Original Article

Investigating the Agent of Temperature into Acute Toxicity (LC_{50} 96h) of Edifenphos in *Rutilus Frisii Kutum* (Kamensky, 1901)

Saeid Shahbazi Naserabad[1], Alireza Mirvaghefi [2], Ghasem Rashidiyan [3], Narges Rostamian [4], Hamed Ghafari Farsani [5]*

Received: 01.11.2016 *Accepted: 04.12.2016*

ABSTRACT

Background: Edifenphos, a kind of organophosphate toxins, is used as agricultural fungicides in rice fields. This study was aimed to investigate the effect of temperature on lethal concentration of exposure to edifenphos on *Rutilus frisii kutum* (Caspian kutum).

Methods: The experiment was carried out in static condition and based on instructions of OECD within 10 d under controlled water physicochemical factors. Dissolved oxygen was fixed on 7-7.5 ppm, pH: 7 to 7.5 and hardness: 200 ppm. Fish were acclimatized in 70*40*30 cm aquarium for 10 d before the test. Treated aquariums with concentrations of 0.01, 0.05, 2, 4, 8, 16 ppm of edifenphos with one control group (no toxic concentration), were performed. In order to test the effect of temperature on acute toxicity, three ranges of 15±1, 20±1 and 25±1 ºC were treated and L_{C1}, LC_{10}, LC_{30}, LC_{50}, LC_{70}, LC_{90} and LC_{99} were calculated for 24, 48, 72 and 96 h. The study was carried out in Laboratory of Aquaculture and Fisheries, University of Tehran in 2016.

Results: LC_{50} value in 25 ºC was lower than 20 and 15 ºC. LC_{50} 96h edifenphos for Caspian kutum in 15±1, 20±1 and 25±1 ºC was 3.70, 3.61 and 3.26, respectively.

Conclusion: Higher temperature increase toxicity rate of edifenphos and the toxin had a positive temperature coefficient on Caspian kutum.

Keywords: Caspian Kutum, Edifenphos, Organophosphorus Fungicide, Lethal Concentration, Temperature.

IJT 2017 (2): 39-44

INTRODUCTION

Application of pesticides in agriculture in order to increase both quality and quantity of food products can directly or indirectly cause contamination in water resources. Indeed, waterborne pesticides are used in farms, orchards and rice paddies; washing into freshwater resources, particularly rivers, after rainfall washed the soil [1, 2]. These contaminants have threatened important ecosystems' survival throughout disturbing ecological relations between organisms and reducing biodiversity. Additionally, they have more adverse effects on non-target animals like fishes rather than target animals (pests) [3], which leads in fish's higher sensitivity and faster mortality rate [4].

Extensive application of agricultural pesticides in Northern provinces of Iran's rice fields and orchards has increased levels of contamination in different kind of aquatic ecosystems including rivers running to the Caspian Sea and the amount of contaminant in estuary has increased consequently [5]. Among them, edifenphos, is a member of organophosphate toxins and used as fungicides in rice agricultural fields in order to eradication rice blast disease (*Pyricularia oryzae*) in soluble form (also known by [O-ethyl S, S-diphenyl phosphorodithioate] and chemical formula C14H15O2PS2) [6, 7]. Organophosphates fungicides poison animals primarily by phosphorylation of acetylcholinesterase enzyme (AChE) at nerve endings, and the results are curb the activity of nervous system [8, 9].

Although, edifenphos is a frequent fungicide, limited data is available on the toxicity of this compound on aquatic animals. The depressive effect of edifenphos is stated on the

1. MSc of Aquatic Ecology, Young Researchers and Elite Club,Yasooj Branch, Islamic Azad University, Yasooj, Iran.
2. Department of Fisheries, University of Tehran, Karaj, Iran.
3. PhD Candidate of Fisheries Aquaculture, Young Researchers and Elites Club, Science and Research Branch, Islamic Azad University, Tehran, Iran.
4. MSc.of Aquatic Ecology, Department of Fisheries, College of Environment Karaj, Karaj, Iran.
5. PhD Candidate of Aquaculture Young Researchers and Elite Club, Shahrekord Branch, Islamic Azad University, Shahrekord, Iran.
*Corresponding Author: E-mail: saeid.shahbazi@alumni.ut.ac.ir

activities of acetylcholinesterase (AChE), adenosine triphosphatase (ATPase) and glutathione-S-transferase (GST) with the elevation of catalase activity in tissues of *O. niloticus* [10]. In addition, edifenphos even at sublethal dose can be considered as a strong toxic pollutant to *O. niloticus* in which it may cause toxicity and mortality for both rice and fish [11].

Caspian kutum (*Rutilus frisii kutum* Kamensky, 1901) from Cyprinidae family only exists in Caspian Sea and some leading rivers to that, but the main habitat is southern and following basin, especially Iranian coasts [12, 13]. This fish due to its tasteful meat and market demands has a huge economic value. This anadromous fish, after sexual maturation gets into leading rivers to Caspian Sea in order to spawn, annually [12, 13]. However, the migration rate has decreased due to aquatic contamination and just a few rivers has used for spawning [12].

On the other hand, more than 200 million fishes (one to two gr) are released to the Caspian Sea, by Iran Fisheries Organization, in order to manage fish resource, annually [13]. Furthermore, Caspian kutum from different ages and weights are exposed to the pesticides entered into aquatic bodies. Sensitivity of various fish species is different on toxic substances, Therefore, toxicology tests are needed for different fish species [14]. For this purpose, LC_{50} 96 h of each ecotoxicology studies is required. Temperature is one of the limitation factors for fish growth, which is affecting the metabolic rate and growth control physiologic mechanisms [15, 16]. Changes in the physical properties of contaminants including pesticides, affecting detoxification rate and biochemical processes of the exposed animals to contaminant are the effects of temperature in the aquatic ecosystems that play a crucial role of life availability and contaminant toxicity [17, 18]. Fish are directly exposed to aquatic environments, so they are sensitive in term of water quality changes both physical and chemical. Thus, the presence of contaminations in different physicochemical conditions like water temperature play a crucial role in level of contaminants toxicity and fish’s tensions and stress. However, the mechanism effect of the temperature for various species and toxicant is different.

The present study was conducted to determine the acute toxicity of the edifenphos in different temperature to *R. frisii kutum*. Finding of this study could be useful in aquatic managements systems especially for fish species.

MATERIALS AND METHODS

Overall, 200 live specimens of *R. frisii kutum* were obtained from Shahid Rajaee Center, Sari, Iran in June 2016. Samples weighted 3±1 gr and acclimatized in 70*40*30 cm aquarium for 10 d. In order to measure biological capability and determine survival, fishes were kept in natural and toxin-free environment to determine natural mortality. Dissolved oxygen was fixed on 7-7.5 ppm, pH: 7 to 7.5 and hardness: 200 ppm. To test the effect of temperature on acute toxicity, three ranges of 15±1, 20±1 and 25±1 ºC were treated. Fish were fed twice daily with Biomar feed at 2% body weight, before the test. Faeces were siphoned every day and toxicant concentration was adjusted after. All experiments were performed 16 h light and 8 h darkness. Fish behavior and clinical signs were recorded.

Static acute toxicity test was performed following guideline the OECD standard method [19]. Six treated aquariums with concentration of 0.01, 0.05, 2, 4, 8, 16 ppm of edifenphos with one control group (no toxic concentration), were performed. Mortality rates were recorded after 24, 48, 72 and 96 h and dead fishes were quickly removed from the aquarium. The nominal concentration of toxin causing mortality (LC_1 ‹LC_{10} ‹ LC_{30} ‹LC_{50} ‹LC_{70} ‹LC_{90} and LC_{99}) within 24, 48, 72 and 96 h for each toxin was calculated separately. LC_{50} values for 24, 48, 72 and 96 h exposures were computed on the basis of probity analysis version 16/0 [14].

RESULTS

No mortality was observed during acclimation period. The results indicated that mortality increase as temperature increase in which LC_{50} determined as 3.70 ppm in 15±1ºC (Table 1, 2), 3.61 ppm in 20±1ºC (Table 3, 4) and 3.26 ppm in 25±1ºC (Table 5, 6). Higher temperature increased toxicity rate of edifenphos and the toxin had a positive temperature coefficient. However, overlay the results indicated that mortality in each three temperature ranges will increase by increasing in toxin concentration and exposure period, though *Rutilus frisii kutum* mortality increased significantly. In other words, the less concentration of toxin is needed to reach 50 percent mortality by longer exposure period. It means that an LC_{50} value in the first 24 h of the experiment always was higher than LC_{50} at 96 h.

Table 1. Cumulative mortality of *Rutilus frisii kutum* (n=30, each concentration) exposed to acute edifenphos (Temperature=15).

Concentration of edifenphos (ppm)	Mortality (No.)			
	24h	48h	72h	96h
0.00	0	0	0	0
1.00	0	0	0	0
2.00	0	2	2	10
4.00	2	4	8	14
8.00	30	30	30	30
16.00	30	30	30	30

Table 2. LC_{10}, LC_{30}, LC_{50}, LC_{70}, LC_{90} and LC_{99} values of edifenphos for *Rutilus frisii kutum* in 15 °C.

Point	Concentration (ppm) (95% of confidence limits)			
	24h	48h	72h	96h
LC_1	3.37 ± 0.33	1.67 ± 0.11	1.32 ± 0.12	-0.32 ± 0.92
LC_{10}	4.31 ± 0.33	3.20 ± 0.11	2.82 ± 0.12	1.48 ± 0.92
LC_{30}	4.98 ± 0.33	4.30 ± 0.11	3.91 ± 0.12	2.79 ± 0.92
LC_{50}	**5.45 ± 0.33**	**5.07 ± 0.11**	**4.66 ± 0.12**	**3.70 ± 0.92**
LC_{70}	5.92 ± 0.33	5.83 ± 0.11	5.41 ± 0.12	4.61 ± 0.92
LC_{90}	6.59 ± 0.33	6.94 ± 0.11	6.50 ± 0.12	5.93 ± 0.92
LC_{99}	7.52 ± 0.33	8.46 ± 0.11	8.00 ± 0.12	7.74 ± 0.92

Table 3. Cumulative mortality of *Rutilus frisii kutum* (n=30, each concentration) exposed to acute edifenphos (Temperature=20).

Concentration of Edifenphos (ppm)	Mortality (No.)			
	24h	48h	72h	96h
0.00	0	0	0	0
1.00	0	0	0	0
2.00	0	2	2	10
4.00	2	4	9	15
8.00	30	30	30	30
16.00	30	30	30	30

Table 4. LC_{10}, LC_{30}, LC_{50}, LC_{70}, LC_{90} and LC_{99} values of edifenphos for *Rutilus frisii kutum* in 20 °C.

Point	Concentration (ppm) (95% of confidence limits)			
	24h	48h	72h	96h
LC_1	3.37 ± 0.33	1.67 ± 0.11	1.26 ± 0.12	-0.29 ± 0.09
LC_{10}	4.31 ± 0.33	3.20 ± 0.11	2.74 ± 0.12	1.46 ± 0.09
LC_{30}	4.98 ± 0.33	4.30 ± 0.11	3.82 ± 0.12	2.73 ± 0.09
LC_{50}	**5.45 ± 0.33**	**5.07 ± 0.11**	**4.56 ± 0.12**	**3.61 ± 0.09**
LC_{70}	5.92 ± 0.33	5.83 ± 0.11	5.30 ± 0.12	4.50 ± 0.09
LC_{90}	6.59 ± 0.33	6.94 ± 0.11	6.37 ± 0.12	5.77 ± 0.09
LC_{99}	7.52 ± 0.33	8.46 ± 0.11	7.85 ± 0.12	7.53 ± 0.09

Table 5. Cumulative mortality of *Rutilus frisii kutum* (n=30, each concentration) exposed to acute edifenphos (Temperature=25).

Concentration of edifenphos (ppm)	Mortality (No.)			
	24h	48h	72h	96h
0.00	0	0	0	0
1.00	0	0	0	0
2.00	0	2	3	12
4.00	2	5	10	18
8.00	30	30	30	30
16.00	30	30	30	30

Table 6. LC_{10}, LC_{30}, LC_{50}, LC_{70}, LC_{90} and LC_{99} values of edifenphos for *Rutilus frisii kutum* in 25 °C.

Point	Concentration (ppm) (95% of confidence limits)			
	24h	**48h**	**72h**	**96h**
LC_1	3.37 ± 0.33	1.56 ± 0.11	0.93 ± 0.11	-0.38 ± 0.10
LC_{10}	4.31 ± 0.33	3.09 ± 0.11	2.50 ± 0.11	1.28 ± 0.10
LC_{30}	4.98 ± 0.33	4.20 ± 0.11	3.63 ± 0.11	2.43 ± 0.10
LC_{50}	**5.45 ± 0.33**	**4.96 ± 0.11**	**4.42 ± 0.11**	**3.26 ± 0.10**
LC_{70}	5.92 ± 0.33	5.73 ± 0.11	5.20 ± 0.11	4.08 ± 0.10
LC_{90}	6.59 ± 0.33	6.83 ± 0.11	6.34 ± 0.11	5.26 ± 0.10
LC_{99}	7.52 ± 0.33	8.36 ± 0.11	7.90 ± 0.11	6.90 ± 0.10

Fish exposed to toxicant showed abnormal behavior as faster opercular activity, swimming erratically with jerky movements, protrusion of the eyes and bruise in the caudal fin. Exposed fish incurred curvature in vertebra and their gill pigmentation was decreased.

According to LC_{50} 96h Maximum Allowable Concentration (MAC) of edifenphos for *R. frisii kutum* in different temperature calculated as 0.37, 0.36 and 0.32 ppm in 15±1, 20±1 and 25±1 °C, respectively.

DISCUSSION

Exposure time is one of the effective factors of organophosphorus toxic ratio [20]. When fish are exposed to a constant concentration of the toxin, fish tolerance is diminishing over time and the toxin is more to affect [21]. However, when the toxin accumulates in fish tissue also increases the adverse effects on the body and thereby causes decreasing in LC_{50} 96 h. According to recent studies [15, 22, 23], LC_{50} decreased by passing time, for instance 5.45 ppm, 4.56 ppm and 3.61 recorded for 24 h, 72 h and 96 h respectively. Reported results from conducted studies to determine acute toxicity of deltamethrin and diazinon in common carp [23, 24], edifenphos and malathion in goldfish [22] and other similar research proof this descending amount of LC_{50} by time passage. In recent study, the LC_{50} 96 h of edifenphos for *R. frisii kutum* in 15, 20 and 25 °C calculated as 3.70, 3.61 and 3.26, respectively. The LC_{50} level will decrease with increase in the temperature and exposure time in case the least LC_{50} was related to 25 °C in fourth day after exposure. Thus, in higher temperature, Caspian kutum is more vulnerable and even lower concentrations of edifenphos can cause adverse effects.

Higher mortality in fishes exposed to chlorpyrifos and endosulfan by increasing in the temperature [25] and also higher mortality observed by increasing in the temperature when *Salmo gairdneri* exposed to mercury [15], *Argyrosomus regius* exposed to NO_3 [26] and also *Oncorhynchus mykiss* and bluegills exposed to roundup [27].

Temperature while affecting the toxicity of essentially all biological endpoints, from the molecular to whole animal level; simultaneously affects the effectiveness of the respiratory surface, which is resulting in respiratory impairment in ectothermic animals such as fish and may increase toxicity of chemical substance at high temperature [28]. Temperature effects on level of contamination and toxicity of contaminant are probably related to some factors including: chemical reactions rate, diffusion, active transition of toxins through cell membrane and metabolism [15]. For instance, higher temperature cause higher body metabolism and oxygen demand will increase consequently, thus higher oxygen demand imposes more toxin absorbance by gills [29].

Several studies including Bhadja and Vaghela [30] implied on the effect of temperature on toxicity of heavy metals such as cadmium, copper, zinc and lead but data on toxicity of organophosphorus in various temperatures are scarce. Increasing temperature, increases acute toxicity of $HgCl_2$ [31]. The increased sensitivity to heavy metals at higher temperature might be the result of increased metabolic activity, respiratory, and cardiac rates, coupled with temperature potentiation of metal ion action on cellular enzymes and cell membrane [32]. The toxicities of the chlorides of lithium and ammonium to *Carassius carassius*, *Pihnephales notatus* and *Notropis blennius* are increased with higher temperature [33]. The effect of temperature on the toxicities of the chlorides of lithium and ammonium does not follow Van't Hoff's rule overall.

Fish’s behavior is a good criterion of their reaction against environmental contaminants [34].

In fact, the behavior is a kind of selective response which is adoptive through continuous directly interaction with physicochemical, social conditions and physiological aspects of environment [34]. Therefore, changing in behavior is one of the most important indicators to show contaminants' toxicity potential in fishes [35]. From clinical signs aspect, respiratory disorders were observed in which operculum opened and closed faster, they had extreme movement and were unrest (inpatient) in which they became more sensitive toward external stimulants and uneven breathing was appeared. Frequent and erratic movements toward water surface might be due to pesticides effects on central nervous system and gills [8]. Higher concentration exposure of edifenphos intensified type, duration and rate of the above-mentioned actions, no changes in behavior and no mortality in the control treatment were observed during the experiment.

Various behaviors such as high locomotion and opercular activity, irregular swimming pattern and paralysis observed through exposure time in clinical test and increased by toxicant concentration. Such behavioral responses were reported by other researchers for different organophosphorus toxicants [36, 37]. In addition, these behavioral changes reported for *Carassius auratus* after exposure to edifenphos [22, 38].

CONCLUSION

Temperature can affect tolerance of fish by causing stress and duplicate the effect of toxicant (edifenphos). Acute toxicity of edifenphos in *R. frisii kutum* increases with higher levels of temperature. Further studies should be carried out on different toxicity of edifenphos in different stage of the life history of the fish, to investigate the ecological toxicity of edifenphos as a popular pesticide.

ACKNOWLEDGMENT

The authors are thankful to the members of Department of Fisheries, Faculty of Natural Resources, University of Tehran, for supporting materials and laboratory and supporting this research. The authors declare that there is no conflict of interests.

REFERENCES

1. Bouldin J, Farris J, Moore M, Smith Jr S, Cooper C. Assessment of diazinon toxicity in sediment and water of constructed wetlands using deployed *Corbicula fluminea* and laboratory testing. Arch Environ Arch Environ Contam Toxicol 2007;53(2):174-82.
2. Konstantinou IK, Hela DG, Albanis TA. The status of pesticide pollution in surface waters (rivers and lakes) of Greece. Part I. Review on occurrence and levels. Environ Pollut 2006;141(3):555-70.
3. Kolpin DW, Thurman EM, Lee EA, Meyer MT, Furlong ET, Glassmeyer ST. Urban contributions of glyphosate and its degradate AMPA to streams in the United States. Sci Total Environ 2006;354(2):191-7.
4. Kreutz LC, Barcellos LJG, de Faria Valle S, de Oliveira Silva T, Anziliero D, dos Santos ED, et al. Altered hematological and immunological parameters in silver catfish (*Rhamdia quelen*) following short term exposure to sublethal concentration of glyphosate. Fish Shellfish Immunol 2011;30(1):51-7.
5. Khanghah MM, Bozorgniya A. Destructive effects of Edifenphos fungicide on *Rutilus rutilus caspicus* gill. Int J Biosci 2014;5:27-33.
6. Wiebe MG, Robson GD, Trinci AP. Edifenphos (Hinosan) reduces hyphal extension, hyphal growth unit length and phosphatidylcholine content of *Fusarium graminearum* A3/5, but has no effect on specific growth rate. Microbiol 1990;136(6):979-84.
7. Chen TS, Kinoshita FK, DuBois KP. Acute toxicity and antiesterase action of O-ethyl-S, S-diphenyl phosphorodithioate (Hinosan®). Toxicol Appl Pharmacol 1972;23(3):519-27.
8. Axelrad J, Howard C, McLean W. Interactions between pesticides and components of pesticide formulations in an in vitro neurotoxicity test. Toxicol 2002;173(3):259-68.
9. Aluigi M, Angelini C, Falugi C, Fossa R, Genever P, Gallus L, et al. Interaction between organophosphate compounds and cholinergic functions during development. Chem-Biol Interact 2005;157:305-16.
10. El-Gendy K, Aly N, Saber N, El Sebae A. Toxicological Effects of Some Pesticides on *Tilapia Nilotica*. Alexandria Sci Exch J 1996;17:243-52.
11. Gaafar A, El-Manakhly E, Soliman M, Soufy H, Zaki M, Mohamed S, et al. Some pathological, biochemical and hematological investigations on Nile tilapia (*Oreochromis niloticus*) following chronic exposure to edifenphos pesticide. J Am Sci 2010;6(10):542-51.
12. Yousefian M, Mosavi H. Spawning of South Caspian Kutum(*Rutilus frisii kutum*) in Most Migratory River of South Caspian Sea. Asian J Anim Vet Adv 2008;3(6):437-42.

13. Enayat Gholampoor T, Imanpoor M, Shabanpoor B, Hosseini S. The Study of Growth Performance, Body Composition and Some Blood Parameters of *Rutilus frisii kutum* (Kamenskii, 1901) Fingerlings at Different Salinities. J Agric Sci Technol 2011;13:869-76.
14. Finney DJ. Probit analysis; a statistical treatment of the sigmoid response curve. Cambridge. 1971.
15. MacLeod J, Pessah E. Temperature effects on mercury accumulation, toxicity, and metabolic rate in rainbow trout (*Salmo gairdneri*). J Fish Res Board Can 1973;30(4):485-92.
16. García-Esquivel Z, Montes-Magallón S, González-Gómez MA. Effect of temperature and photoperiod on the growth, feed consumption, and biochemical content of juvenile green abalone, *Haliotis fulgens*, fed on a balanced diet. Aquaculture 2007;262(1):129-41.
17. Boone MD, Bridges CM. The effect of temperature on the potency of carbaryl for survival of tadpoles of the green frog (*Rana clamitans*). Environ Toxicol Chem 1999;18(7):1482-4.
18. Lau ETC, Karraker NE, Leung KMY. Temperature-dependent acute toxicity of methomyl pesticide on larvae of 3 Asian amphibian species. Environ Toxicol Chem 2015;34(10):2322-7.
19. OECD. OECD Guidelines for the Testing of Chemicals: Organization for Economic; 1994.
20. Larkin DJ, Tjeerdema RS. Fate and effects of diazinon. Rev Environ Contam Toxicol 2000;166:49-82.
21. Peña-Llopis S, Ferrando MD, Peña JB. Fish tolerance to organophosphate-induced oxidative stress is dependent on the glutathione metabolism and enhanced by N-acetylcysteine. Aquat Toxicol 2003;65(4):337-60.
22. Naserabad SS, Mirvaghefi A, Gerami MH, Ghafari H. Acute toxicity and behavioral changes of the gold fish (*Carassius auratus*) exposed to malathion and hinosan. Iran J Toxicol 2015;8(27): 1203-8.
23. Svoboda M, Luskova V, Drastichova J, Žlabek V. The effect of diazinon on haematological indices of common carp (*Cyprinus carpio* L.). Acta Vet Brno 2001;70(4):457-65.
24. Svobodova Z, Luskova V, Drastichova J, Svoboda M, Žlábek V. Effect of deltamethrin on haematological indices of common carp (*Cyprinus carpio* L.). Acta Vet Brno 2003;72(1):79-85.
25. Patra RW, Chapman JC, Lim RP, Gehrke PC. The effects of three organic chemicals on the upper thermal tolerances of four freshwater fishes. Environ Toxicol Chem 2007;26(7):1454-9.
26. Kir M, Topuz H, Sunar MC, Topuz M. Effect of Temperature on Acute Toxicity of Nitrite to Meagre, *Argyrosomus regius* (Asso, 1801). J World Aquacult Soc 2015;46(5):564-8.
27. Folmar LC, Sanders H, Julin A. Toxicity of the herbicide glyphosate and several of its formulations to fish and aquatic invertebrates. Arch Environ Contam Toxicol 1979;8(3):269-78.
28. Gordon CJ. Temperature and toxicology: an integrative, comparative, and environmental approach: CRC press; 2005.
29. Masud S, Singh I. Temperature dependent toxicity and behavioural responses in the freshwater fish *Cyprinus carpio* exposed to pyrethroid pesticide, cypermethrin. J Environ Sci Water Resour 2013;2(10):375-81.
30. Bhadja P, Vaghela A. Effect of temperature on the toxicity of some metals to *Labeo bata* (Hamilton, 1822). Int J Adv Life Sci 2013; 6(3): 252-4.
31. Rathore RS, Khangarot B. Effects of temperature on the sensitivity of sludge worm Tubifex tubifex Müller to selected heavy metals. Ecotoxicol Environ Saf 2002;53(1):27-36.
32. Shimaila AMA. Effect of Some Additives on the Toxicity of Certain Rodenticides against White Rat Fed on Different Diets. [Ph.D. Thesis] High Institute of Public Health, Alexandria University, Egypt, 1989.
33. Powers EB. Influence of Temperature and Concentration on the Toxicity of Salts to Fishes. Ecology 1920;1(2):95-112.
34. Weis JS, Candelmo A. Pollutants and fish predator/prey behavior: a review of laboratory and field approaches. Curr Zool 2012;58(1):9-20.
35. Mohanambal R, Purvaneswari S. A study of the Acute toxicity of Lead Nitrate (Pb (NO3) 2) on the fresh water fish *Catla catla* (Hamilton, 1862). Int J Curr Res 2013;5(8):2151-5.
36. Köprücü SŞ, Köprücü K, Ural MŞ, İspir Ü, Pala M. Acute toxicity of organophosphorous pesticide diazinon and its effects on behavior and some hematological parameters of fingerling European catfish (*Silurus glanis* L.). Pestic Biochem Physiol 2006;86(2):99-105.
37. Patil VK, David M. Behaviour and respiratory dysfunction as an index of malathion toxicity in the freshwater fish, *Labeo rohita* (Hamilton). Turk J Fish Aquat Sci 2008;8(2).
38. Naserabad SS, Mirvaghefi A, Gerami MH, Farsani HG. Acute toxicity and behavioral changes of Caspian kutum (*Rutilus frisii Kutum* Kamensky, 1991) and Caspian roach (*Rutilus rutilus caspicus* Jakowlew, 1870) exposed to the fungicide hinosan. Afr J Biotechnol 2015;14(20):1737-42.