



## The acute toxicity of herbicide roundup ultra in mosquito fish *Gambusia affinis*

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### Abstract

Roundup Ultra it is a general non-selective herbicide were used widely by Iraqi farmers ,we here in determined the median lethal concentration (LC<sub>50</sub>) of Roundup Ultra herbicide in mosquito fish ( *Gambusia affinis*) by using several concentrations for studying acute effects of the herbicide (5,8,11,14,17,20,23,26,29,32) mg/l for 72 hours using the automatic transfer method using the SPSS program and using the probite method to assign the LC<sub>50</sub> of the pesticide then recording the behavioral changes that were showed on exposed fish . The results determined the value of LC<sub>50</sub> for Roundup Ultra herbicide in mosquito fish was (17.82) mg/l and show that the dead's fish increased with increasing concentrations. The results show also that behavioral changes as erratic swimming, hyperactivity, loss of equilibrium, increased in speed of gills operculum and touching the aquarium walls, increased with increasing concentrations. Due to the unsafe and excessive use of pesticides in the Iraqi environment, the present research was conducted to detect changes which are caused by the herbicide Roundup Ultra in mosquito fish.

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## Introduction

Pesticides are defined as a substance or a mixture of substances used to control agricultural pests (insects and Weeds) that are harmful to humans, animals and plants that affect the environment and health of society (Nordberg, 2009). There are many types of pesticides that vary according to the nature of their work, such as Insecticide, Herbicide, Fungicide, Rodenticide and other species such as Nematicide and Acaricide (WHO, 2006). Weed killers are commonly used to kill unwanted plants and herbicides are an selection to kill special targets, while the target crop is usually left without damage (Yamamoto and Nakamura, 2003) and also eliminates the diseases of beneficial plants. 98% of the insecticides sprayed as well as 95 % of herbicides reach distances beyond target areas (Miller, 2004). Ventura and others (2008) noted that pesticides in the aquatic environment can affect aquatic organisms in a variety ways, causing significant damage to plant and animal habitats, depleting the main sources of the fish food chain and the bioaccumulation of herbicides. Their concentration is high enough to cause a toxic response in fish (Hu, 2012). Recent decades have witnessed a significant increase in the production of pesticides in the world. In general herbicides are one of the technological advances in pest control and increase or maintain agricultural production. In the last decade the widespread use of pesticides in water pollution (Shiogiri, 2012). Herbicide Roundup Ultra is a general non-selective herbicide sprayed on leaves in the post-growth phase to eradicate the annual and perennial weeds and is used in the orchards of tree crops, field crops, paddy, industrial lands and aquatic areas. Effective substance Isopropyl Amine Glyphosate 480 g/l. Mosquito fish *Gambusia affinis* This mosquito fish is named because of its importance in controlling mosquitoes insect of the anovilus, the carrier of malaria (Deateneo, 2010). It feeds on mosquito larvae when they are released from mosquito eggs placed on the surface of the water, where they can eat several hundred larvae per day (Berthou, 1999). The mosquitoes fish are Carnivorous predators, which feed on different sources of food Omnivorous (Lockwood *et al*, 2007).

Mosquito fish prefer to live in warm, shallow, slow-moving, highly vegetative, highly mineralized and nutrient-rich waters; so they growing in freshwater, and also found in ponds, lakes, canals, streams, and rivers (Wydoski & Whitney 2003). They can also live in high salinity water, with low oxygen content waste water contaminated with oil stains and some dairy residues, therefore mosquito fish widespread in the world especially in the equator and under the equator (Al-Haefedh, 2007). Because of These characteristics of mosquito fish they are preferred for many researches.

Due to the unsafe and excessive use of pesticides in the iraqi environment, the present research was amid to detect changes which are caused by the herbicide Roundup Ultra in mosquito fish.

## Materials and methods

### *Experiment design*

The experiment lasted for 5 months from December 2015 until May 2016. 22 glass aquarium were used in dimensions (20 x 20x40) cm and the water volume in each aquarium was 14 liters. The glass aquarium were placed on iron racks with a height 100 cm and a width 100 cm and a length 180 cm. These aquariums are based on a layer of 10 cm cork to reduce the impact of the shocks on the glass aquariums. Coverings were placed on the glass aquariums made of a plastic clip surrounded by wooden frames to prevent the splashing of fish outside the aquariums during the experiment period. The aquariums were equipped with continuous oxygen through air pumps Installed on appropriate heights of glass aquariums, from which flexible plastic pipes to the bottom of the glass aquariums and are attached to a small stone to fix them close to the bottom to outfit the aquariums by oxygen. The aquariums were supplied with water by a water pump attached to a water tank of 500 liters to keep water in for 48 hours before use to dispose of chlorine in water. The water is manually changed by a plastic pipette and in a siphon way. The aquariums are cleaned by a mechanical pipette, the temperature of the water in the glass aquarium is controlled by 50 watts of china-made heaters when needed to keep the water temperature at the required degree 25-22 ° C.

*Fish experiment*

The mosquito fish were collected from the Jadriya channels/University of Baghdad. A fishing net, 5.2 mm diameter, and 900 fish were collected, using 730 fish and weighing between 0.3 - 0.4 g and 30-40 mm tall, the rest of the fish retained for compensation in the event of damage before the start of the experiment and during the period of acclimatization.

*Fish acclimatization*

The experimental fish after collected were put at 1% of the saline solution for 1 hour for purification from external parasites. The fish randomly and equally were distributed, the duration of the acclimatization period lasted two weeks before the experiment. The fish were distributed after the end of the acclimatization period at 10 fish per aquarium, approximately fish weights must be equal as much as possible in all experimental units, with three replicates per treatment. Fish were fed with dry chips at 5% of body weight and were given two meals at morning and evening during the duration of the experiment. Clean the ponds daily before and after the food to get rid of the waste and food residues by a mechanical pipette.

The aquarium water was switched on regular times and in a siphon way. The fish was weighed by a sensitive balance, and the lighting system was used 12 hours light and 12 hours darkness. Flake food, commonly known as Basic Flake, is available in the market and consists of: protein 38%, fat 3%, fiber 5%.

*Stock solution*

Prepare the stock solution for the pesticide by taking 100 ml of the pesticide and completing the volume to 1000 ml with distilled water. This one-time solution is prepared for all treatments to avoid the degradation of the pesticide and also to protect the solution from light and temperature rise. This method was used to prepare the required concentrations based on the active ingredient in the pesticide (AL-Azawi, 2010).

*Acute toxic effects*

Behavioral changes: These include monitoring and recording the observations that led to change in the fish behavior due to acute exposure to pesticide for 72 hours, and including: movement, swimming, movement of gills, breathing, food intake and balance.

*Median Lethal Concentration LC<sub>50</sub>*

The median lethal concentration of the pesticide was measured by using 10 parameters (each parameters contain 10 fish) and three replicates For each treatment, the concentrations of the pesticide Roundup Ultra 5, 8, 11, 14, 17, 20, 23, 26, 29 and 32 mg/l. The treatments which are exposed to the pesticide, are noticed for 72 hours and the results are recorded, including the concentration used, the number of losses per concentration and the number of fish remaining, using the automatic transfer method using the SPSS program and using the probite method to assign the LC<sub>50</sub> of the pesticide (AL-Samrri, 2011) and draw the diagram and determine the mean concentration.

**Results and discussion***Median lethal concentration LC<sub>50</sub> of herbicide Roundup Ultra*

The value of median lethal concentration of the mosquito fish when exposed to the acute exposure of the herbicide Roundup Ultra was determined for 72 hours by calculating the percentage of losses for each group of exposed fish, as shown in Table (1) using the SPSS program and the Probite method, the (LC<sub>50</sub>) of the pesticide, as shown in Fig. (1) the (LC<sub>50</sub>) of the pesticide was set at 17.82mg/l for the half of exposed fish. Studies (Sadeghi and Hedayati, 2014; Vera, 2012) showed variation in the value of LC<sub>50</sub> when exposed to different species of fish for the Roundup and glyphosate pesticide. The meanLC<sub>50</sub> concentration in female mosquito fish was 16.6 mg/l compared to the absolute glyphosate in the study of (Al Jowari, 2005) this value approximates the LC<sub>50</sub> value of the Roundup Ultra herbicide in the present study.

**Table 1.** The toxicity of the herbicide Roundup Ultra in mosquito fish with different concentrations over 72 hours.

Concentration mg/l	Dead's fish%	Total numbers of fish	Concentration logarithm	Probability units
0	0	10	0	0
5	0	10	0.69	0
8	0	10	0.9	0
11	20	10	1.04	4.16
14	30	10	1.14	4.48
17	40	10	1.23	4.75
20	50	10	1.30	5
23	70	10	1.36	5.52
26	80	10	1.41	5.84
29	90	10	1.46	6.28
32	100	10	1.50	7.33

*Behavioral changes in exposed fish*

The results of the present research showed a disturbance in fish behavior due to the acute exposure to herbicide Roundup Ultra, as showed in Table (2).

The toxicity of the Roundup Ultra in the fish is attributed to the compound poly oxyethylene amine (POEA) because of the direct exposure of fish and other aquatic organisms to the higher concentrations of the pesticide results in physiological effects and adverse effects (Jiraungkoorskul *et al.*, 2003).

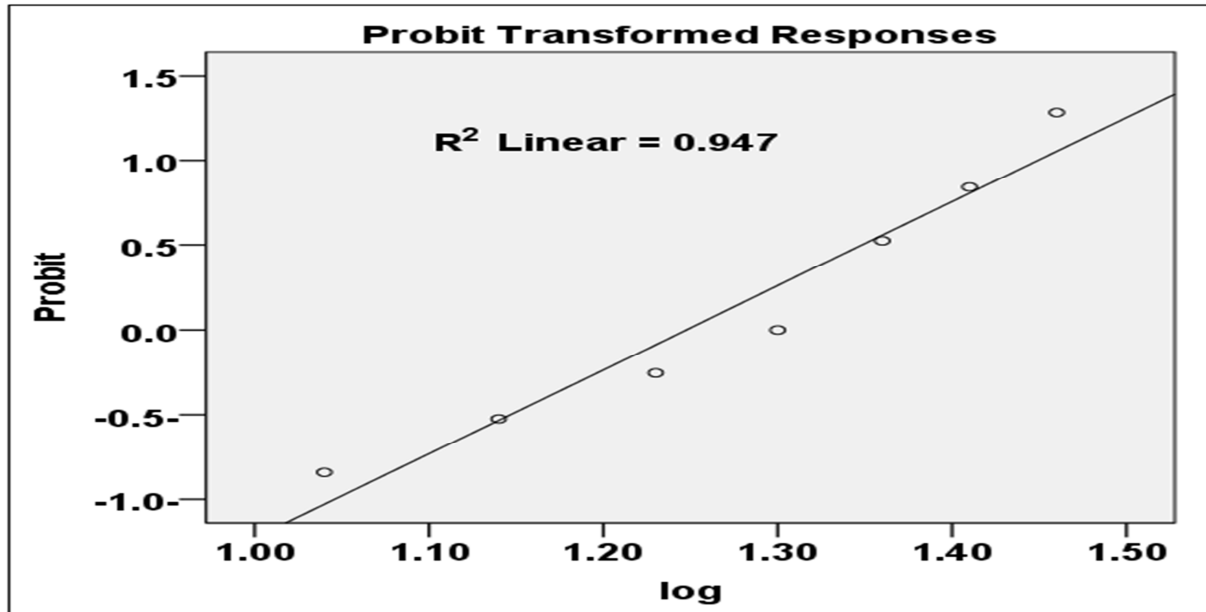
**Table 2.** Behavioral changes of mosquito fish exposed to different concentrations of the herbicide Roundup Ultra for 72 hours.

Concentration mg/l	Behavioral changes
0	Behavior and patterns of swimming are normal and the number of losses(0)
5	Behavior and patterns of swimming are normal and the number of losses(0)
8	Behavior and patterns of swimming are normal and the number of losses(0)
11	Random swimming, such as jumping up and down the aquarium quickly and stillness for a short time ( loos 2 fish)
14	They will jump back several times and then settle in the bottom of the aquarium then fumbling the walls of the aquarium and the loss of (3 fish)
17	The rotation of the fish around itself, its tendency to assemble at the corners of the aquarium, and increase the velocity of the operculum movement and the loss of (4 fish)
20	Increase the velocity of the operculum movement, loss of equilibrium and non-response fish to external effects and loss of (5 fish)
23	The same changes with difficulty of breathing, low speed of the operculum movement and loss of (7 fish)
26	The previous changes are the same with the idle observation, the reversal of fish on her back and the loss of (8 fish)
29	The long-term stagnation of the fish, and the incidence of congestion and erection of fins, loos (9 fish)
32	Loss of the whole fish after 72 hours of exposure

*The results agree with*

Ayoola (2008) when exposing the *Clarias gariepinus* fish to different concentrations of the herbicide glyphosate, so the fish were made randomly movements and had severely damaged by hitting the walls of the aquarium, and that causing its destruction. The increasing in the velocity of the mosquito fish, that may be associated with sudden response to fish led to the shock due to exposure to chemicals (Chindah *et al.*, 2004).

The rotational movement of the affected fish and fumbling the aquarium sides in the experiment , abnormal in fish behavior may be it's the failure to produce energy and fail to extract stored metabolic energy that may cause stress, causing severe stress, the results agree with (Okogwu *et al.*, 2015).



**Fig.1.** LC<sub>50</sub>-72h for herbicide Roundup Ultra in *Gambusia affinis*.

The loss of equilibrium of mosquito fish in the current research may be due to the inhibition of the brain's cytochrome C oxidase which causes damage to areas responsible for maintaining brain balance, the results agree with (David *et al.*, 2007). Random swimming, shivering and sudden irritation, of registered mosquito fish in the experiment possibly due to the accumulation of acetylcholine esterase (AChE) enzyme in neuromuscular ravel (Rao ,2005). The acetylcholine is the chemical responsible for the transfer of nerve impulses from neurons to specific receptors that may be other neurons or muscle cells, as the end of the transfer of nerve guidance when the formation of (AChE) breakdown of acetylcholine to choline, acetic acid and without the work of this enzyme, the acetylcholine accumulates in the neural connections between nerve cells and receptors leading to the cut off the transmission of nerve signaling, which may be due to the work of pesticides inhibiting the work of neurotransmitter enzymes, resulting in change in fish behavior. This explains the abnormal behavior of mosquito fish exposed to the pesticide Roundup Ultra, or may be due to fish fatigue, which is due to the attempts of many fish to escape from the poisonous medium or upward to the surface of the water to get oxygen, the results agree with (Okayi, 2010).

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