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Acute toxicity effects of 2,4-D herbicide on common carp(*Cyprinus carpio* Linnaeus, 1758) and grass carp (*Ctenopharyngodon idella* valenciennes,1844)

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Abstract

The acute toxicity effects of (2,4-D) pesticide to the common carp and grass carp was determined through 24, 48, 72 and 96 hr. Group of eight Common carp (Cyprinus carpio L., 1758; Pisces, Cyprinidae) and group of eight of grass carp (*Ctenopharyngodon idella*) were exposed to the 2,4-d. Lethal concentration (LC_{50}) was determined with probit analysis. The median lethal concentration (LC50) values for common and grass carp were (157.77, 157.25, 156.41 and 152.62) and (115.92, 114.92, 112.94 and 96.52) respectively. Behavioral changes of the above mentioned species were examined for various herbicide concentrations.

Keywords: Herbicide, Common carp, Grass carp, 2,4-D.

التأثيرات السمية الحادة لمبيد الاعشاب 2,4-د على اسماك الكارب الاعتيادي *Cyprinus carpio* (Linnaeus,1758) و الكارب العشبي(*Ctenopharyngodon idella* valenciennes,1844)

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الخلاصة:

حددت التاثيرات السمية الحادة لمبيد (2، 4 – د) لاسماك الكارب الاعتيادي واسماك الكارب العشبي خلال 24، 48، 72و 96 ساعة. عرضت مجموعة من ثمانية اسماك الكارب الاعتيادي ومجموعة من ثمانية اسماك الكارب العشبي لمبيد 2 ،4 –دي . حدد التركيز القاتل لنصف العدد بطريقة الاحتمالية . كانت قيم التركيز القاتل لنصف العدد لاسماك الكارب الاعتيادي والعشبي (157.77 ، 157.25، 156.41 و 152.62) و (115.92,114.92,112.94و96.52)للمعاملات المختلفة على التوالي . اختبرت التغيرات السلوكية للانواع المذكورة اعلاه بالتراكيز المختلفة للمبيد العشبي .

Introduction:

Large amounts of foreign substances enter the environment due to anthropogenic activities, the fate of these substances are in surface water and aquatic organisms [1]. Aquatic environment is the ultimate sink for all pollutants where they are going to affect the zoans more than their counterparts in the two environs of land and water [2]. Freshwater is highly vulnerable to pollution since they act as immediate sinks for the consequences of human activity always associated with the danger of

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accidental discharges or criminal negligence [3]. Some of these pollutants are directly discharged by industrial plants and municipal sewage treatment plants; others come from polluted runoff in urban and agricultural areas [4]. One of the important factors contaminating the natural habitat is agricultural pesticides. Pesticides can migrate from agricultural fields into the aquatic environment through surface, subsurface, and groundwater flows and subsequent river transport [5]. Regular inflow and high persistence can result in high pesticide concentrations in surface waters over weeks and months [6]. Herbicides, also commonly known as weed killers, are pesticides used to kill unwanted plants. These herbicides may enter from agricultural run-off, industrial terrestrial ecosystems effluent and other sources into aquatic media and produce undesirable side effects on biological and functional properties by changing the species composition of an algal community [7].2,4-D is a chlorophenoxy acetic acid herbicide, Toxicity to fish and aquatic invertebrates varies widely depending on chemical form, with esters being the most toxic [8 and 9]. Toxicity tests allow the determination of pollution effects, providing direct evidence of the biological response of aquatic organisms to contaminants [10]. Acute toxicity is widely used in order to identify the dose or exposure concentration and the time associated with death of 50 percent of the fish exposed to toxic materials which is expressed as LC_{50} in parts per million (ppm) or milligrams per liter (mg/L) [11]. In the acute toxicity test, juvenile fish are exposed to a range of toxicant concentrations in a static system for 96 h. A toxic effect is determined by a statistically significant decrease in the survival rate of fish exposed to the toxicant relative to the survival of fish in a control (i.e., without toxicant) [12]. In acute toxicity, pollutant toxicity to fish was tested by using lethal concentrations and determining the LC50 (lethal concentrations for 50% of fishes) [13].

Materials and methods

Experimental fish: Live specimens of the common (*Cyprinus carpio* L., 1758) and grass carp (*Ctenopharyngodon idella*, Val., 1844) (30±5 g) were purchased from hatchery in Al-Musayyib city , Iraq. The characteristics of experimental fish shown in Table-1. Fish were transferred to aquarium for acclimated to the laboratory conditions for one week. Fish were fed commercial food during acclimation period under continuous aeration condition and were fasted for 24 hrs before the start of the experiments. There was a simultaneous control group together with the actual experiments. The control group was kept in experimental water without adding the 2,4-D pesticide keeping all other conditions constant.

Table 1- characteristics of experimented fish

Species	Weight	Age
C. carpio	30±5 g	6 months
C. idella	30±5 g	6 months -1 year

Water: Water (dechlorinated tap water) was used in the experiment in glass aquarium. The temperature, dissolved oxygen (DO), and the pH of the water in the aquaria were checked before the experiment and kept constant through the experiment and its value show in Table-2. The water temperature was 21-25 C° .Also the experimental media was aerated in order to keep the amount of oxygen not less than 4mg/l (using portable pH/temp meter, oxygen meter) during acclimation period for measuring pH ,temperature and DO.

Table 2- Physical and chemical properties of the experiment water

physical and chemical properties	Value
Temperature (C°)	21-25
Dissolved oxygen (DO) (mg/l)	5-7
Hydrogen ion concentration(pH)	7.3- 7.5
Electrical conductivity (µs/cm)	900- 1150

Toxicant: The toxic compound used was 2,4-d (2,4-dichlorophenoxyacetic acid), the esteric toxic compound**.**

Acute Toxicity Experiment: The concentrations that used in the experiments were calculated depending on the following equation: $C1V1 = C2V2$ in mg/l units. The acute toxicity test was conducted under static (non-renewable) test conditions [14]. Uniform sized rectangular [6] glass aquaria (60×30×30 cm) were used for acute toxicity test. In each glass aquarium total volume of water was maintained at 30 L and was provided with contineous aeration. Eight fish (for each species) were exposed to 0, 130, 140, 145, 155, 160, 165 and 170 ppm (common carp) and 0, 70, 95, 115,130 and 140 ppm (grass carp) from 2,4-d (2,4- dichlorophenoxy acetic acid) . Fish mortality was recorded at an interval of 24 hrs over a period of four days. Dead fish were immediately removed by dip net to avoid possible deterioration of the water quality [15]. LC_{50} values were calculated from the data obtained in acute toxicity bioassays, by Finney's (1971) method of "probit analysis" and with SPSS computer statistical software [16]. In this method the experimental solution (i.e. 2,4-d herbicide) and the samples (i.e. fish) are put in a suitable experimental cell (i.e. aquarium) and kept like that for a certain period. Since the decreased amount of oxygen and increased metabolic waste become a problem in long term experiments, the duration of such experiments are usually kept at 96 hr. or less [17].

RESULTS AND DISCUSSION

The physico-chemical characteristics of the test water are given in Table-2. Water quality parameters throughout the acclimation and experiments period were similar and relatively stable. For common carp, an increase in number of mortalities with an increase in concentration of the herbicide was observed and has been presented in Tables -3. There was 100% mortality at 170 ppm concentration within the 24hrs after dosing for all fishes and no mortality at 130 ppm within the exposure times. LC_{50} at 24,36,72,96 hrs was 157.77, 157.25, 156.41 and 152.62 shown in figure-1. No mortality observed in the control group during the 96hr of the experiment. The changes in behavioral response are the most sensitive indicators of potential toxic effects [18]. Fish in the control experiment appeared active and healthy throughout the test period. The changes in behavioral response started 2hrs after dosing. The lowest concentration (130 ppm) showed behavior similar to the control group. The higher concentrations (over 130 ppm) showed abnormal behavioral responses (rapid gill movement, gulping air at the surface, erratic swimming, loss of equilibrium,) in all fishes. At the highest concentration 170 ppm , all these responses were observed at high intensities. The abnormal behavioral responses increased with increasing concentration and exposure time. Similar behavioral responses determined in this study have been observed with the spotted snakehead *Channa Punctatus* exposed to various concentrations of atrazine herbicide [19].

Median lethal concentration (LC_{50}) is the most widely accepted basis for acute toxicity test and it is the concentration of a test chemical which kills 50% of the test organisms after a particular length of exposure, usually 96 hrs [20]. The effects of herbicides depend upon depth of water reservoir, rate of flowing water, fish densities and chemical composition of the herbicide [21]. Hardness of water, pH and temperature may also be key factors [22]. While grass carp, also the mortality rate increase with increase the concentration of the herbicide and that shown in Table -4. At the concentration 140 ppm the mortality rate was 100% concentration within the 24hrs after dosing for all fishes and at 70 ppm there was no mortality within the exposure times. LC_{50} values was 115.92, 114.92, 112.94 and 96.52 show in figure-2.

The control group stays normal and no mortality. The changes in behavioral response started 1hrs after dosing. The lowest concentration 70 ppm showed behavior similar to the control group. The higher concentrations (over 70 ppm)showed abnormal behavioral responses which represented by hyperventilation, jumping and sudden startling, abnormal swimming , vertical and downward swimming patterns, balance loss, the fish were observed to have breathing difficulties and tried to breathe air from the surface in all experimental fishes. The LC_{50} values of both carp species were shown in Table-5.

Table 3- Cumulative mortality of common carp after 24, 48, 72 and 96 hrs of exposure to different 2,4-d concentrations

Concentrarions ppm	Log of Conc.	24 hr. Probit unit	48 hr. Probit unit	72 hr. Probit unit	96 hr. Probit unit
130	2.113	0	0	0	0
140	2.146	0	0	0	3.85
145	2.161	3.85	4.33	4.33	4.65
155	2.190	4.65	5.00	5.00	5.32
160	2.204	5.32	5.32	5.67	6.10
165	2.217	5.67	5.67	6.10	6.10
170	2.230	7.33	7.33	7.33	7.33

Table 4- Cumulative mortality of grass carp after 24, 48, 72 and 96 hrs of exposure to different 2,4-d concentrations

Concentration ppm	Log of Conc.	24 hr. Probit unit	48 hr. Probit unit	72 hr. Probit unit	96 hr. Probit unit
70	1.845	0	0	0	3.85
95	1.977	3.85	3.85	4.33	4.65
115	2.060	4.65	4.65	5.00	5.32
130	2.114	5.32	5.67	5.67	6.10
140	2.146	7.33	7.33	7.33	7.33

Table 5- comparative between LC_{50} of two experimental carp fish

Species	Herbicide	Median lethal concentration LC_{50}			
		24 hr.	48 hr.	72 hr.	96 hr.
C. carpio	2,4-d	157.77	157.25	156.41	152.62
C. idella	2,4-d	115.92	114.92	112.94	96.52

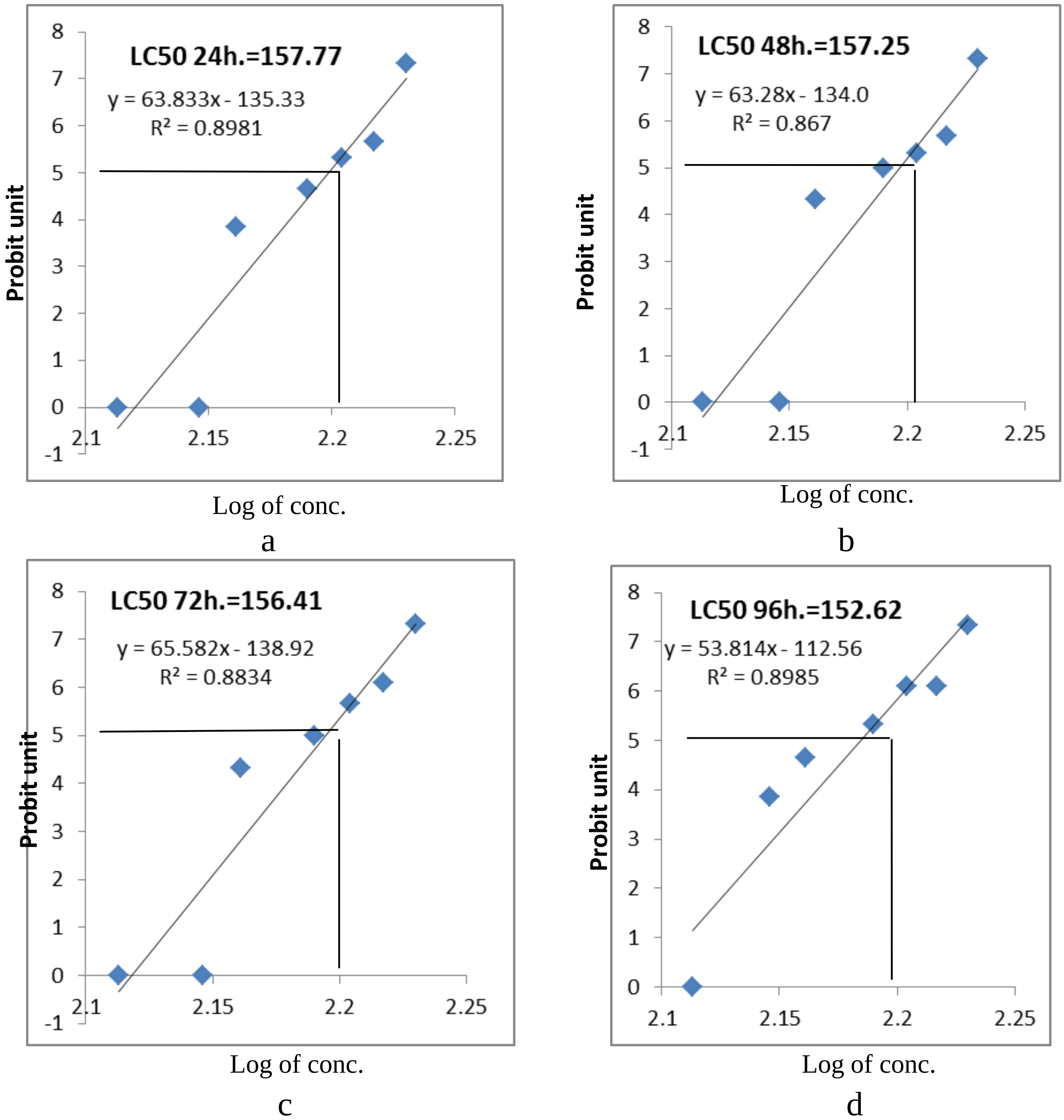

Figure 1- Median lethal concentration value of 2,4-D herbicide in *Cyprinus carpio* through:
a- 24 hr of exposure to 2,4-D pesticide, the LC_{50} was 157.77
b- 48 hr of exposure to 2,4-D pesticide, the LC_{50} was 157.25
c- 72 hr of exposure to 2,4-D pesticide, the LC_{50} was 156.41
d- 96 hr of exposure to 2,4-D pesticide, the LC_{50} was 157.62

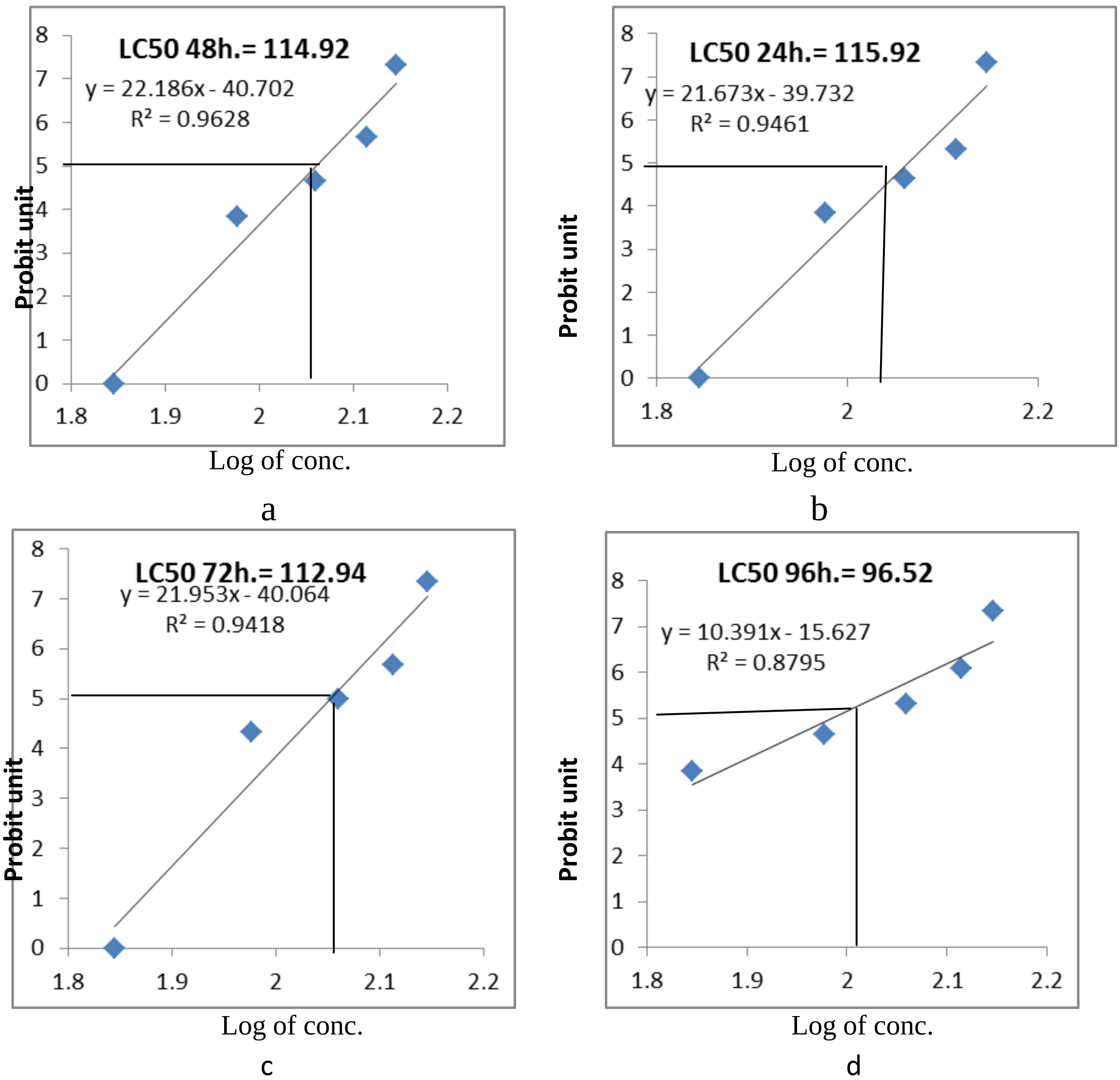

Figure 2- Median lethal concentration value of 2,4-d herbicide in *Ctenopharyngodon idella* through:
a- 24 hr of exposure to 2,4-D pesticide, the LC_{50} was 115.92
b- 48 hr of exposure to 2,4-D pesticide, the LC_{50} was 114.92
c- 72 hr of exposure to 2,4-D pesticide, the LC_{50} was 112.94
d- 96 hr of exposure to 2,4-D pesticide, the LC_{50} was 96.52

This study is focused on the acute toxic effects of 2,4-D on *C. carpio* (L., 1758) *C. idella* (Val., 1844)and estimating LC_{50} values as well as the behavioral changes in the fish subjected to different concentrations of 2,4-D. The influences of herbicide on aquatic species include direct killing [23, 24], functional disorders and reproductive abnormalities [25].Fish and aquatic animals are exposed to pesticides in three primary ways (1) dermally, direct absorption through the skin by swimming in pesticide-contaminated waters, (2) breathing, by direct uptake of pesticides through the gills during respiration, and (3) orally, by drinking pesticide-contaminated water or feeding on pesticide-contaminated prey [26]. Behavioral abnormalities of two experimental carp species those subjected to various concentrations of 2,4-D were the anxiety, sudden jerks, loss of balance, swimming upside down or vertical manner, respiratory difficulties, gathering at the surface for breathing, and hitting to the side walls of the aquaria. Ferrando *et al*. [27], in their study on the effects of eight selected organochloride pesticides such as endosulphane, diazinone, phenyltrithian and methylparathion on eels, they determined their 96-h LC_{50} values and reported behavioral changes in fish. They also observed anxiety, disorders in swimming pattern, loss of balance, excessive mucus secretion and lightening in color. Although the modes of function of these insecticides are markedly different than 2,4-D, behavioral changes observed are similar to ours. Another study in the acute effect of 2,4-d on

C.carpio showed similar behavioral changes [28]. A pesticide's capacity to harm fish and aquatic animals is largely a function of its (1) toxicity, (2) exposure time, (3) dose rate, and (4) persistence in the environment. The widespread use of chlorophenoxyacetic acid as an herbicide and a growth regulator in agriculture, forestry, and gardening has increased the damage incurred by these toxic compounds on environment and human health [29].The agricultural chemicals contaminate all abiotic media particularly water and soil. The contamination of underground waters and other water sources by agricultural chemicals poses a potential threat to aquatic organisms and fish. Toxic compounds have an effect on fish, which are the last part of the food chain in aquatic ecosystem, and have an effect on other animals, which feed on fish, to be exposed to the same toxic effect. It also accumulates in tissues and causes acute poisoning [30].

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