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Toxicological Evaluation of Dimethoate 30% EC to A Freshwater Fish, *Heteropneustes fossilis* (Bloch) at Two Different Selected Levels of pH

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Abstract: The current investigation was aimed to evaluate the acute toxicity (LC_{50} values) of Dimethoate 30% EC to a freshwater fish, *Heteropneustes fossilis* (Bloch) at two different selected levels of pH (8.32±0.3 and 6.32±0.3) for 96 hours by Finney's Probit analysis statistical software method.The 96 hours LC_{50} values and its Confidence Ratio were analysed as 25.933 ppm and 1.332; and 19.109 ppm and 1.290 at pH 8.32±0.3 and 6.32±0.3 respectively during an experimental routine static bioassay test. However, the Safe dischargeable concentration were estimated as 1.0664 and 1.0716 ppm which are too low as compared to Safe or harmless concentration, noticed as 8.9164 and 6.3751 ppm at both the selected levels of pH 8.32±0.3 and 6.32±0.3 respectively. There are significant declines in Dissolve oxygen (DO) content (ppm) has been seen from 0 to 96 hours, indicates that test fish utilize more oxygen under stress condition. The highest percentage (%) alteration in DO content was noticed as 17.56 ppm at 21.0 ppm Concentration of Dimethoate 30% EC (Highest Concentration at pH 8.32±0.3). The behavioral response exhibited by exposed test fishes has been noticed during course of full scale static bioassay. Group analysis (One way ANOVA) between LC_{50} values of Dimethoate 30% EC for 24, 48, 72 and 96 hrs to *Heteropneustes fossilis* (Bloch) at pH (8.32±0.3) verses and pH (6.32±0.3); and pH (6.32±0.3) verses pH (8.32±0.3) has been also analyzed by using SPSS statical software. The current investigation recommends that Dimethoate 30% EC is highly toxic to a freshwater fish, *Heteropneustes fossilis* (Bloch) at both the experimental pH strength but it is more toxic at pH 6.32±0.3 since LC_{50} values were recorded too low.

Key words: Acute Toxicity · LC_{50} Value · Dimethoate 30% EC and *Heteropneustes Fossilis* (Bloch)

INTRODUCTION

Pollution in aquatic bodies is mainly due to widespread use of synthetic pesticides in agriculture and public health programme [1]. Since due to less persistent and more effectiveness, the organophosphate insecticide are preferred in agriculture, households and public health programme for controlling various kind of pest instead of organochloride which exhibits high environmental persistent [2]. This enhanced the concentration of organophosphate insecticide in aquatic ecosystem which causes the acute and chronic toxicity to the itchthyofaunal species has been reported [3-5].

Dimethoate is an organophosphate insecticide, found to be highly toxic to fish and other aquatic invertebrates. It is used for controlling a variety of insect's pests viz; a mite, aphids, flies and plant hoppers and also reported as inhibitor of acetylcholine cholinesterase enzyme activity which causes nerve damage and finally death [6]. Dimethoate is biodegradable insecticide and its rapid degradation occurs in the environment and; in sewage treatment plants [7]. It has been reported that the activity of acetylcholinesterase enzyme is directly inhibited by organophosphate insecticide in fishes and invertebrates [8-10]. Aquatic Contamination by pesticide causes acute and chronic toxicity to the fish and other organism [11] which leads to severe damage to its vital organs [3, 12]. Therefore, an attempt has been made to assess the acute toxicity (LC_{50} values) of Dimethoate 30% EC to a freshwater fish, *Heteropneustes fossilis* (Bloch) at two different selected levels of pH (8.32±0.3 and 6.32±0.3) for 96 hours.

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MATERIALS AND METHODS

Experimental Fish: Approximately equal sizes (5.0±0.4 cm) of healthy fishes, *Heteropneustes fossilis* (Bloch) were collected from local water sources, acclimatized separately in tank (500 liters capacity) for 15 days, were selected for the experimental routine bioassay tests.

Stock Solution: Stock solution were prepared for Dimethoate 30% EC by applying formula of $N_1V_1 = N_2V_2$, Where, N_1 = Concentration of available pesticide, V_1 = Volume of available pesticide, N_2 = Required concentration of pesticide to be prepared, V_2 = Volume of solution required for application. Different concentrations (in ppm) of toxicant solution were prepared by adding the stock solution into the measured diluents water with the help of micropipette. The series of different concentrations of toxicant solution applied in the full scale bioassay tests were based on the progressive bisection of intervals on logarithmic scales [13]. The full scale static bioassay test (up to 96 hrs) for selected toxicant to the experimental fish, *Heteropneustes fossilis* (Bloch) were runs separately in test container (five liter water capacity), filled with three litre of toxicant solution, containing two different selected levels of pH, 8.32±0.3 and 6.32±0.3. The tap water of pH, 8.32±0.3 and water of pH strength, 6.32±0.3 (prepared by adding HCl solution with the help of micropipette) were utilized as experimental water during course of bioassay.

Preliminary or Screening Tests: The test concentrations range of Dimethoate 30% EC was taken between the lowest and highest concentrations at which most of the test fishes died or survived within a specified period of exposure, i.e. 24, 48, 72 and 96 hrs, since it provide a clues for the full scale bioassay.

Full Scale Bioassay Test: The test container with five liter water capacity, filled with three litre toxicant solution were placed in three rows and each container was labeled with the details of the experiment *viz*; date and time of the experiment, pH strength, concentration, replicate number. The acclimatized experimental fish, *Heteropneustes fossilis* (Bloch) of approximately equal sizes (5.0±0.4 cm) were added to these containers after about 30 minutes of the preparation of toxicant solutions. The bioassays for test fishes were carried out for Dimethoate 30% EC separately at two different selected level of pH strength (i.e. 8.32±0.3 and 6.32±0.3). There are ten acclimatized test fishes were transferred to each experimental test containers (filled with three litre toxicant solution) and a controls were also run simultaneously for the specified period of exposure i.e. 24, 48, 72 and 96 hrs. Fishes were not provided any food items during course of bioassay [14, 15]. The test solutions were replaced with renewed and fresh toxicant solutions subsequently after each 24 hrs and the experiments were continued for 96 hrs. The number of test fishes died in each concentration of toxicant solution was removed carefully at the time intervals of 24, 48, 72 and 96 hrs. The LC_{50} values and the 95 per cent confidence limits for different concentration and time intervals (24, 48, 72 and 96 hrs) for Dimethoate 30% EC were calculated by Probit analysis statistical software methods [16]. The presumable safe or harmless and safe dischargeable concentrations of selected organophosphates for experimental fishes, *Heteropneustes fossilis* (Bloch) were analyzed by using the formula of Hart *et al*. [17]. The change in behavioural response in the studied fishes was also documented carefully during the full scale bioassay.

Dissolve Oxygen (DO) Content: Dissolve oxygen (DO) content (ppm) of the toxicant solution were measured at the interval of 0, 24 and 96 hrs by Winkler Method.

Statistical Analysis: The data's has been subjected to SPSS statical software (Version 16) to analyse the LC_{50} values, Upper and Lower Confidence Limits and One way ANOVA.

RESULTS AND DISCUSSION

The series of test concentrations of Dimethoate 30% EC (in ppm) used in full scale static bioassays (96 hr) for a freshwater fish, *H. fossilis* (Bloch) at two different selected levels of pH (8.32±0.3 and 6.32±0.3) were based on the progressive bisection of intervals on logarithmic scales, described in APHA [13] (Table 1). The 24, 48, 72 and 96 hrs LC_{50} Value and; Confidence Ratio (R=UCL/LCL) of Dimethoate 30% EC to *H. fossilis* (Bloch) were documented as 38.445, 33.803, 27.813 and 25.933 ppm and; 3.004, 1.810, 1.383 and 1.332 respectively at pH of 8.32±0.3 (Table 2), whereas at pH strength 6.32±0.3 these values were analyzed as 28.045, 24.420, 22.261 and 19.109 ppm and; 2.281, 1.571, 1.416 and 1.290 respectively (Table 3).

Table 1: Test concentrations of Dimethoate 30% EC (in ppm) used in full scale static bioassays (96 hr) for a freshwater fish, *H. fossilis* (Bloch) at two selected levels of pH (8.32±0.3) and (6.32±0.3)

	pH (8.32±0.3)	pH (6.32±0.3)
S. No.	Dimethoate 30% EC (ppm)	Dimethoate 30% EC (ppm)
1	37.0	28.0
2	32.0	24.0
3	28.0	21.0
4	24.0	18.0
5	21.0	15.5

* Based on logarithmic scale (APHA, 2005)

Table 2: Median lethal concentrations (LC_{50} Value) of Dimethoate 30% EC (in ppm) for 24, 48, 72 and 96 hrs to *H. fossilis* (Bloch) at pH of 8.32±0.3.

Duration (hrs)	Dimethoate 30% EC (ppm)	LCL	UCL	R
24	38.445	32.685	98.192	3.004
48	33.803	28.837	52.222	1.810
72	27.813	23.246	32.164	1.383
96	25.933	21.704	28.923	1.332

UCL = Upper Confidence Limits; LCL = Lower Confidence limits; and
R = Confidence Ratio (UCL/LCL)

Table 3: Median lethal concentrations (LC_{50} Value) of Dimethoate 30% EC (in ppm) for 24, 48, 72 and 96 hrs to *H. fossilis* (Bloch) at pH of 6.32±0.3.

Duration (hrs)	Dimethoate 30% EC (ppm)	LCL	UCL	R
24	28.045	24.082	54.945	2.281
48	24.420	21.081	33.123	1.571
72	22.261	18.958	26.850	1.416
96	19.109	16.375	21.126	1.290

UCL = Upper Confidence Limits; LCL = Lower Confidence limits; and
R = Confidence Ratio (UCL/LCL)

Table 4: Safe or harmless and safe dischargeable concentrations of Dimethoate 30% EC (in ppm) for *H. fossilis* (Bloch) at pH of 8.32±0.3and 6.32±0.3.

Concentrations	pH 8.32±0.3	pH 6.32±0.3
Safe or harmless (as ppm)	8.9164	6.3751
Safe dischargeable (as ppm)	1.0664	1.0716

Table 5: Dissolve oxygen (DO) content (ppm) and percentage (%) alteration (after 96 hrs) during the course of bioassay test.

	Dissolve oxygen (DO) content (ppm)			
Concentration of Dimethoate 30% EC	0 hrs	24 hrs	96 hrs	Percentage (%) Alteration (After 96 hrs)
Control (0.00 ppm)	7.40	7.30	7.00	5.40
15.5 ppm (Highest Concentration at pH 6.32±0.3)	7.40	6.90	6.15	16.89
21.0 ppm (Highest Concentration at pH 8.32±0.3)	7.40	6.80	6.10	17.56

Table 6: One way ANOVA (Group analysis) between Median lethal concentrations (LC_{50} Value) of Dimethoate 30% EC (in ppm) for 24, 48, 72 & 96 hrs to to *H. fossilis* (Bloch) at (a) pH (8.32±0.3) verses and pH (6.32±0.3) and (b) pH (6.32±0.3) verses pH (8.32±0.3)

One way ANOVA	Sum of Squares (SS)	df	Mean Square (MS)
Between Groups	98.122	3	32.707
Within Groups	0.000	0	
Total	98.122	3	

Table 6(a):

One way ANOVA	Sum of Squares (SS)	df	Mean Square (MS)
Between Groups	42.313	3	14.104
Within Groups	0. 000	0	
Total	42.313	3	

Table 6(b):

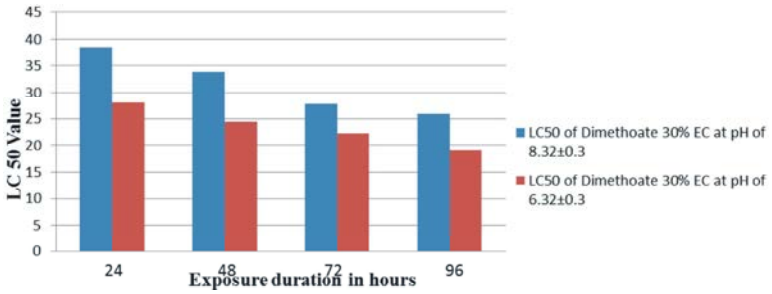

Fig. 1: Comparison of Median lethal concentrations (LC_{50} Value) of Dimethoate 30% EC (in ppm) for 24, 48, 72 and 96 hrs to *H. fossilis* (Bloch) at pH of 8.32±0.3 and 6.32±0.3

The safe dischargeable concentrations were estimated to be 1.0664 and 1.0716 ppm; whereas safe or harmless concentrations were noticed as 8.9164 and 6.3751 ppm at the pH of 8.32±0.3 and 6.32±0.3 respectively (Table 4). Dissolve oxygen (DO) content for control were measured as 7.40, 7.30 and 7.00 ppm in the time interval of 0, 24 and 96 hrs and; its percentage (%) alteration (after 96 hrs) were estimated as 5.40. Dissolve oxygen content and percentage (%) alteration (after 96 hrs) were also measured as 7.40, 6.90 and 6.15 ppm and; 16.89 in the time interval of 0, 24 and 96 hrs for 15.5 ppm (Highest Concentration at pH 6.32±0.3), however these values were noticed as 7.40, 6.80 and 6.10 ppm and; 17.56 for the highest Concentration (21.0 ppm) at pH 8.32±0.3 (Table 5).

One way ANOVA (Group analysis) between Median lethal concentrations (LC_{50} Value) of Dimethoate 30% EC (in ppm) for 24, 48, 72 & 96 hrs to *H. fossilis* (Bloch) for the pH 8.32±0.3 verses and pH 6.32±0.3 and pH 6.32±0.3 verses pH 8.32±0.3 has been analyzed by SPSS software (16.0).Sum of Squares (SS) and Mean Square (MS) values between the Groups were analyzed as 98.122 and 32.707 for pH 8.32±0.3 verses pH 6.32±0.3, however these value between the Groups were estimated as 42.313 and 14.104 for the pH 6.32±0.3 verses pH 8.32±0.3 (Table 6 a and b).

In the current investigation, 96 hrs LC_{50} Value were analyzed as 25.933 (at pH 8.32±0.3, Table 2) and 19.109 ppm (at pH 6.32±0.3, Table 3) for Dimethoate 30% EC to a freshwater fish, *H. fossilis* (Bloch). Whereas, the 96-hour LC_{50} value for Dimethoate to rainbow trout was reported as 6.2 ug/l and 48-hour LC_{50} value to Daphnia magna (a small freshwater crustacean) was found to be 2.5 ug/l [7]. The LC_{50} value for Dimethoate was also reported in the range from 40 to 60 mg/l in mosquito fish [18]. The 96 hrs LC_{50} values of Dimethoate were estimated as 65 mg/L in air-breathing teleosts, *Clarias batrachus* [19]. A very high LC value of dimethoate was reported as 26.11mg/L for 96 hrs in fry (size 20 - 34 mm) of *Cyprinus carpio* [20]. But, the LC_{50} value were noticed as 1.84, 1.78, 1.68 and 1.61 mg/L for 24, 48, 72 and 96 hrs respectively to the fingerlings of common carp (*Cyprinus carpio*), exposed to dimethoate [21]. The 96 h LC_{50} value of Dimethoate was found to be 21.64 µg/l for fingerlings and 60.00 µg/l for adults Zebrafish, *Danio rerio* [22], whereas these value were reported in the juvenile of *Cyprinus carpio* var. *communis* as 1.1 ppm in static bioassay system by Probit analysis method for dimethoate [23]. Previous report for the other organophosphates like Chlorpyriphos 20% EC, the 96 hr LC_{50} value were also cited to juveniles, males, females and mixed population of *Poecilia reticulata* (Peters, 1859) as 7.009, 14.575, 130.777 and 51.924 ppb [24], whereas for combined formulation of organophosphates and pyrethroid based insecticide (Chlorpyriphos 50% + Cypermethrin 5% EC), the 96 hr LC_{50} value were estimated to be 13.396, 18.845, 261.866 and 106.255 ppb respectively [25]. The malondialdehyde (MDA) and catalase (CAT) in the liver and kidney and; alkaline phosphatase (ALP) and catalase (CAT) in gill were found to be increased but lactate dehydrogenase (LDH), glucose 6-phsphate dehydrogenase (G6PDH) and glycogen were reported to be decreased in liver of a common carp, *Cyprinus carpio,* exposed to 16 and 32 μg/l of dimethoate for 14 days [26]. The 96 hrs LC_{50} values for Dimethoate 30% EC were noticed as 12.545 and 10.673 ppm at pH 8.74±0.3 and 6.74±0.3 respectively, but these value were documented as 18.259+6.633 and14.20+4.510 ppm for combination of two organophosphates (Monocrotophos 36% SL+Dimethoate 30% EC) at the pH 8.74±0.3 and 6.74±0.3 respectively to the females of a freshwater fish, *Poecilia reticulata* [27]. Thus, the LC_{50} value noticed in present investigation are also corroborating the literature cited by previous authors, since LC_{50} value exhibits variation for the same species and toxicant depending on age, size and condition of test species along with various experimental factor [28]. The safe dischargeable concentrations were analyzed as 1.0664 and 1.0716 ppm,

whereas safe or harmless concentrations were reported to be 8.9164 and 6.3751 ppm at the pH of 8.32±0.3 and 6.32±0.3 respectively (Table 4). The Safe dischargeable concentrations were found to be very low as compared to safe or harmless concentrations, which are in agreement to the findings of previous researchers.

Dissolve oxygen content (ppm) were found to be significantly decline in the time interval of 0, 24 and 96 hrs for the highest concentration of Dimethoate at both the selected pH strength (Table 5). Total consumption of Dissolve oxygen has not been observed, it may be due to shifting to aerial respiration of an air breathing fish, *H. fossilis* (Bloch) under stress condition. It has been reported that the hypoxic condition is created mainly due to damage of gills in pesticide exposed test fish which reduced the oxygen uptake [3] and it also bring about to increase surfacing [29]. There are a more or less similar patterns of the results has been observed during present investigation in context of Dissolve oxygen content as reported by Pandey *et al*. [5] and Singh *et al*. [21]. In current investigation, Dimethoate exposed test fishes exhibits abnormalities in behavior such as abundant mucus secretion around the body, restlessness, erratic and jerky movements, more excitement and try to jump out of test container, increase in opercular movement, settled down on bottom after death with belly upside. Approximately, a similar pattern of behavioral response has been observed in other organophosphate exposed fishes [5, 12, 23, 24, 27, 30]. Thus, Dimethoate is highly toxic to a freshwater air breathing fish, *Heteropneustes fossilis* (Bloch) which alter its behavioral response under stress condition.

CONCLUSIONS

It has been concluded that *Heteropneustes fossilis* (Bloch) is highly sensitive to Dimethoate 30% EC at both the selected pH strength as it alter the Dissolve oxygen content in water and behavioral response of the exposed fishes. Therefore, it should be used in controlled way to manage the health of aquatic fauna, particularly fishes during aquaculture, worldwide.

REFERENCES

1. Cope, W.G., R.B. Leidy and E. Hodgson, 2004. A Text Book of Modern Toxicology. 3rd Edition, John Wiley and Sons Publication, New Jersey, USA.
2. Oruc, O.E., N. Uner, Y. Sevgiler, D. Usta and H. Durmaz, 2006. Sublethal effects of organophosphate diazinon on the brain of Cyprinus carpio. Drug and Chemical Toxicology, 29(1): 57-67.
3. Velmurugan, B., M. Selvanayagam, E.I. Cengiz and E. Unlu, 2007. The effects of monocrotophos to different tissues of freshwater fish Cirrhinus mrigala. Bulletin of Environmental Contamination and Toxicology, 78(6): 450-454.
4. Aker, W.G., X. Hu, P. Wang and H.M. Hwang, 2008. Comparing the relative toxicity of malathion and malaoxon in blue catfish Ictalurus furcatus. Environmental Toxicology, 23(4): 548-554.
5. Pandey, R.K., R.N. Singh, S. Singh, N.N. Singh and V.K. Das, 2009. Acute toxicity bioassay of dimethoate on freshwater airbreathing catfish, Heteropneustes fossilis (Bloch). Journal of Environmental Biology, 30(3): 437-440.
6. Van Scoy, A., A. Pennell and X. Zhang, 2016. Environmental fate and toxicology of Dimethoate. Reviews of Environmental Contamination and Toxicology, 237: 53-70.
7. Cheminova Agro, A.S., 1991. Material Safety Data Sheet: Dimethoate. Cheminova, Lemvig, Denmark. (June 11).
8. Fulton, M.H. and P.B. Key, 2001. Acetylcholinesterase inhibition in estuarine fish and invertebrates as an indicator of organophosphorus insecticide exposure and effects. Environmental Toxicology and Chemistry, 20(1): 37-45.
9. Rao, J.V., G. Begum, V. Sridhar and N.C. Reddy, 2005. Sublethal effects of monocrotophos on locomotor behavior and gill architecture of the mosquito fish, Gambusia affinis. Journal of Environmental Science and Health B, 40(6): 813-825.
10. Agrahari, S., K. Gopal and K.C. Pandey, 2006.Biomarkers of monocrotophos in a fresh water fish Channa punctatus (Bloch). Journal of Environmental Biology, 27: 453-457.
11. Heger, W., S.J. Jung, S. Martin and H. Peter, 1995. Acute and prolonged toxicity to aquatic organism of new and existing chemicals and pesticides. Chemosphere, 31(2): 2707-26.
12. Omitoyin, B.O., E.K. Ajani, B.T. Adesina and C.N.F. Okuagu, 2006. Toxicity of Lindane (Gamma Hexachloro-CycloHexane) to Clarias gariepinu (Burchell 1822). World Journal of Zoology, 1(1): 57-63.

13. American Public Health Association (APHA), 2005. American Water Works Association (AWWA) and Water Pollution Control Federation (WPCF), Sandard methods for the examination of water and wastewater, 21st Edn, American Public Health Association, Washington D.C.
14. Ward, G.S. and P.R. Parrish, 1982. Toxicity tests. In: Manual of methods in aquatic environment research part 6. FAO. Fish. Tech. Pap., 185: 1-23.
15. Reish, D.J. and P.S. Oshida, 1987. Short term bioassay. In: Manual of methods in aquatic environment research part 6. FAO. Fish. Tech. Pap., 247: 1-62.
16. Finney, D.J., 1971. Probit Analysis, University Press, Cambridge, pp: 333.
17. Hart, W.B., P. Doudoroff and J. Greenbank, 1945. The evaluation of the toxicity of industrial wastes, chemical and other substances to freshwater fishes. Atlantic Refining Co. (Phill), pp: 317.
18. Meister, R.T., 1992. Farm Chemicals Handbook '92. Meister Publishing Company. Willoughby, OH.
19. Begum, G. and S. Vijayaraghavan, 1995. *In vivo* toxicity of dimethoate on protein and transaminase in the liver tissue of freshwater fish Clarias batrachus (Linn). Bulletin of Environmental Contamination and Toxicology, 54: 370-375.
20. De Mel, G.W.J. L.M.V.T.M. and A. Pathiratne, 2005. Toxicity assessment of insecticides commonly used in rice pest management to the fry of common carp, Cyprinus carpio, a food fish culturable in rice fields. Journal of Applied Ichthyology, 21: 146-150.
21. Singh, R.N., R.K. Pandey, N.N. Singh and V.K. Das, 2009. Acute Toxicity and Behavioral Responses of Common Carp, Cyprinus carpio (Linn.) To an Organophosphate (Dimethoate).World Journal of Zoology, 4(2): 70-75.
22. Ansari, S. and B.A. Ansari, 2011. Embryo and Fingerling Toxicity of Dimethoate and Effect on Fecundity, Viability, Hatchability and Survival of Zebrafish, Danio rerio (Cyprinidae).World Journal of Fish and Marine Sciences, 3(2): 167-173.
23. Qayoom, I., F.A. Shah, M. Mukhtar, M.H. Balkhi, F.A. Bhat and B.A. Bhat, 2016. Dimethoate Induced Behavioural Changes in Juveniles of Cyprinus carpio var. communis under Temperate Conditions of Kashmir, India. Scientific World Journal, 16: 1-6.
24. Wast, N., K. Tiwari, A.K. Gupta, M.M. Prakash and S. Gaherwal, 2015. Assessment of Acute Toxicity of Chlorpyriphos 20% EC to the Guppy, Poecilia reticulata (Peters, 1859). Global Veterinaria, 14(2): 239-243.
25. Wast, N., A.K. Gupta, M.M. Prakash and S. Gaherwal, 2014. Acute Toxicity of Chlorpyriphos 50% + Cypermethrin 5% EC to the Guppy, Poecilia reticulata (Peters, 1859). Global Veterinaria, 12(3): 393-398.
26. Shadegan, M.R. and M. Banaee, 2018. Effects of dimethoate alone and in combination with Bacilar fertilizer on oxidative stress in common carp, Cyprinus carpio. Chemosphere, 208: 101-107.
27. Prakash, M.M., R. Rajput, N. Wast and R. Verma, 2020. Comparative Assessment of Acute Toxicity of Two Organophosphates (Monocrotophos 36% SL and Dimethoate 30% EC) to a Freshwater Fish, Poecilia reticulata (Peters) at Two Different Selected Level of pH. World Applied Sciences Journal, 38(2): 153-161.
28. Sprague, J.B., 1969. Measurement of pollutant toxicity to fish I: Bioassay methods for acute toxicity. Water Research, 3(11): 793-821.
29. Radhaiah, V. and K. Jayantha Rao, 1998. Behavioral responses of fish, Tilapia mossambica exposed to fenvalerate. Environment Ecology, 6(2): 2-23.
30. Johal, M.S., M.L. Sharma and Ravneet, 2007. Impact of low dose of organophosphate, monocrotophos on the epithelial cells of gills of Cyprinus carpio communis Linn. - SEM study. Journal of Environmental Biology, 28(3): 663-667.