Original Article

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

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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.

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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

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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.

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