IJVAR International Journal of Veterinary and Animal Research
Uluslararası Veteriner ve Hayvan Araştırmaları Dergisi
E-ISSN:2651-3609 1(3): 46-48, 2018

Comparative Toxicity of Paraquat and 2, 4-Dichlorophenoxy Acetic Acid in Adult *Artemia franciscana*

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Abstract
Herbicides are being used widely in agriculture and aquaculture for controlling noxious weeds. Paraquat and 2, 4-Dichlorophenoxyacetic acid (2,4-D) have been the most widely used herbicide during the past three decades. Toxicological properties of Paraquat are attributed to its ability to produce reactive oxygen species such as superoxide anion that may directly or indirectly cause cell death. 2,4-Dichlorophenoxyacetic acid (2,4-D) is a broad-leaf, systemic, phenoxy herbicide used as the active ingredient in several commercially available aquatic herbicide products. Bioassay technique has been the cornerstone of programs on environmental health and chemical safety. The application of environmental toxicology studies on non-mammalian vertebrates is rapidly expanding. So the present study investigated the acute toxicity of Paraquat and 2,4- Dichlorophenoxy acetic acid (2,4-D) as aquatic ecosystems pollutants on *Artemia franciscana*. Artemia is one of the most suitable test organisms available for ecotoxicity testing and research and most commonly used live food in aquaculture. Acute toxicity (48 h LC50) of two herbicides (Paraquat, 2, 4-dichlorophenoxy acetic acid) was determined. *A. franciscana* exposed to Serial concentrations of both mentioned herbicides. Mortalities at 12, 24, 36 and 48 hours after exposure were recorded and LC50 were calculated using Probit software. The results obtained indicate that the acute toxicity of these herbicides is significantly different in adult *A. franciscana*. The lethal concentration of Paraquat and 2,4-D were calculated 2.701, 14.475 mg/L in *A. fransiscana* respectively. So The LC50 of two examined herbicides was significantly different and the mortality rate was increased by increasing exposure time. Finally, these data support the hypothesis the possible risks associated with the presence of herbicides particularly Paraquat residues in the aquatic animals and their environment.

Keyword: *Artemia franciscana*, herbicides, toxicity

INTRODUCTION

Unfortunately, most aquatic ponds in some country (i.e.: Iran) are located close to agricultural areas. Large amounts of herbicides are commonly used in agricultural practices to control unwanted weeds [1]. However, they are of ecological concern since they are toxic to non-target species at low concentrations [2]. Herbicides may reduce environmental quality and influence essential ecosystem functioning by reducing species diversity and community structures, modifying food chains changing the stability of ecosystems. Utilization of herbicides in agricultural purposes represents 49% of total consumption of agrochemicals in the world [3].

Paraquat is one of the most popular herbicide, which acts fastly on a broad spectrum of weeds and it has been shown to be a highly toxic compound for humans and animals, and many cases of acute poisoning and death have been reported over the past few decades. 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 [4]. Severe Paraquat poisoning is characterized by multi organ involvement, mainly the lungs, kidneys, liver, myocardium, and adrenal cortex. Absorbed paraquat is distributed via the blood to all organs and tissues of the fish. Due to the lipophilic property of this herbicide, it accumulates mainly in fatty tissues [5].

2,4-Dichlorophenoxyacetic acid (2,4-D) is widely used in agriculture as herbicide/pesticide, plant growth regulator and fruit preservative agent [2] and [6]. It progressively accumulates in the environment including surface water, air and soil. It could be detected in human food and urine, which poses great risk to the living organisms. It has a high water solubility and low soil binding affinity, increasing its transport to aquatic environments [6]. In addition, 2,4-D is widely used by federal and private agencies as an aquatic herbicide for the control of invasive aquatic plants in lakes, ponds, and natural waterways. It is used in its ester, acid, and amine salt formulations globally; but in aqueous environments it is typically found in its free anion form through either disassociation (amine salts) or hydrolyzation (ester forms) [7].

The brine shrimp Artemia is zooplankton, like copepods and daphnia, which are used as live food in the aquarium trade and for marine finfish and crustacean larval culture [8], [9] and [10]. Artemia is subdivided into six generally recognized bisexual species and a large number of parthenogenetic populations, is characterized by common features such as adaptability to wide ranges of salinity (5-250 g L^{-1}) and temperature (6-35°C), short life cycle, high adaptability to adverse environmental conditions, high fecundity, bisexual/parthenogenetic reproduction strategy (with nauplii or cysts production), small body size, and adaptability to varied nutrient resources as it is a non-selective filter feeder [8], [9] and [11]. There are few studies on comparison of different herbicide toxicity in aquatic animals [12]. The aim of the present work is to assess the adverse effect of of Paraquat and 2, 4-Dichlorophenoxy Acetic Acid in Adult *Artemia franciscana.*

MATERIALS AND METHODS

Standard artificial seawater of (35 ± 1‰) was used for the culture to *Artemia* as well as for the toxicity test. Cysts were hatched in seawater (38 g/l), at 28 °C, under conditions of continuous illumination and aeration [12]. Then, adult Artemia were transferred into a multi-well test plate with the respective concentrations of the tested formulations, prepared in artificial seawater. The toxicity of the two herbicides on *Artemia fransiscana* adults was tested at 12, 24, 36 and 48 h of exposure. The rate of toxicity, based on LC50, was calculated. The lethal concentration (LC50) was tested by exposing 30 adults *Artemia fransiscana* per group (in triplicates), the control group was kept in experimental water without herbicides with all of the other conditions kept constant. Then the adult Artemia was exposed to 4-6 sequential rising concentrations of each herbicide (in triplicates)

in a way that zero and 100% mortality yield after 48 hours in selected concentrations. The mortality rate was recorded every 6 hours and until 48 hours.

The concentration of herbicides to induce 10-100 percent mortality was estimated after 12, 24, 36, and 48 hours using probit software version 1.5 designed by U.S. EPA [13].

Table 1: Experimental design table: concentration range tested on the adult *A. franciscana*

Tested herbicides	Concentration range (mg/L)	Number of treatments	Number of replicating	Total exposed Artemia
Paraquat	0.6, 1.25, 2.5, 5, 10, 20, 40	7	3	30
2, 4 - D	0.3, 0.6, 2.5, 5, 25, 50, 100, 200	8	3	30

Table 2: Specifications of the survey 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

RESULTS AND DISCUSSION

The Artemia mortality rate following the exposure to increasing concentrations of herbicides after 12, 24, 36, and 48 hours showed that the higher the herbicide concentration, the greater the mortality rate. 48 hours LC50 value was calculated at 2.701and 14.475 mg/l in *Artemia fransiscana.* In this study both tested herbicides were toxic for *A. fransiscana* but Paraquat was more toxic than 2, 4 – D.

In Khuzestan province of Iran, most fish ponds are built close to agricultural areas with shared water sources [1]. Water contamination by agricultural herbicides is a potential threat to productivity and a major cause of fish mortality. The main objective of this study was to investigate the effects of sub-lethal concentrations of paraquat and 2, 4 – D on adult *Artemia fransiscana.* The toxicity of various echotoxicants such as herbicides and their detrimental consequence on aquatic animals was mostly assayed by 48h LC50 in bio indicators. The results show differences toxicity varied between the two herbicides which used in our experiment. These results are in agreement with those reported in the literature for crustaceans [14] and [15] and pointed out the resistance of the two Artemia species to acute exposure to these herbicides. Among the evaluated herbicides Paraquat was more toxic than 2, 4 – D.

Besides, the acute toxicity (96 h LC50) of the herbicide paraquat for *Oreochromis niloticus* was 12.25 mg l-1 [16]. Deivasigamani et al. [12] 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 [12]. Based on the findings, the amount of lethal concentration of 2,4-D for 50 percent of Artemia was determined to be 14.475 mg/l after 48 hours. In toxicity studies, the sensitivity of organisms

can be different, even using the same product [17]. The results indicated although toxicity of two tested herbicides was different for *A. fransiscana*, their toxicity revealed positive correlation to herbicide concentration and exposure duration. Alishahi et al., [1] have reported that toxicity of herbicides in *L. esocinus*, and the mortality rate of exposed fish to herbicides enhanced either by increasing herbicides concentration or duration of exposure like our result too. Barahona and Sa´nchezFortu´n [18] reported on the exposure of three different life stages of *A. salina* to several phenolic compounds. These data show that animals of clearly defined age classes must be used for toxicity assays; since the toxicity expression is directly influenced by the development stage of the test organisms [18].

For sustainable agricultural activity, especially in areas that fish ponds and agriculture farms use the same water sources, it is highly recommended to use Paraquat as an alternative to 2,4-dichlorophenoxy-acetic acid.

CONCLUSION

This study supports the study’s hypothesis because sub-lethal concentrations of paraquat had significant effects adult *Artemia franciscana*. Continuous exposure of adult *A. franciscana* to sub-lethal concentrations of paraquat leads to great mortality.

Also, for unwanted weeds, especially in areas that fish ponds and agriculture farms use the same water sources, it is highly recommended to use 2, 4-Dichlorophenoxy Acetic Acid as an alternative to Paraquat.

Acknowledgment

Financial support of research deputy of Shahid Chamran University, Ahvaz, Iran is appreciated.

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