations of indoxacarb technical grade on the fish *Labeo rohita* are given in the Table 6 and represented in figure.3. In sublethal concentrations of indoxacarb technical grade it was observed that fish showed similar tendency of increase in oxygen consumption during the initial time of exposures i.e.1 to 6 hours and a gradual decrease was observed during the subsequent period of study. The presence of sublethal concentration of toxicants is inevitable. The toxicant stress in oxygen consumption along with depletion in oxygen in aquaculture practices makes them less fit and reduces growth due to lack of proper metabolism.

In control also, the rate of oxygen consumption gradually decreased and this can be attributed to the reduced metabolic rates in starved conditions. In exposed fish, the reduction in oxygen uptake can be correlated to the extent of damage of gill epithelium. Throughout the experimental period, the fish showed severe respiratory distress and rapid opercular movements leading to the higher amount of toxicant uptake, increased secretion of mucus higher ventilation volume, decrease in oxygen uptake efficiency, labored breathing and engulfing of air through the mouth. The increased oxygen consumption in the present study is in agreement with[17] ;[18,19] in which an elevation in oxygen uptake is observed during initial stages of exposure i.e., 1-4 hours followed by decrease in subsequent hours.

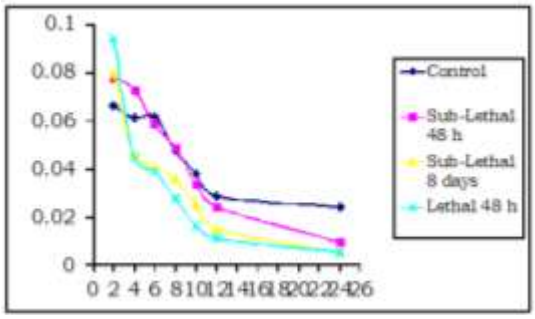

Fig. 3: The amount of oxygen consumed in mg/g body weight/h of the fish.

Table 6: The amount of oxygen consumed in mg/g body weight/h of the fish, *Labeo rohita*

Hours	Control	Sub lethal 48 h Oxygen consumption ± SD	Sub lethal 8 days Oxygen consumption ± SD	Lethal 48 h Oxygen consumption ± SD
2	0.0662	0.07795^{a} ±0.002	0.08^{a} ±0.029	0.09444^{b}±0.004
4	0.0611	0.0730^{c}±0.002	0.0461^{a} ±0.004	0.0444^{b}±0.029
6	0.0620	0.0584^{a} ±0.004	0.0410^{b}±0.003	0.0388^{b}±0.002
8	0.0477	0.0487^{b}±0.002	0.0358^{c}±0.002	0.0277^{a}±0.001
10	0.0381	0.0341^{a} ±0.001	0.0256^{b}±0.004	0.0166^{c}±0.004
12	0.0286	0.0243^{c} ±0.004	0.0153^{a} ±0.001	0.0111^{a}±0.004
24	0.0243	0.0097^{b} ±0.001	0.0051^{c} ±0.042	0.0055^{b}±0.044

Values are means ±SD (n=3) for oxygen consumption in a row followed by the same letters and are not significantly different (*P*<0.05) from each other

Hypoxic conditions prevail under toxic conditions and a number of poisons existing in low concentrations in the medium (which were not toxic earlier) become more toxic to the organism. Hence, the fish breathe more rapidly and the amplitude of respiratory movements will increase [20] Observed that the lack of oxygen increases the ventilation volume of fishes and the cardiac output is reduced. This reduces the rate of passage of blood trough the gills, thus allowing a longer period of time for uptake of oxygen and also conserves oxygen by reducing muscular work. The zone of resistance is reached when the oxygen tension in the medium is so low that homeostatic mechanisms of the fish are no longer able to maintain the oxygen tension in the afferent blood and the standard metabolism begins to fall.

CONCLUSION

The symptoms induced by the indoxacarb insecticide in fish can also be attributed to an increase in physiological stress. Physiological stress may have occurred in the form of neural excitation, which apparently might have resulted in the continuous synthesis and destruction of neurotransmitting enzymes.

All the studies mentioned above indicate a considerable effect of insecticides on oxygen consumption in different species of fish in lethal as well as sublethal concentrations. The present study revealed alterations in the oxygen consumption of the fish *Labeo rohita*, exposed to sublethal and lethal concentrations of Indoxacarb.

ACKNOWLEDGEMENTS

The authors are thankful to Head, Department of Biochemistry & Zoology, Acharya Nagarjuna University, and Guntur, India for providing laboratory facilities for the study.

REFERENCES

1. Palaniappan, P.L.R.M and karthikeyan. Bioaccumulation and depuration of chromium in the selected organs and whole body tissues of freshwater fish Cirrhinus mrigala individually and in binary solutions with nickel.J.Environ.sci, 2009, 21,229-236.
2. Subramani lavanya, Mathan Ramesh, Chokkalingam kavitha. Annamalai Malarvizhi. Hematological, biochemical and ionoregulatory responses of Indian major carp Catla catla during chronic sublethal exposure to inorganic arsenic; Chemosphere, 2011,82:977-
3. Nagaraju Bantu, Venkata rathnamma Vakita, Somaiah Karra. Effect of Chlorantraniliprole on Biochemical and Certain Biomarkers in Various Tissues of Freshwater Fish Labeo rohita (Hamilton). Environment and Ecology Research, 2013, 1, 205-215.doi: 10.13189/eer.2013.010403.
4. Nagaraju. Bantu and Venkatarathnamma. Gas liquid chromatography-flame ionization detector (GLC-FID) residue analysis of carbamate pesticide in freshwater fish Labeo rohita, Toxicology Research, 2014, 3, 3, 177-183.
5. Doudoroff P; Anderson B. O; Burdick G. E; Galtsoff P. S; Hart W. B; Parick R; Strong E. R; Surber E. W; Vanhorn W. M. Bioassay methods for the evaluation of acute toxicity of industrial wastes to fish, Sewage Industry Wastes, 1951,23:1380.
6. APHA. American Public Health Association. Standard methods for examination of water including bottom sediments and sludges.Standard Methods, 2005, (19th ed.), p. 874.
7. Finney D. J. Probit Analysis, 3rd edn.Cambridge University Press, Cambridge, 1971, 20.
8. U. S. EPA Pesticide Fact Sheet: Indoxacarb. Litster S. Mathematical modelling of fuel cells for portable devices. M.A.Sc. Thesis, University of Victoria, 2005.

9. Johnson,W.W and M.T.Finely..Handbook of acute toxicity of chemicals to fish and aquatic invertebrates.U.s.Fish wilddl.serv.Resour.publication unit, U.S. fish and wildlife service, Washington, Dc 1980, 20240.
10. Muthukumaravel, K., Sukumaran, M and Sathick, O.Studies on the acute toxicity of pesticides on the freshwater fish Labeo rohita. J. Pure Appl. Zool. 2013, 1(2): 185-192.
11. Patil V. K. and David M. Behaviourand respiratory dysfunction as an index of malathion toxicity in the freshwater fish, Labeorohita (Hamilton). Turk. J. Fish. Aquat. Sci. 2008, 8,233-237.
12. Bilal Ahmad Bhat, Alok Varma and Geeta saxena. Comparative study on the acute toxicity of plant based pesticide, Kethrin and an organophosphate pesticide, Dichlorvos to (Hamilton). Indian J.L.Sci. 2012,2 (1): 95-98.
13. Ram nayan Singh. Acute toxicity of an organophosphate, dimethoate to an air breathing fish, Colisa fasciatus (Bl. & Schn.), Indian J.Sci.Res. 2013, 4(1): 97-100
14. Nagaraju Bantu and Rathnamma vakita.Effect of Dimethoate on mortality and Biochemical changes of Freshwater fish Labeo rohita (Hamilton). Journal of Biology and today's world,2013, 2(10):456-470.
15. Koprucu S.S., Koprucu K., Ural M.S., Ispir U.and Pala M. Acute toxicity oforganophosphorus pesticide Diazinon and itseffects on behavior and some hematologicalparameters of fingerling European catfish(Silurus glanis L.). Pesticide Biochemistry and Physiology,2006, 86, 99-105.
16. Parithabhanu A. Pesticide toxicity and behavioural responses in the fish Orechromismossambicus. International Journal of Fisheries and Aquaculture Sciences, 2013,3(2), 161-164.
17. Kalavathy, K., Sivakumar, A.A. and Chandran, R. Toxic effects of the pesticide dimethoate on the fish,Sarotherodon mossambicus. J. Ecol. Res. Bio, 2001, 2: 27-32.
18. Katja, S., Georg, B.O.S., Stephan, P. and Christian, E.W.S. Impact of PCB mixture (Aroclor 1254) and TBT and a mixture of both on swimming behaviour, body growth and enzymatic biotransformation activities (GST) of young carp, Cyprinus carpio. Aquatic Toxicology, 2005, 71: 49-59.
19. Magare, S.R. and Patil, H.T. Effect of pesticides on oxygen consumption, red blood cell count and metabolites of a fish, Puntius ticto. Environ. Ecology,2000,18: 891-894.
20. Vineetkumar K. Patil1, M. David. Behaviour and Respiratory Dysfunction as an Index of Malathion Toxicity in the Freshwater Fish, Labeo rohita (Hamilton). Turkish Journal of Fisheries and Aquatic Sciences, 2008, 8: 233-237.