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Original Article

Acute toxicity effect of glyphosate on survival rate of common carp, *Cyprinus carpio*

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Abstract

Background: Herbicides are usually used to control weeds and some of them like glyphosate are non-selective herbicides. Aquatic environments are usually the last destinations of agricultural pesticides, which disrupt the metabolic processes of organisms. Thus, the aim of this study was to evaluate acute toxicity of glyphosate on the survival rate of common carp, *Cyprinus carpio*.

Methods: A total of 135 common carp averaged 7 ± 0.8 g in weight were exposed to 0, 25, 50, 75, 100, 125, 150, 175 and 200 $mL.L^{-1}$ glyphosate (15 fish in each treatment, with triplicates) for 96 hours. The aquariums capacity was 98 L in volume (80 × 35 × 35 cm) and physicochemical parameters were the same for all groups (pH 7.4-8, temperature = 26 ± 1°C, DO = 7 $mg.L^{-1}$ and total hardness of 190 mg $CaCO_3$). LC_{10}, LC_{20}, LC_{30}, LC_{40}, LC_{50}, LC_{60}, LC_{70}, LC_{80}, LC_{90} and LC_{95} of glyphosate were calculated at 24, 48, 72 and 96 hours after adding glyphosate using probit test.

Results: Mortality was observed in all treatments which exposed to higher than 50 $mL.L^{-1}$ after 96 hours. The results showed that 96-hour LC_{50} of glyphosate for common carp was 92.71 $mL.L^{-1}$. The fish exposed to different concentrations of glyphosate showed clinical signs such as increased mucus secretion, skin darkening and death with mouth open.

Conclusion: Glyphosate disrupts the synthesis of amino acids in plants by inhibiting enzymatic activity of 5-enolpyruvylshikimate-3-phosphate synthase (EPSP). This enzyme is absent in animals. However, glyphosate is toxic for common carp.

Keywords: Animals, Herbicides, Glyphosate, Carps, Excitatory postsynaptic potentials

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Introduction

In recent years, the use of agricultural pesticides has been increased significantly (1). Various types of agricultural pesticides such as herbicides, insecticides and fungicides are commonly used globally (2); these pesticides usually spread in the aquatic environments through various ways such as rainfall and soil erosion (3). In other words, aquatic ecosystems are the ultimate destinations of agricultural pesticides (4). Aquatic organisms contact with their environment through physiological surfaces and pesticides can penetrate through the skin, gills and mouth into their body, and affect liver, kidneys, gonads and other organs (5). Contamination and accumulation of pesticides in the aquatic environment can lead to food poisoning and mortality in humans (1,2).

Herbicides are commonly used in farms to control weeds (6). In the meantime, glyphosate (glyphosate 41% SL) is one of the most widely used herbicides in many countries, especially developing countries (1,7). The use of glyphosate had the fastest growth rate compared to other herbicides (8). Two-thirds of the total volume of glyphosate has been sprayed in the recent decades (9). Glyphosate inhibits the 5-enolpyruvylshikimate-3-phosphate synthase (EPSP) activity in plants and impairs the synthesis of the aromatic amino acids (10). EPSP is found only in plants and some bacteria (11).

Glyphosate is highly soluble in water (10500 $mL.L^{-1}$) and its half-life, depending on the environmental conditions, is between 3.5 and 70 days (12). However, Souza et al (13) pointed out that the half-life of glyphosate in freshwater was between 28 and 87 days. It was thought that since EPSP enzyme was not found in animals, so glyphosate is not toxic to them. However, the results of previous studies showed that it was toxic to fish species (10,14). Loro et al (15) reported that glyphosate-based herbicide was toxic to *Rhamdia quelen* and *Leporinus obtusidens*. Sandun et al (16) reported that 96-hour LC_{50} of glyphosate to *Oreochromis niloticus* was 16.7 $mg.L^{-1}$; Da Cruz et al (17) also pointed out that glyphosate-based herbicide was toxic to fish as an aquatic organism.

Fish, as aquatic organisms, can be used as biomarkers in toxicology and biological studies (18). So fish often have been used as experimental biological models for exhibiting environmental impacts of toxins (19). Toxicological studies on lethal concentration of toxins are very important (20), and one of the methods is to determine 96-hour LC_{50} of pesticides on fish (21,22). Toxicological studies like those determining 96-hour LC_{50} of toxins on fish can provide better management of water resources as a method for detection of biological effects of pollutants (23). Finally, these studies can also be useful in determining the limits of toxins in nature and human food safety (24).

Common Carp, *Cyprinus carpio* Linnaeus, 1758, as a commercially- important fish species in aquaculture (25) is found in most temperate regions of the world (26). It is omnivorous (27) and often searches bottom of the pond for getting food (28). Feeding habits, high growth rate, reproduction in captivity, adaptation to commercial diets and density are reasons for the common carp popularity in aquaculture industries (29). However, human activities and environmental pollutants can disrupt the life cycle of common carp (30). The use of herbicides such as glyphosate in addition to the destruction of macrophytes may affect non-target organisms such as common carp through destruction of habitats and food (31,32). For example Zebrafish, *Danio rerio*, needs a substrate full of plants for spawning and reproduction (33), so the pollutants may have negative effects on breeding of this species through the destruction of non-target aquatic plants.

There is limited information about the toxicity of glyphosate. It is also true for the maintenance of carp in ponds and reservoirs as well as its long half-life in the aquatic environments. So, the aim of the present study was to assess acute toxicity of glyphosate on survival rate of common carp, *C. carpio* as an animal model.

Methods

The experiment was performed according to Yalsuyi and Vajargah (2) and Montajami et al (34).

Sample preparation

200 common carp averaged 7 ± 0.8 g in weight were prepared from some farms in Guilan province and transferred to the laboratory in Faculty of Natural Resources, University of Guilan, Guilan province, Iran, and divided into four 250-L tanks (50 fish in each tank). Fish were maintained in these tanks for two weeks in order to acclimate with laboratory conditions. Fish were fed a commercial diet (produced by Faradaneh Co. Tehran, Iran) at 3% of body weight 3 times a day. Physicochemical parameters of water in all tanks were the same (pH 7.4-8, temperature = 26 ± 1°C, DO =7 $mg.L^{-1}$ and total hardness = 190 mg $CaCO_3$).

Toxicity tests

After adaptation, 135 fish were selected randomly and divided into 9 treatment groups (0, 25, 50, 75, 100, 125, 150, 175 and 200 $mL.L^{-1}$ glyphosate) in triplicates into 27 aquariums (80 × 35 × 35 cm, capacity 85 l). Fish were exposed to different concentrations of glyphosate (Glyphosate Aria 41% SL) for 96 hours. Mortality rates of fish were recorded at 24, 48, 72 and 96 hours after adding glyphosate. Animals then were transferred into aquariums 16 hours before the toxicity test and feeding was stopped 24 hours before the experiment. Water physicochemical parameters were the same as the adaptation time (pH 7.4-8, temperature = 26 ± 1°C, DO = 7 $mg.L^{-1}$ and total hardness = 190 mg $CaCO_3$).

Data analysis

Toxicity of glyphosate was estimated using the method proposed by Vajargah and Hedayati (35). Lethal concentrations of glyphosate for 50% of the population at 24, 48, 72 and 96 hLC50 were calculated using probit test (with a 95% CI) by IBM SPSS 20. Spearman test (two-tailed test) was used to determine the correlation between different concentrations of glyphosate and fish mortality.

Results

Mortality was not observed in the adaptation period. The results of toxicity tests showed that there was a significant correlation ($P < 0.01$) between concentration of glyphosate and fish mortality. All fish died after 96 hours exposure to the concentrations higher than 150 $mL.L^{-1}$ glyphosate, while in concentration of 200 $mL.L^{-1}$, all fish died after 24 hours (Table 1).

96-h LC_{50} of glyphosate for common carp was 92.711 $mL.L^{-1}$. LC_{10}, LC_{20}, LC_{30}, LC_{40}, LC_{50}, LC_{60}, LC_{70}, LC_{80}, LC_{90} and LC_{95} of glyphosate within 24, 48, 72 and 96 hours are demonstrated in Table 2. The fish exposed to different concentrations of glyphosate showed clinical signs such as increased mucus secretion, skin darkening, fast swimming, increased movements of operculum and death with mouth open.

Discussion

As it is estimated that the world population increases up to 9 billion persons by 2050 (36), therefore, food supply needs to be increased by productivity (37); and

Table 1. Mortality rate of common carp, *Cyprinus carpio* exposed to different concentrations of glyphosate (n=15 in each treatment)

Concentration ($mg.L^{-1}$)	Number	No. of mortality			
		24 h	48 h	72 h	96 h
0	15	0	0	0	0
25	15	0	0	0	0
50	15	0	0	0	1
75	15	1	3	4	6
100	15	2	5	7	9
125	15	2	6	8	12
150	15	3	8	13	15
175	15	6	10	14	15
200	15	15	15	15	15

All fish died at concentrations higher than 150 $mL.L^{-1}$.

Table 2. Lethal concentration of glyphosate for common carp, *Cyprinus carpio*

Point	Concentration ($mg.L^{-1}$)			
	24 h	48 h	72 h	96 h
LC_{10}	109.420	71.438	63.006	52.731
LC_{20}	128.898	93.337	79.563	66.455
LC_{30}	142.943	109.130	91.501	76.352
LC_{40}	154.944	122.624	101.702	84.808
LC_{50}	**166.161**	**135.236**	**111.237**	**92.711**
LC_{60}	177.378	147.848	120.771	100.615
LC_{70}	189.379	161.342	130.972	109.071
LC_{80}	203.425	177.135	142.911	118.967
LC_{90}	222..903	199.036	159.467	132.691
LC_{95}	238.988	217.122	173.40	144.025

agricultural growth requires an increase in productivity through the use of a variety of nutrients for plants and soil, improving agricultural techniques, and the use of high-yielding plant varieties and pesticides (38). Glyphosate is widely used in agriculture (9). According to the World Health Organization (WHO) (39), application of glyphosate has increased annually. It has also long half-life in aquatic environments (13) and its residues can remain stable in foods for a year or even more, even if the foods are frozen, dried or processed (40). It also damages macrophytes as well as non-target organisms such as common carp through deterioration offish habitats and food (31). Hence, one of the main challenges concerning the widespread application of agricultural pesticides can be related to their devastating impact on non-target organisms (41).

Akinsorotan (42) reported that the 96-h LC_{50} of dizensate (glyphosate) on fingerlings of *Clarias gariepinus*, as an aquatic organism, was 18.07 $mg.L^{-1}$. It was also found that dizensate can reduce the survival rate of the fish, which is consistent with the results of the present study.

Micah et al (43) studied behavioral responses of *Heteroclarias* (hybrid) exposed to two types of glyphosate and found that it was toxic to *Heteroclarias* (hybrid) as an aquatic organism and its 96-hour LC_{50} was 6.838 $mg.L^{-1}$. They also reported that glyphosate had some clinical signs such as increased opercular ventilation and also caudal fin damage, which was consistent with the results of the present study.

Da Cruz et al (17) investigated sensitivity, ecotoxicity and histopathological effects on some Neotropical fish exposed to glyphosate alone and with surfactant. Their results showed that glyphosate was toxic to these species and there was significant correlation between mortality rate of fish and concentration of glyphosate ($P<0.01$). It also reduced the survival rate of fish as non- target organism and had harmful effects on fish, which was also similar to the results of the present study.

Jofré et al (12) studied toxicity of two commercial glyphosate on survival rate of two fish species (*Danio rerio* and *Poecilia reticulata*). The results of their study indicated that both herbicides may produce potential environmental damages, which is similar to the results of the present study.

Henao Muñoz et al (44) studied acute toxicity and sub-lethal effects of the mixture glyphosate (Roundup® Active and Cosmo-Flux 411F) to anuran embryos and tadpoles of four Colombian species. The results showed that 96-hour LC_{50} of glyphosate to embryos and tadpoles was between 2.2-3.9 $\mu g.L^{-1}$ and 1.4-2.8 $\mu g.L^{-1}$, respectively. Tadpoles of

Hypsiboas crepitans was the most sensitive and glyphosate was toxic to aquatic organisms, which is consistent with the results of the present study.

By comparing the results of the present study and Jofré et al (12), Akinsorotan (42), Micah et al (43) and Henao Muñoz et al (44), it was found that the toxicity of glyphosate depends on the size, species and physicochemical parameters of the environment.

Conclusion

According to the results, although glyphosate inhibits the enzyme EPSP and this enzyme has been observed only in plants and some bacteria, but glyphosate was toxic to *C. carpio* as a non-target organism. The results of the present study can be useful for further ecotoxicological studies. On the other hand, agricultural pesticides are associated with materials which have different combinations such as salts and a surfactant called polyethylene amine (POEA). These compounds in some cases, can increase the toxicity of pesticides. For example, Peyote®, which is one of the glyphosate compounds, is more toxic than glyphosate. POEA is also toxic to aquatics (45). The present study can also be useful for further investigations on the toxicity of POEA to the other aquatics.

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Ethical issues

Fish was used as a model of aquatic organism to study the toxicity of different pollutants. In term of ethical issues, the procedures described in this paper are in accordance with the ecological effects of test guidelines (46) published by the United States Environmental Protection Agency (US EPA).

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

Authors were participated in all aspects of this research work, like data collection, analysis, interpretation and manuscript approval.

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