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Acute toxicity evaluation of five herbicides: paraquat, 2,4-dichlorophenoxy acetic acid (2,4-D), trifluralin, glyphosite and atrazine in *Luciobarbus esocinus* fingerlings

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Abstract:

BACKGROUND: Evaluation of herbicide pollution in aquatic environments needs the great concern and the most important echo-pollutant effects of herbicides are related to their effects on non target aquatic organisms. Native fish can serve as a proper bio-indicator for evaluation of pollution on aquatic ecosystems. **OBJECTIVES:** To find environmentally friendly herbicides, in this study the acute toxicity of five widely used herbicides in Iran as aquatic ecosystems pollutants on *Luciobarbus esocinus* were investigated. **METHODS:** Acute toxicity (96 h LC50) of five herbicides (Paraquat, 2,4-dichlorophenoxy acetic acid, Trifluralin, Glyphosite and Atrazine) were determined via OECD standard method. *L.esocinus* exposed to Serial concentrations (more than 6 in triplicates) of each herbicide. Mortalities at 24, 48, 72 and 96 hours after exposure were recorded and the LC50 were calculated using Probit software. **RESULTS:** Results showed that acute toxicity of these herbicides are significantly different in *L. esocinus*. The 96 h LC50 of Paraquat, 2,4-D, Trifluralin, Glyphosite and Atarzine in *L.esocinus* were 54.66, 138.8, 1.09, 716.83 and 44.30 mg/l respectively. Glyphosite showed lowest toxicity in *Luciobarbus esocinus* among the five herbicids. The highest toxicity of herbicides in *L. esocinus* belongs to Trifluralin. The mortality rate of exposed fish to herbicides enhanced either by increasing herbicides concentration or duration of exposure. Mortality patterns during 96 hours of toxicity evaluation were similar in all five herbicides. **CONCLUSIONS:** Regarding the high application and similar efficacy of herbicides in most of the cane farms of Khouzestan province, and based on different toxicities of these five herbicides for fish as a non targeting organism, Glyphosite is highly recommendable as a proper alternative to Trifluralin, Atrazine, Paraquat and 2,4-dichlorophenoxy acetic acid.

Introduction

Unfortunately, most cyprinid ponds in Iran are located close to agricultural areas. Because of modern pest management practices, large amounts of herbicides are used in these areas for crop protection. Herbicides are actively used in terrestrial and aquatic ecosystems to control unwanted weeds, and their use has generated serious concerns about the potential adverse effects of these chemicals on the environment and human health (Oleh et al., 2009).

Herbicides may reduce environmental quality and influence essential ecosystem functioning by reducing species diversity and community structures, modifying food chains, changing patterns of energy flow and nutrient cycling and changing the stability and resilience of ecosystems.

In aquatic toxicology, laboratory experiments are normally used to estimate the potential hazard of chemicals and to establish ‘‘safe’’ levels of pollutants (Cattaneo et al., 2011).

Paraquat dichloride is a non-selective contact herbicide, used in controlling pests of cultivated farmlands of sugar cane, rice, fruit, and vegetable. It quickly kills a wide range of annual grasses, broad leaves, weeds and some perennial grasses when sprayed directly onto leaves. More so, the active ingredient is rapidly absorbed by clay and silt particles in the soil and does not leave any effective soil residue. It has a long half life in the environment and poses a threat to aquatic organisms and human health because of its bioavailability, resistance to microbial degradation, and resistance to decomposition in the presence of light (Yao et al., 2013). 2,4-dichlorophenoxyacetic acid (2,4-D) is a common and worldwide herbicide that is employed for post-emergence foliar spray and is also used for weed control of sugar cane, wheat, rice, maize and aquatic weeds (Farah et al., 2004). As a phenoxyherbicide, 2,4-D may cause an array of adverse effects to the nervous system such as myotonia, disruption of nervous system activity and behavioral changes (Bortolozzi et al., 2004).

Trifluralin is a dinitroaniline-type preemergence herbicide for control of grass and broadleaf weeds. Trifluralin has also been used to control larval mycosis in penaeid shrimp culture. It is extensively accumulated in the environment and slowly eliminated. (Schultz and Hayton, 1993). Its adverse effects on biotic components of a freshwater ecosystem were reported in chronic concentrations (Poleksic and Karan, 1999).

Glyphosate is one of the widely used herbicides that could be persistent and mobile in soil and water, and it is known to be one of the most common terrestrial and aquatic contaminants. It is used as a non-selective herbicide and for control of a great variety of annual, biennial, and perennial grasses, sedges, broad-leaved weeds, and woody shrubs. They are also used in fruits orchards, vineyards, conifer plantations and many plantation crops. It is perhaps the most important herbicide ever developed (Ayoola, 2008).

Atrazine (2-Chloro-4-ethylamino-6-Isopropylamino-s-triazine) is one of the most commonly used herbicides found in the rural environments. It is extensively used for corn, sugar cane, sorghum, and to some extent in landscape vegetation. Rated as moderately toxic to aquatic species, atrazine is mobile in the environment and is among the most detected pesticides in streams, rivers, ponds, reservoirs and ground water (Battaglin et al., 2008).

One of the largest sugar cane farms (over 100,000 hectares) of Asia is located in Khouzestan Province, Iran, in which large amounts of herbicides (hundreds of tonnes) are being used annually. More than 30% of Iran's running water flows in the rivers of Khuzestan province (Haji Sharafi and Shokuhfar, 2009).

The toxicity of a chemical is totally dependent on the concentration of the chemical in organisms or even the concentration at the target receptor in the organism. Herbicides at high concentration are known to reduce the survival, growth and reproduction of fish, and produce many visible effects on fish (Ladipo and Dohetry, 2011). There are few works on comparison of different herbicide toxicity in aquatic animals (Deivasigamani, 2015).

Besides, toxicity testing of chemicals on animals has been used for a long time to detect the potential hazards posed by chemicals to environment and human. Bioassay technique has been the cornerstone of programs on environmental health and chemical safety (Moraes et al., 2007). Aquatic bioassays are necessary in water pollution control to determine whether a potential toxicant is dangerous to aquatic life and if so, to find the relationship between the toxicant concentration and its effect on aquatic animals (Olaifa et al., 2003). The application of environmental toxicology studies on non-mammalian vertebrates is rapidly expanding (Ayoola, 2008), thus in this study the toxicity of five common herbicides in Iranian agriculture were compared in *Luciobarbus esocinus* to find which herbicides induce less impact on aquatic animals and environment.

Materials and Methods

Fish: 1200 fingerlings *Luciobarbus esocinus* were purchased from Native Fish Breeding and Cultivation Center in Hamidieh, Khouzestan. Fish were transferred to aquarium room of Veterinary Faculty, Shahid Chamran University of Ahvaz, Iran. Fish were acclimatized for 2 weeks in 300 L indoor fiberglass tanks and were fed with standard diet.

Fish were kept in continuously aerated water in a static system and with a natural photoperiod (12 h light - 12 h dark). During acclimation, fish were fed once a day with commercial fish pellets. For the LC determinations, 528 fingerlings were uniformly distributed in 21 40-L plastic aquaria. Each herbicide was tested using 6 to 9 different concentrations, with 2 repetitions. Two aquaria were kept as control (without herbicide). Fingerlings were observed at 24 h intervals, for 96h (acute toxicity) when the test was concluded. During the experimental period, fingerlings were not fed and water exchange was stopped. Later (after adaptation) they were transferred into 20 liter aquaria (10 fish weighing about 3-5 g in each aquarium. All products used were purchased from local stores. The generic, commercial, and chemical names, and pesticide group of each product tested are shown in Table 1, and the concentrations used are shown in Table 2. Before addition, each product was mixed in a small volume of water from each aquarium and then added to the water using a glass pipette. Fingerlings were then observed for 96h and the mortality recorded; swimming behavior (normal, erratic swimming, lethargy) was checked and compared to the control group.

Feeding was terminated 48 h prior to the initiation of the experiment. All experiments were carried out for a period of 96 h and the number of dead fish were counted every 24 h. During the acute toxicity test of experiment, water in each aquarium was aerated

Table 1. Specifications of the test herbicides.

Herbicide	Chemical Name	Supplier	Purity Rate	Acute Oral LD50 For Rat	Statue
Paraquat	1,1-dimethyl-4,4‘-bipyridinium	Aria Shimi Co, Iran	20%	129-157 MG/KG	water soluble green liquid.
2, 4 - D	2-(2,4-dichlorophenoxy)-acetic acid	Shimagro Co, Iran	67.5%	2100 MG/KG	water soluble brown liquid
Trifluralin	α-trifluoro-2,6-dinitro-N,N-dipropyl-p-toluidine),	Aria Shimi Co, Iran	48%	5000 MG/KG	water soluble orange liquid
Glyphosate or Roundup	N- phosphonomethyl glycine	Sinochem Co, China	41%	5600 MG/KG	water soluble yellow liquid
Atrazine	Chloro-4-ethylamino-6-isopropylamino-1-3-5-triazinre	Moshkfam Co, Iran	80%	1350 MG/KG	water soluble white powder

Table 2. The selected herbicides concentrations to determine their acute toxicity for *L. esocinus*.

Herbicide	Number of treatments	Number of replicates	Total exposed fish	Concentrations (mg/l)
Paraquat	7	3	210	,128 ,100 ,64 ,32 ,16 ,0 256
2,4-D	7	3	210	,200 ,100 ,50 ,25 ,0 800 ,400
Trifluralin	9	3	270	,2.5 ,1.25 ,0.65 ,0.32 ,0 15 ,10 ,7.5 ,5
Glyphosite	8	3	240	,640 ,320 ,160 ,80 ,0 5120 ,2560 ,1280
Atrazine	6	3	180	160 ,80 ,40 ,20 ,10 ,0

and had the same conditions as follow: dissolved oxygen 8.1±0.5 mg/l, temperature 25±1ºC,

pH 7.8± 0.2, water total hardness 640 mg/l as CaCO3, NH3 and NO2 <0.01 mg ml^{-1}.

The lethal concentration (LC50) was tested by exposing 10 fish per group according to Table 2 herbicides per liter of water for 96 hours. The control group was kept in experimental water without herbicides with all of the other conditions kept constant. The movement and the behavior of the fish were examined during the study. Motionless fish and those without any opercula movement were considered dead and were removed from the tanks.

Then fish were exposed to 6-9 sequential concentrations of each herbicide in a way that zero and 100% mortality yield after 96 hours. The mortality rate was recorded after 24, 48, 72, and finally 96 hours. The concentration of herbicides to induce 10, 15, 50, 85, 95 and 100 percent mortality (LC10, LC15, LC50, LC85, LC95, LC100) was estimated after 24, 48, 72, and 96 hours using Probit software verion 1.5 designed by U.S. EPA. This software estimates LC concentration based on regression between mortality rate and log of toxin concentration. Estimation was presented with lower and upper range with 95% confidence level (Aydin and Kuprucu, 2005). The selected herbicides concentrations for estimating their acute toxici-

Table 3. Lethal concentrations of herbicides (mg/l) (95% confidence intervals) depending on exposure time for *L. esocinus*.

Type of Toxicant		Exposure time (h)			
	Lethal concentra- (tion (mg/l	h 24	h 48	h 72	h 96
Paraquat	LC10	68.78	61.47	41.3	31.44
	LC15	73.33	65.51	45.4	34.95
	LC50	96.17	85.74	67.72	54.66
	LC85	126.12	112.22	101.02	85.5
	LC95	147.88	131.43	127.76	111.17
	LC100	176.73	156.87	166.2	149.18
2,4-D	LC10	226.22	173.09	121.52	60.44
	LC15	245.48	190.14	134.68	70.86
	LC50	346.83	282.9	208.02	138.82
	LC85	490.02	420.90	321.32	271.95
	LC95	600.23	531.45	414.75	403.57
	LC100	753.38	690.11	552	627.1
Trifluralin	LC10	0.413	0.46	0.36	0.39
	LC15	0.56	0.6	0.47	0.47
	LC50	2.17	1.76	1.45	1.09
	LC85	8.3	5.15	4.43	2.52
	LC95	18.26	9.68	8.5	4.12
	LC100	44.13	19.61	17.72	7.13
Glyphosite	LC10	846.43	532.9	410.04	226.3
	LC15	988.06	637.012	487.25	282.14
	LC50	1900.54	1354.44	1010.62	716.83
	LC85	3655.72	2879.87	2096.14	1821.22
	LC95	5367.18	4484.29	3216.71	3148.33
	LC100	8251.8	7363.97	5196.85	5812.30
Atrazine	LC10	86.81	38.54	28.65	25.58
	LC15	90.91	41.48	31.61	28.41
	LC50	110.53	56.56	47.93	44.30
	LC85	134.39	77.14	72.67	69.06
	LC95	150.72	92.55	92.77	89.62
	LC100	171.39	113.50	121.97	120.01

ty in *L. esocinus* are brought in Table 2.

Results

None of the herbicides, even at the highest concentration, altered the water quality parameters. Lethargy, swimming at the water surface and erratic swimming (mainly vertical swimming) were the main behavioral changes observed throughout the experiment, in the presence of herbicides. The behavioral changes were observed with different herbicides, usually at the high concentrations tested.

The fish mortality rate following the exposure to increasing concentrations of herbicides after 24, 48, 72, and 96 hours showed that the higher the herbicide concentration, the greater the mortality rate. The mortality rate increased along with increasing the exposure time. Acute toxicity of Paraquat, 2,4-D, Trifluralin, Glyphosite and Atarzine was determined in *L. esocinus* after 24, 48,

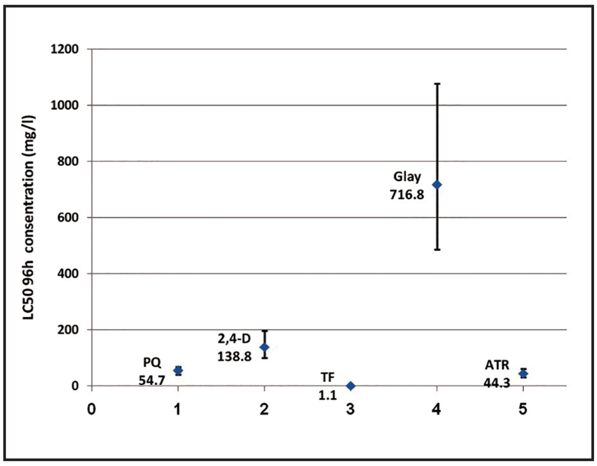

Figure. 1. Comparison of LC50 96h concentrations of selected herbicides in *L. esocinus*.

72 and 96 hours of exposure. 96 hours LC50 value (Median lethal concentration) was calculated at 54.66, 138.82, 1.09, 716.83 and 44.30 mg/l in *L. esocinus*, (Table 3) and MAC of these herbicides was 5.46, 13.88, 0.1, 71.68 and 4.43 mg/l respectively. The greatest LC50 was 716.83 mg/l for Glyphosate and the least was 1.09 mg/l for Trifluralin. Therefore, the most toxic herbicides for *L. esocinus* were Trifluralin, Atrazine, and Paraquat, respectively.

The glyphosate showed significantly lesser lethality (higher LC50 concentration) than other tested herbicides. Trifluralin and Atrazine showed the highest lethality (lower LC50 concentration) in *L.esocinus* among the examined herbicides. (Fig 1).

Discussion

In Khouzestan province of Iran, most cyprinid fish ponds are built close to agricultural areas with shared water sources. Water contamination by agricultural herbicides is a potential threat to productivity and a major cause of fish mortality. However, water contamination with herbicides at non-lethal concentrations might pass unnoticed including loss in growth, health and resistance to diseases which, in most cases, might be difficult to assess (Nwani et al., 2010). From an ecotoxicological point, contamination of water sources poses a potential threat to aquatic organisms including fish. Since fishes are the last chain of the food web in aquatic ecosystems there may be bioaccumulation problems in addition to acute effects (World Health Organization, 1984). The toxicity of different echotoxicants such as herbicides and their adverse effects on aquatic animals was mostly assayed by 96h LC50 (median lethal concentration) in native fish. The 96-h LC50 of several commonly used agricultural herbicides were determined in this study. *Luciobarbus esocinus* fingerlings were used because this fish species is an ubiquitous native species in rivers, wetlands and ponds in southwest Iran and has been newly cultivated in polyculture system of cyprinid farms.

In this study all tested herbicides were toxic for *Luciobarbus esocinus*, such that Median lethal concentration after 96 h exposure (96 h LC50) of five herbicides: Paraquat, 2,4-D, Trifluralin, Glyphosite and Atrazine in *Luciobarbus esocinus* was 54.66, 138.82, 1.09, 716.83 and 44.30 mg/l respectively. The results showed although toxicity of five tested herbicides was different for *L. esocinus*, their toxicity revealed positive correlation not only to herbicide concentration, but also to exposure duration. The most toxic herbicide tested was Trifluralin, which is used in cane farms for controlling the unwanted plant growth. Among the evaluated herbicides Trifluralin and Atrazine were more toxic than the others, and Trifluralin, which was used in large scale in cane (over one hundred thousand hectares farms) for controlling weed growth was the most toxic. All environmental parameters, herbicides concentration and examined fish source were quite similar in this study based on OECD regulation, then the differences in herbicides toxicity just refer to each herbicide toxicity mechanism in fish. The results of this work, like other toxicity assessment studies, showed somehow different results compared to a similar report in other fish, but it is worth considering that toxicity of chemicals to aquatic organisms has been shown to be affected by age, size and health of the species. Physiological parameters like quality, temperature, pH, dissolved oxygen and turbidity of water, concentration and formulation of chemical and its exposure also greatly influence such studies.

The data obtained for acute toxicity of trifuralin in this study were in the range of LC50 concentrations established for juvenile rainbow trout and bluegill sunfish 0.01-0.04 and 0.02- 0.09 mg/l, respectively. Poleksic and Karan (1999) showed that Median lethal concentration (LC50) on Carp, after 24h and 48h exposure was 0.185 mg/l and 0.066 mg/l, and 0.045 mg/l after 96 h (Poleksic and Karan, 1999). The herbicide trifrluralin has been classified in the group of highly toxic substances, according to its LC50 values to fish (OECD, Chemical Group and Management Committee, 1992). Trifrluralin affects fish health, making them more sensitive to environmental changes and less resistant to diseases.

Water contamination with trifluralin may occur via agricultural farms leaching after raining or irrigation. Nevertheless, only 0.5% of the quantity applied to the soil in field conditions is leached and may consequently contaminate water sources. This percentage means rather low water contamination, representing smaller concentrations than 1.0 $\mu g\ l^{-1}$. (Grover et al., 1997).

LC50 (48h) of trifluralin was reported 19 $\mu g\ l^{-1}$ in bluegill fish (Lepomis macrochirus), 19 $\mu g\ l^{-1}$ in Mola mola, 1 mg/l in Cyprinus carpio and 0.56 mg/l in pollutant biomarker, Daphnia magma , respectively. Also, the amount of LC50 after 96 hours in *Oncorhynchus mykiss* was 0.21 mg/l and 10-90$\mu g\ l^{-1}$ in *Lepomis macrochirus* (Gangolli ,1999).

Toxicity mechanisms of trifluralin include genotoxicity effects which cause DNA breaks, loss of genetic material, immunotoxicity and mutations which lead to cell death. Besides, trifluralin causes hemoglobin oxidation (by forming metahemoglobin), red blood cell destruction, damage to kidney and the liver.

Existing reports characterize trifluralin as a highly acute toxic substance to fish, but there are not enough descriptions of its chronic toxicity and cytotoxic effect. Studies mainly related to its genotoxic, mutagenic and carcinogenic potential are mostly inconclusive or even contradictory.

Paraquat is a chlorinated herbicide which is mainly used to control weeds in agriculture, however, once it enters surface waters it may affect other organisms such as fish as a non-target organism either in natural or culture conditions. In the present study the 96 h LC50 of paraquat was estimated to be 54.66 ppm in *Lusiobarbus esocinus* fingerling. Yeo (1967) reported that paraquat at 1.0 and 3.0 ppm was toxic to small mouth bass (Micropterus dolomieui) and to mosquito fish (Gambusia affinis) in 180-gallon plastic pools that had an average pH of 9.4. The 96-hour LC50's for the blue gill and channel catfish were 13 ppm and greater than 100 ppm, respectively. Besides, the acute toxicity (96 h LC50) of the herbicide paraquat for *Oreochromis niloticus* was

12.25 mg l^{-1} (Babatunde and Oladimeji, 2014). Deivasigamani et al. (2015) reported that common carp weighing 300-400 g died within 15 min after exposure to paraquat at the concentration of 100 ppm, but they did not describe the source of the test chemical (Deivasigamani et al., 2015).

Paraquat-induced endothelial cell toxicity occurs by NO synthase which causes NADPH oxidation, moreover, Paraquat-induced cytotoxicity is potentiated in cytokine-activated macrophages in a manner that correlates with its ability to block NO formation.

The difference in toxicity between pure chemical and formulated commercial products may be attributable to the other ingredients supplemented into the formulated products. The difference in toxicity rate of paraquat in various fish species can be ascribed to species sensitivity, age and weight of fish as well as environmental parameters.

Based on the findings, the amount of lethal concentration of 2,4-D for 50 percent of fish was determined to be 138.82mg/l after 96 hours. The maximum value equals 13.89 mg/l which is also called NOEC (or ineffective concentration). The minimum concentration level (LOEC) which is also called LC10 96h was 60.44 mg/l (Table 3).The lethal concentration allowing 50 percent of the fish to perish was determined to be 346.83, 282.9, 208.02, and 138.82 after 24, 48, 72, and 96 hours respectively (Table 3). It reveals an increase in the herbicide toxicity following an increase in exposure time.

2-4-D herbicides toxicity in fish mostly induced by chromosomal damage including chromosome breakage, sister chromatid exchange and micronuclei anisocytosis as well as altered nuclear morphology. Disrupted chromosome material and erythrocytes with several micronuclei present is a conventional effect of 2-4-D in fish. There are some bacteria and fungi that use 2-4-D as a source of carbon and energy (Daugherty and Karei, 1995). The widespread use of chlorophenoxy acetic acid as a herbicide and a growth regulator in agriculture, forestry, and households has compounded the damage incurred by these toxic compounds on the environment and human health.

Abdelghani et al. (1997) found 96 h LC values of 2,4D as 181.2 for catfish, 266.3 for bluegill, and 750.1 mg/l for crawfish, respectively. Farah et al. (2004) found the LC50 values of 2,4-D as 81 ppm for *Heteropneustes fossilis*, 122 ppm for Clarias batrachus, 107 ppm for Channa punctatus and 302 ppm for Culex pipiens fatigans. Sarikaya et al. (2002), found LC50 value of 2,4-D on tench (Tinea tinea) as 48 mg/l. Sarikaya and Yilmaz (2003) reported 96 h LC50 value for Cyprinus carpio as 63.24 mg/l. In the current study, we found that acute toxicity of 2,4D (LC50) significantly increased in accordance with the exposure time from 346.83 mg/l at 24 h to 138.82mg/l at 96 h.

In the present study the 96-h LC50 determined for the glyphosate in *Luciobarbus esocinus* was 716.83 mg l^{-1}. It is much higher than that reported by other authors in other fish species as 17.8 mg/l in Gambusia yucatana (Rendsn-Van-Osten et al., 2005) and 120 mg/l in *Oncorhynchus mykiss* (GIESY et al., 2000). Glyphosate (Roundup®) exhibited very slight toxicity in *Luciobarbus esocinus* which was the lowest toxicity among 5 highly used herbicides in Iran. In other works different results reported about the toxicity of glyphosate in various fish species, 96-h LC50 of glyphosate for *Huso huso*, *Acipenser stellatus*, *A. persicus* and *Oncorhynchus mykiss* were reported 26.4, 23.2 ,27.5 and 86 mg l^{-1} respectively. Sturgeon fries exposed to concentrations of 60 to 100 mg l^{-1} showed an increase in mortality. Glyphosate exhibited a slight to moderate toxicity in sturgeon species (Filizadeh and Rajabi Islami, 2011). Most of the studies indicated a slight or moderate toxicity for fish species. Fish exposed to 10 mg/l of glyphosate for two weeks were found to have gill and liver damage. For commercial formulations, the LC 50 found varied from 13 to 33mg/l in coho salmon. Even et al., report that the toxicity of glyphosate-based herbicides might be attributed to the presence of the surfactant (POEA), which is more toxic than glyphosate itself, however Rodeo® has no surfactant, and is registered for aquatic use (Fryer, 1977).

Glyphosite toxicity causes some oxidative stress in high concentration in fish Hence, after exposure, an increase in antioxidative stress enzymes was seen.

Despite the toxicity levels of glyphosate in mentioned works, Hildebrand et al. (1980) found that Roundup® treatments at concentrations up to 220 kg/ha did not significantly affect the survival of Daphnia magna or its food base of diatoms under laboratory conditions. It appears that under most conditions, rapid dissipation from aquatic environments of even the most toxic glyphosate formulations prevents build-up of herbicide concentrations that would be lethal to most aquatic species.

The results of the LC50 (median lethal concentration) of the present study at 96 h was 44.30 mg/l for atrazine. This result indicated moderate toxicity of atrazine among the tested herbicides. In other works different results reported about the toxicity of atrazine in various fish species. In tilapia (*Oreochromis mossambicus* and *Oreochromis niloticus*) the LC50 in 96 h was 8.8 and 5.02 mg/l, respectively. This indicates , that the species used in this study are less sensitive to atrazine than those used in other studies.

Although the 96 h LC50 of 44.30 mg/l reported for *L. esocinus* exposed to atrazine in the present study differed from the report of Bathe et al. (1973), Neskovic et al. (1993) and Hussein et al. (1996) who reported LC50 of 16.0, 18.8 and 9.37 mg/l for *Lepomis macrochirus* (Bluegill sunfish), *Cyprinus carpio* and *Oreochromis niloticus* respectively exposed to atrazine, the present findings seem to be consistent with other research which found 37 mg/l (LC50 96h) for grass carp fingerlings. On the other hand, we can showthat fingerlings are less sensitive to some chemicals and toxins than adult or juvenile fishes.

In toxicity studies, the sensitivity of organisms can be different, even using the same product (Botelho et al., 2012).

It has been reported that atrazine-induced apoptosis, defined by both morphological and biochemical criteria, in carp cells occurred in a dose- and time-dependent manner. Intracellular free Ca2+ is thought to act as an important messenger in a variety of cellular signaling pathways and metabolic processes and has a crucial role in the apoptotic process. Recent evidence suggests that intracellular Ca2+ involved in atrazine-induced apoptosis in fish cells (Mizuhashi et al., 2000).

In conclusion, there is evidence that trifluralin and Atrazine tend to be more toxic to *L. esocinus* than other herbicides. Respective industries should be encouraged to look into the possibility of reducing the rate of these herbicides because of their potency to harm non target aquatic organisms. Also, for sustainable agricultural activity, especially in areas that fish ponds and agriculture farms use the same water sources, it is highly recommended to use glyphosate as an alternative to Trifluralin and Atrazine. To the best of our knowledge, this work is the first study to compare the toxicity of different common herbicides on a high risk native fish.

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مجله طب دامی ایران، ۱۳۹۵، دوره ۱۰، شماره ۴، ۳۳۰-۳۱۹

ارزیابی سمیت حاد علفکش‌های: پاراکوات، توفوردی، ترفلان، گلایفوزیت و آترازین در بچه ماهی عنزه (*Luciobarbus esocinus*)

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چکیده

زمینه مطالعه: توجه به آلایندگی علف‌کش‌ها در محیط‌های آبی ضروری به نظر می‌رسد و مهم‌ترین اثر آلایندگی علف‌کش‌ها مربوط به اثر آنها بر آبزیان غیر هدف می‌باشد. ماهیان بومی به عنوان یک شاخص زیستی مناسب برای بررسی آلاینده‌های اکوسیستم آبی می‌باشند. **هدف:** برای دستیابی به سمی که کمترین اثرات سوء زیست محیطی را دارد، سمیت حاد پنج علف کش رایج در صنعت کشاورزی ایران به عنوان آلاینده های محیط زیست در ماهی عنزه ارزیابی گردیدند. **روش کار:** سمیت حاد (LC۵۰) پنج سم علف‌کش (شامل پاراکوات، توفوردی، ترفلان، گلایفوزیت و آترازین) با استفاده از روش استاندارد OECD اندازه گیری شد. به این منظور ماهی عنزه در مجاورت حداقل ۶ غلظت افزایشی هر سم در سه تکرار قرار داده شد و تلفات روزانه ثبت گردید، سپس LC۵۰ ۹۶ ساعته هر سم با استفاده از نرم افزار Probit مشخص گردید. **نتایج:** نتایج نشان داد که سمیت حاد این علف کش‌ها در ماهی عنزه به طور معنی‌داری متفاوت است (p<۰/۰۵). LC۵۰ ۹۶ ساعته پاراکوات، توفوردی، ترفلان، گلایفوزیت و آترازین در ماهی عنزه به ترتیب برابر با mg/l ۵۴/۶۶، ۱۳۸/۸، ۱/۰۹، ۷۱۶/۸۳ و ۴۴/۳۰ بود. گلایفوزیت کمترین سمیت را در مقایسه با سایر سموم علف‌کش دارا بود. بیشترین سمیت در بچه ماهی عنزه مربوط به ترفلان بود. میزان مرگ و میر ماهیان هم با افزایش غلظت سموم و هم با افزایش مدت مجاورت با سم افزایش یافت. الگوی مرگ و میر ایجاد شده توسط تمام این علف کش‌ها در طی ۹۶ ساعت مشابه بود. **نتیجه‌گیری نهایی:** با توجه به کاربرد وسیع و اثر بخشی مشابه علف کش‌ها در اغلب مزارع نیشکر استان خوزستان و با توجه به تفاوت سمیت این ۵ علف کش در ماهی عنزه به عنوان یک موجود غیر هدف، توصیه می‌شود گلایفوزیت که سمیت کمتری دارد، به عنوان یک جایگزین مناسب برای ترفلان، آترازین، پاراکوات و توفوردی استفاده گردد.

واژه‌های کلیدی: سمیت حاد، آلاینده‌های اکوسیستم، عنزه، علف کش، LC۵۰

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