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EFFECT OF JASPER HERBICIDE ON SOME IMMUNOLOGICAL AND PHYSIOLOGICAL ASPECTS OF NILE TILAPIA "*Oreochromis niloticus*"

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ABSTRACT

Recently, excessive use of herbicides appeared to boost agricultural crops production. This caused many health problems for farmed fish and led to poor production. Therefore, this study was conducted to evaluate the toxicity of herbicide (Jasper) on Nile tilapia as one of the most recently used herbicides. The 96-h LC_{50} was determined and found to be 3.80 ppm. While setting this dose, the fish showed irregular swimming movements. Mucus secretion and buildup on the gills increased, and fish showed breathing disorders while swimming on the surface of the water, with their mouths opened with rapid and frequent exhalation.

The immunological and physiological assays were evaluated for one month under exposure to 1/4 and 1/8 of the 96-h LC_{50} (0.95 and 0.48 ppm). These sublethal concentrations of jasper caused significant reduction in the activity of plasma superoxide dismutase **(**SOD**)**, catalase (CAT), glutathione peroxidase **(**GPX) and acetylcholinestrase (AChE). While, plasma uric acid, creatinine and malondialdehyde (MDA) were elevated significantly at all periods of exposure. The activities of alanine transferase (ALT), aspartate transferase (AST) and alkaline phosphatase (ALP) were increased significantly through the 4^{th} and 15^{th} day of exposure and declined at the end of the experimental period. Total plasma protein was decreased through all periods, while total lipids and glucose was significantly increased in all treated fish groups on the 4^{th} and 15^{th} day of exposure and significantly decreased at the end of exposure time.

In the light of the present study, we can conclude that judicious use of jasper herbicide should be done by the fish farmers to avoid haphazard water pollution and obtain good fish production**.**

Keywords: Nile tilapia, Jasper herbicide, Immunity, physiology, biomarkers.

INTRODUCTION

Most of the water bodies have become polluted due to haphazard and extravagant pouring of wastes from farms, industries and domestic uses which gradually find their way into the aquatic environment and making it unfavorable for aquaculture (Chindah *et al.,* 2008). The aquatic environment is not only the ultimate recipient of pollutants, but also the place where some chemicals are applied directly. Consequently, aquatic organisms; including fish are subjected to different toxic agents which adversely affect the immunological and physiological state of fish through multiple pathways (Wester, 1988). Accordingly, the effect of various pollutants on fish has been researched and is still under study. The most numerous contaminants are heavy metals and pesticides (Khoshnood, 2016). Pesticides are substances used to control organisms, including insects, water weeds, and plant diseases. Pesticides usage in agricultural fields to control pests is extremely toxic to non target organisms like fish and affect fish health through impairment of metabolism, sometimes leading to mortality (Shankar *et al*., 2013). During those days, increased human population with rapid pace of industrialization induced problem of disposal of waste waters. The domestic wastes and untreated or partially treated industrial effluents, supplemented with pollutants like heavy metals, pesticides and many organic compounds, heave greatly contributed to massive fish death of aquatic ecosystems (Pazhanisamy and Indra, 2007). Pesticides toxicity in fish has been studied by several authors who have shown that at chronic level, it causes diverse effects including oxidative damage, inhibition of AChE activity as well as dysfunctions of liver and kidney. Since pesticides present in the environment with other similar organophosphate compounds, may induce lethal or sublethal effects in fish (Mathur, 1999). Many pesticides have been banned and others are under strict regulations for use. Now, pesticides are more species-specific, less motile and less persistent for reducing their effects on non-target organisms (Khoshnood *et al*., 2014).

Herbicides are widely used for the control of unwanted water plants. While, the direct effect of herbicides addition is the loss of macrophytes, non-

target organisms including fish may also be affected through loss of habitat and food supply (Ervnest, 2004). Bai and Ogbourne (2016) recorded that the fish species have many health problems due to the continuous subjection to the herbicides residues due to their increasing use in agriculture.

Jasper 520 EC herbicide is used as a non-selective herbicide. It is produced as haloxyfop-R-methyl ester and is used as post-emergence selective herbicide. It controls annual and perennial grasses in sugar beet, oil seed, potatoes, leaf vegetables, onions, sunflowers, strawberries, and other crops. Haloxyfop-R-methyl should not be allowed to be used in aquatic environments, except for limited time periods, in specific discrete areas, where there is a biosecurity emergency and where no alternative herbicide is available for specific waterweed eradication (EPA, 2012).

Nile tilapia (*Oreochromis niloticus*) is one of the most important commercially cultured tilapia species in Egypt. However, no reports have described the effects of jasper herbicide on Nile tilapia (*O. niloticus*) and few for other freshwater organisms.

Velkova-Jordanoska *et al*. (2008) and Lopez-Lopez *et al*. (2011) confirmed that many environmental pollutants or their metabolites are capable of inducing liver and kidney dysfunctions as well as oxidative stress in fish. Antioxidants such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX) and malondialdehyde (MDA) have been proposed as biomarkers of contaminant or seasonally mediated oxidative stress in a variety of marine and freshwater organisms and their induction reflects a response to pollutants (Borković *et al*., 2005). Hamed and El-Sayed (2018) and Abd-Allah *et al*. (2019) recorded different alterations in activities of SOD, CAT, MDA and GPX in liver and gills tissues of glyphosate, pendimethalin and cadmium toxicated Nile tilapia as a result of oxidative stress. Some biochemical parameters also represent fine tools for evaluating the effects of contaminants and for environmental monitoring (Ahmad *et al*., 2004). These biomarkers include acetyl-cholinesterase (AChE).

Gonado-somatic index (GSI) is a bioindicator that supply structural information, more than functional to respect of health and gonadal maturation status. There is evidence that the majority of the species undergo reproductive cycle and, frequently, variation in the gonadal size is observed across of the cycle (Sadekarpawar and Parikh, 2013). Consequently, calculating the GSI has been used for determining the reproductive maturity, as well as responses to environmental dynamics (e.g., exposition to contaminants and exogenous stress). Now, there is clear evidence that exposition to several environmental pollutants can result in gonad alterations like reduction of GSI, (Sakamoto *et al*., 2003). Billiard and Khan (2003) observed alterations in hepato-somatic index (HSI) of fish exposed to different pollutants.

So, this study aimed to evaluate the toxicity of jasper herbicide for Nile tilapia; *Oreochromis niloticus* because no data available about that.

MATERIALS AND METHODS

Herbicide:

Jasper 520 EC herbicide with active constituent 520 g/l haloxyfop-R-methyl ester.

Fish:

Apparently healthy Nile tilapia; *Oreochromis niloticus* with an average body weight 40.0±3.0 g were collected from the fish farm of Central Laboratory for Aquaculture Research (CLAR), Abbassa, Abu-Hammad, Sharqia, Egypt. Fish were acclimatized in laboratory conditions for 15 days prior the experiment.

1- Determination of 96 h-LC_{50}:

Eight groups of fish were conducted in 30 L glass aquaria, 10 fish per aquarium, containing commercial form of jasper in dechlorinated tap water to the following concentrations: 0.0 (control group), 1, 2, 3, 4, 5, 6 and 7mg/l. Each treatment had 3 replicates. All laboratory conditions were maintained constant. Deaths and abnormal behavior fish were recorded every day for 4

days. Then the value of 96-h LC_{50} was calculated according to Behreus and Karbeur (1953).

Table 1. Determination of the 96-h LC_{50} = Biggest concentration-∑ab/n).

Group	Number of fish in each group	Herbicide Concentration (mg/l)	Dead Fish in each group	(a) The difference between each two consecutive concentrations	(b) Average of dead fish in each two consecutive groups	(ab)
1	10	0.0	0.0	0.0	0.0	0.0
2	10	1	1	1	0.5	0.5
3	10	2	3	1	2	2
4	10	3	3	1	3	3
5	10	4	5	1	4	4
6	10	5	7	1	6	6
7	10	6	8	1	7.5	7.5
8	10	7	10	1	9	9
				∑ab = 32.00		

96 h-LC_{50} = 7- 32.00/10 = 7.00 - 3.2 = 3.80 mg/l

2- Experimental Design.

Three groups of fish were set, each of 30 fish divided into three replicates of ten fish and maintained in glass aquaria (each 90 liter capacity) with dechlorinated and aerated tap water. All were kept in constant and suitable lab conditions (temperature of 27±1 ^{o}C, pH 7.1±0.2 and dissolved oxygen 4.5 ± 0.5 mg/l). The 1st group was kept as control. The 2nd was exposed to the $^{1}/_{4}$ LC_{50} (0.95 ppm) and the 3rd was exposed to the $^{1}/_{8}$ LC_{50} (0.48 ppm) of the used herbicide, and left for 30 days to determine the effects of this herbicide on some immunological and physiological assays. The feeding rate was 3% of the body weight.

Blood samples were collected by syringe contained EDTA solution as anticoagulant from the caudal veins of anesthetized fish at the end of the 4th, 15th and 30th day of exposure. The blood samples were centrifuged to obtain the plasma which was kept in deep freezer till biochemical analyses. Superoxide dismutase **(**SOD**)** activity was determined by the method of Flohé and Otting (1984). Catalase (CAT) activity was determined according to the

technique described by Beutler (1975). Glutathione peroxidase (GPX) activity was determined by the method of Flohé and Gunzler (1984). Malondialdehyde (MDA) was determined according to Ohkawa *et al.* (1979). Acetyl-cholinesterase (AChE) activity was measured as described by Ellman *et al.* (1961). Alanine transferase (ALT) and aspartate transferase (AST) were determined according to Reitman and Frankel (1957). Alkaline phosphatase (ALP) was determined according to the method of Bergmeyer (1974). Uric acid was determined according to Barham and Trinder (1972). Glucose was determined according to Trinder (1969). Total protein and creatinine were determined according to Henry (1964). Total lipids was determined according to Schmit (1964).

Statistical analysis

The obtained results were subjected to analysis of variance (ANOVA). Duncan multiple range test (Duncan, 1955) was further used to evaluate the mean differences at $P \leq 0.05$ significant levels.

RESULTS AND DISCUSSION

Herbicides are substances used to control unwanted herbs causing crop damage and prevent loss of plant production. Pesticides (including herbicides) usage in agricultural fields is extremely toxic to non target organisms like fish and affect fish health through impairment of metabolism, sometimes leading to mortality (Shankar *et al*., 2013). A Pesticides capacity to harm fish and aquatic animals is largely a function of its toxicity, exposure time, dose rate, and persistence in the environment. A lethal dose is the amount of pesticide necessary to cause death because not all animals of a species die at the same dose, a standard toxicity dose measurement, called a lethal concentration 50 (LC_{50}), is used. This concentration of pesticide that kills 50% of a test population of fish within a set period of time is usually determined after 24 to 96 hours. According to Choudhury (2018), hazard ratings ranging from minimal to super toxic and LC_{50}'s for commonly used insecticides, herbicides, and fungicides are presented as follow:

Minimal toxic >100, Slightly toxic = 10-100, Moderately toxic = 1-10, Highly toxic = 0.1-1.0, Extremely toxic = 0.01-0.1 or Super toxic < 0.01mg/l.

In this study, the 96 h-LC_{50} of jasper for Nile tilapia (40±3 g) was found 3.80 ppm. So, jasper herbicide could be consider moderately toxic for tilapia. The Agrochemicals Handbook (1994) stated that the 96 h-LC_{50} of jasper herbicide for fathead minnows is 0.54 mg/l, for bluegill sunfish is 0.28 mg/l, for rainbow trout is 1.8 mg/l and is 0.3 mg/l for *Lepomis macrochirus*. Tooby (1971) recorded many differences in the toxicities of different herbicides even between different forms of the same herbicide. During determination of the 96-h LC_{50}, the fish exhibited erratic swimming movements. The mucus secretion appeared to increase and accumulated on the gills and the fish exhibited a respiratory disorder with surfaced swimming, opening their mouth with rapid and frequent exhalation.

Environmental pollutants such as herbicides are one of the most exogenous resources that lead to oxidative stress by causing excess production of reactive oxygen species (ROS) within the cell. These are known as rather reactive agents that lead to oxidative disruption in biomolecules like lipids, proteins, and nucleic acids. The negative imbalance between ROS generation and antioxidant defense capacity is called as oxidative stress. This fact has been confirmed by Monteiro *et al.* (2006), Dogan *et al.* (2011) and Jin *et al.* (2011) who are stated that reactive oxygen species (ROS) led to disturbance in physiological cell processes due to attack lipids, proteins and DNA in the living cells. Burella *et al.* (2018) mentioned that in order to maintain reactive oxygen species (ROS) amount in the cells at a normal level, the antioxidant superoxide dismutase (SOD) work together with antioxidant enzymes like catalase (CAT), peroxidase (GPx) and malondialdehyde (MDA) with other antioxidants. Superoxide dismutase is an enzyme that alternately catalyzes the dismutation of the superoxide (O_2^-) radical into two less damaging species: either molecular oxygen (O_2) or hydrogen peroxide (H_2O_2). Superoxide is produced as a by-product of oxygen metabolism and, if not regulated, causes many types of cell damage. Malondialdehyde (MDA) is a highly reactive three carbon dialdehyde

produced as a byproduct of polyunsaturated fatty acid peroxidation and arachidonic acid metabolism.

Data presented in Table 2 showed that jasper herbicide caused significant reduction in the activity of SOD, CAT, GPX through the different periods of exposure to both sublethal concentrations for tilapia. On the other hand, the activity of MDA was increased significantly at all periods of exposure. The reduction in SOD activity was reported also by Kathya and Martinez (2010) after exposure of neotropical fish; *Prochilodus lineatu* to the roundup herbicide for 6 hours and he attributed the decreasing to the production of oxidants. Excess of hydrogen peroxide may reduce SOD activity, while the superoxide anion may be responsible for decreased CAT activity (Bagnyukova *et al*., 2006). It is known that there is a complex pathway of interaction among the enzymes involved in the animal's antioxidant system and that the activity of one enzyme influences the activity of others. It is also known that the substrate of product of some of the antioxidant enzymes can also influence the activity of others. So, it is reasonable to assume that hydrogen peroxide is responsible for the reduction observed in the SOD activity. This is confirmed by the reduction of CAT activity in fish after exposure to the herbicide due to the accumulation of hydrogen peroxide in the cell. Similarly, this reduction in CAT activity may be due to superoxide ions, which are probably not being neutralized efficiently by SOD. The SOD–CAT system is the first line of defense against oxygen toxicity, due to the inhibitory effects on the formation of oxy-radicals (Pandey *et al*., 2003), and these enzymes are frequently used as biomarkers, indicating the production of ROS (Monteiro *et al*., 2006). Also, Hamed and El-Sayed (2018) showed reduction in activity of SOD and CAT but significant increasing of MDA in liver tissues of Nile tilapia after exposition to pendimethalin for 28 days as a result of oxidative stress. Abd-Allah *et al*. (2019) recorded different alterations in these antioxidants in gills and liver tissue of glyphosate and cadmium toxicated Nile tilapia. Many studies gave similar results to that of the present study such as increase activity of MDA of Nile tilapia after exposure to the 96 h-LC_{50} concentration of fenitrothion insecticide for 4 days (Abu Zeid *et*

al., 2014). Gills of *Labeo rohita* exposed to cadmium chloride at 96 hrs led to oxidative stress where activity of catalase decreased (Dabas *et al*., 2012). Yonar and Sakin (2011) found that CAT activity has been inhibited in fish species exposed to pesticide deltamethrin. Modesto and Martinez (2010) confirmed that herbicides induced lipid peroxidation in various fish species causing oxidative stress. MDA readily combines with several functional groups on molecules including proteins, lipoproteins, and DNA. MDA-modified proteins may show altered physico-chemical behavior and antigenicity. It is one of the lipid peroxides (LPO) products deriving from oxidative attack on cell membrane phospholipids and circulating lipids, and its level directly reflects the degree of oxidative damage induced by contaminants (Banerjee *et al*. 1999). The measurement of MDA content (an index of lipid peroxidations) provides a relative measure of the potential for pollutants to cause oxidative injury (Vlahogianni *et al.* 2007). The elevated MDA level was considered as a result of oxidative stress from xenobiotics. Similar results were obtained by Gu¨l *et al*. (2004) in case of cyprinidae in polluted areas. Ahmed *et al*. (2014) found that MDA activity in liver of cadmium toxicated tilapia was significantly increased at all experimental periods but in gills, was significantly increased at 42 days. Exposed liver cells of *Oreochromis niloticus* to diclofenac (DCF) for 30 days showed activity of CAT inhibited and the activities of SOD increased (Pandey *et al*., 2017).

Table 2. Changes in plasma SOD, CAT, GPX and MDA of Nile tilapia "*Oreochromis niloticus*" through different periods of exposure to sublethal concentrations of jasper herbicide**.**

Parameters	SOD (U/ml)			CAT (U/L)			GPX (U/L)			MDA (nmol/ml)		
Time	4th day	15th day	30th day	4th day	15th day	30th day	4th day	15th day	30th day	4th day	15th day	30th day
Control	3.67^{A} ±0.24	3.70^{A} ±1.11	3.56^{A} ±0.07	180.66^{A} ±2.61	180.33^{A} ±3.76	182.33^{A} ±3.28	89.66^{A} ±2.44	90.33^{A} ±2.23	85.66^{A} ±1.76	10.36^{C} ±0.54	11.30^{B} ±0.75	11.16^{B} ±0.20
1/4 LC50	2.74^{B} ±0.31	2.36^{B} ±0.17	2.58^{B} ±0.02	110.00^{B} ±2.93	158.66^{B} ±3.76	120.00^{C} ±0.57	71.60^{C} ±3.31	76.00^{C} ±3.13	79.00^{B} ±1.15	17.50^{A} ±0.56	15.50^{A} ±0.50	13.13^{A} ±1.53
1/8 LC50	2.16^{C} ±0.38	1.70^{C} ±0.28	2.24^{B} ±0.54	100.00^{C} ±3.22	157.00^{B} ±1.52	128.66^{B} ±0.33	84.00^{B} ±1.76	86.00^{B} ±1.16	84.66^{A} ±2.25	15.40^{B} ±0.65	13.76^{AB} ±0.61	13.13^{A} ±0.54

Data are expressed in means ± SE.

Means with the same letter within the same column are not significantly different at $P<0.05$.

Data in Table 3 revealed the changes in acetylecholinesterase (AChE), uric acid and creatinine in plasma of *O. niloticus* subjected to sublethal concentrations of jasper herbicide. AChE plays an important role in neurotransmission at cholinergic synapses and neuromuscular junctions by rapidly hydrolyzing acetylcholine to choline and acetate. The exposure concentrations of pesticides that are not lethal to fish may affect their physiology and behavior, ultimately decreasing survival, reproduction, metabolic disturbances, and growth (Kegley *et al*., 1999). Herein jasper herbicide caused inhibition in AChE activities. These findings agree with those of Modesto and Martinez (2010) and Kathya and Martinez (2010) who found that several herbicides inhibit AChE activity in fishes. Inhibition of AChE specially in the brain produces alterations in behavior, and in muscle leads to hyper stimulation of muscle fibers, which may cause titania, paralysis and death (Kirby *et al*., 2000).

Table 3. Changes in plasma AChE (uM), Uric Acid (mg/dl) and Creatinine (mg/dl) of Nile tilapia "*Oreochromis niloticus*" through different periods of exposure to sublethal concentrations of jasper herbicide**.**

Parameters	AChE (uM)			Uric Acid (mg/dl)			Creatinine (mg/dl)		
Time	4th day	15th day	30th day	4th day	15th Day	30th day	4th day	15th day	30th day
Control	15.16^{A} ±0.29	17.40^{A} ±0.80	15.46^{A} ±0.43	4.77^{C} ±0.08	4.83^{C} ±0.18	4.65^{C} ±0.14	0.89^{C} ±0.01	0.97^{C} ±0.01	0.92^{C} ±0.01
1/4 LC50	10.23^{C} ±0.65	10.30^{B} ±0.78	11.26^{B} ±0.34	8.16^{A} ±0.38	7.10^{A} ±0.34	5.86^{A} ±0.03	1.62^{A} ±0.04	1.87^{A} ±0.02	1.89^{A} ±0.02
1/8 LC50	12.50^{B} ±0.69	11.70^{B} ±0.91	11.40^{B} ±0.59	6.83^{B} ±0.59	6.70^{B} ±0.25	5.11^{B} ±0.08	1.58^{A} ±0.01	1.44^{B} ±0.05	0.99^{B} ±0.01

Data are expressed in means ± SE.

Means with the same letter within the same column are not significantly different at P<0.05.

The muscle hyperactivity leads to intracellular ATP depletion and enhances the generation of reactive oxygen free radicals which may play an important role as mediators of skeletal muscle damage and inflammation (Yang *et al.*, 1996). Accordingly, accumulation of oxygen free radicals might be a consequence of AChE inhibitor-induced muscle hyperactivity.

The levels of uric acid and creatinine (Table, 3) were increased in fish exposed to both concentrations of jasper. These could be suggested that jasper herbicide had nephrotoxic and hepatotoxic effects. This increase might be induced by damage in the glomeruli, increased muscle tissue catabolism or impairment of general metabolism as previously reported by El-Ashram and Mohammed (2011). Plasma biomarkers uric acid, creatinine, alanine transferase (ALT), aspartate transferase (AST) and alkaline phosphatase (ALP) have been used to detect cellular damage in kidneys and liver and measure the responses to any pollutant (Yang and Chen, 2003). Their measurements can be useful as a diagnostic tool in fish toxicology to identify their general health status and target organs affected by toxicants (McDonald and Grosell, 2006).

In the present study, exposure of Nile tilapia to sublethal concentrations of jasper herbicide increased the plasma activity of ALT, AST and ALP at 4th and 15th and decreased at the 30th day of exposure (Table, 4). The increased

concentration of transaminases in blood may be attributed to the hepatocellular damage and release the stored enzymes or cellular degradation induced by the toxic effect in the liver, heart or muscles (Öner *et al*., 2008).The decrease of these enzymes at the 30th day may be attributed to the exhaustion of the fish liver and other organs and the inhibitory effects of the pesticides which are dependent on their binding capacity to the enzyme active site and by their rate of phosphorylation (El-Ashram and Mohammed, 2011). These results were in agreement with Mousa (2004) and Mastan and Shaffi (2010).

Table 4. Changes in plasma ALT (U/L), AST (U/L) and ALP(U/L) of Nile tilapia "*Oreochromism niloticus*" through different periods of exposure to sublethal concentrations of jasper herbicide**.**

Parameters	ALT (U/L)			AST (U/L)			ALP (U/L)		
Time	4th day	15th day	30th day	4th day	15th Day	30th day	4th day	15th day	30th day
Control	22.73 C ±1.06	21.43 C ±0.96	22.80 A ±1.85	17.67 B ±0.89	18.13 C ±1.12	18.60 A ±1.26	52.00 C ±3.05	54.00 C ±3.46	56.00 A ±0.58
1/4 LC50	30.03 A ±1.08	28.46 A ±0.51	15.78 B ±0.86	31.33 A ±2.01	31.53 A ±1.48	10.13 B ±0.68	76.66 A ±1.20	125.0 A ±3.60	53.00 B ±1.52
1/8 LC50	25.36 B ±0.66	23.33 B ±0.43	15.50 B ±0.20	32.16 A ±0.84	24.26 B ±1.92	12.10 B ±2.28	59.66 B ±0.89	64.66 B ±1.45	51.33 B ±1.76

Data are expressed in means ± SE.
Means with the same letter within the same column are not significantly different at P<0.05.

Quantitative determination of total plasma protein reflects the liver capacity of protein synthesis and refer to the osmolality state of the blood as well as the renal impairments. So, it is of valuable effect in the diagnosis of the toxicity in the fish.

The decrease in total plasma protein in this study (Table 5) might be attributed to either a stage of hydration and change in water equilibrium and/or disturbances in the liver protein synthesis resulted due to the toxicity (Salah El-Deen and Rogers, 1993 and Mousa, 2004). The same trend was recorded by Choudhury *et al*. (2017) after long term exposure of *Channa punctatus* and *Oreochromis mossambicus* to hexaconazole 5% SC and Azoxystrobin 23% SC fungicides.

The increase of total plasma lipids (Table 5) may be due to the increase of lipids peroxides formation induced by the effect of toxic effect of jasper as showed before and previously reported for other pollutants by Arias *et al.* (1990). Otherwise, the destruction of liver cells and other organs due to the effect of the pollutant, increase the levels of total lipids in plasma. On the other hand, the depletion in the plasma lipids on the 30th day might be due to the increase of energy demand due to the stress factor of toxicity (Mousa, 2004).

Alteration of blood sugar levels revealed a stress response of fish (Nemcsok *et al.*, 1987). These results are in agreement with those of Mousa (2004). The hyperglycemia induced by any toxicant might be explained by the inhibition of the neuroeffector sites in the adrenal medulla leading to hypersecreation of adrenalin, which stimulates the breakdown of glycogen to glucose (Gupta, 1974). Moreover, Pickering (1981) recorded that the increase in the blood glucose might have resulted from an increase in plasma catecholamines and cortecosteroid hormones and the state of hypoglycemia that occurred on the 30th day in the field treated fish group, might be due to the complete depletion in liver glycogen due to continuous exhaustion.

Table 5. Changes in plasma T. Protein (g/dl), T. Lipids (g/dl) and Glucose of Nile tilapia "*Oreochromis niloticus*" through different periods of exposure to sublethal concentrations of jasper herbicide**.**

Parameters	T. Protein (g/dl)			T. Lipids (g/dl)			Glucose (mg/dl)		
Time	4th day	15th day	30th day	4th day	15th day	30th day	4th day	15th day	30th day
Control	5.75^{A} ±0.11	5.23^{A} ±0.06	5.64^{A} ±0.06	1.54^{C} ±0.01	1.61^{C} ±0.24	1.47^{A} ±0.09	113.33^{C} ±1.22	114.00^{C} ±2.64	109.00^{A} ±1.73
1/4 LC50	3.33^{B} ±0.11	2.46^{B} ±0,18	2.44^{C} ±0.18	2.40^{A} ±0.02	2.69^{A} ±0.12	1.23^{B} ±0.07	134.66^{A} ±0.88	173.66^{A} ±2.16	77.00^{B} ±2.00
1/8 LC50	3.45^{B} ±0.12	2.94^{B} ±0.02	3.13^{B} ±0.27	2.17^{B} ±0.06	2.26^{B} ±0.02	1.34^{B} ±0.15	128.66^{B} ±0.89	129.00^{B} ±8.33	79.00^{B} ±3.60

Data are expressed in means ± SE.

Means with the same letter within the same column are not significantly different at P<0.05.

Data in Table 6 revealed that the exposure of tilapia to both sublethal concentrations of jasper reduce the liver and gonad weights significantly at the 15th and 30th day of exposure. The effect was concentration-dependent. Choudhury *et al*. (1993) observes a decreased in the GSI values in *Mystusvittatus* after exposure to organophosphate. Sakamoto *et al*. (2003) observed lowest GSI values for *Cyprinus carpio* from dioxins contaminated zone compared to the reference zone at Hikiji River in Japan. The relative size of the liver should be correlated with the nutritional state of the fish and with the growth rate. So, the decrease in the hepato-somatic index in this study indicate to the disturbances in the nutrition and growth of treated fish as previously mentioned by Mousa *et al*. (2004).

Table 6. Changes in hepato-somatic index (HSI%) and gonado-somatic index (GSI%) of Nile tilapia "*Oreochromis niloticus*" through different periods of exposure to sublethal concentrations of jasper herbicide.

Parameters	HSI%			GSI%		
Time	4th day	15th day	30th day	4th day	15th day	30th day
Control	0.89 A ±0.02	0.92 A ±0.03	0.92 A ±0.17	1.16 A ±0.15	1.17 A ±0.01	1.24 A ±0.08
1/4 LC50	0.85 A ±0.093	0.65 B ±0.08	0.60 B ±0.01	1.15 A ±0.06	0.60 B ±0.04	0.39 B ±0.01
1/8 LC50	0.86 A ±0.11	0.65 B ±0.06	0.61 B ±0.07	0.97 B ±0.14	0.52 B ±0.02	0.39 B ±0.01

Data are expressed in means ± SE.
Means with the same letter within the same column are not significantly different at $P<0.05$.

CONCLUSION

From these findings, it could be concluded that the continuous exposure of fish to the herbicide jasper means a continuous their health hazards. So, we should take the necessary precautions during the application of jasper herbicide by using the appropriate machines that reduce environmental pollution for protection of fish and water quality.

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تأثير مبيد الأعشاب "جاسبر"على بعض النواحي المناعية والفسيولوجية في سمكة البلطي النيلي "*أوريوكرومس نيلوتيكس*"

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الملخـــص العربـــي

ظهر في الآونة الأخيرة الاستخدام المفرط لمبيدات الحشائش لتعزيز إنتاج المحاصيل الزراعية. وهذا تسبب في العديد من المشاكل الصحية للأسماك المستزرعة وأدى إلى ضعف الإنتاج. لذلك أجريت هذه الدراسة لتقييم سمية مبيد الحشائش (جاسبر) على البلطي النيلي باعتباره واحدا من مبيدات الحشائش المستخدمة بكثرة مؤخرا. حيث تم تعيين الجرعة المميتة للنصف خلال ٩٦ ساعة، وقد وجدت تساوي ٣،٨٠ جزء في المليون. وأثناء تعيين هذه الجرعة، أظهرت الأسماك حركات سباحة غير منتظمة. كما ازداد إفراز المخاط وتراكم على الخياشيم، وأظهرت الأسماك اضطرابات في التنفس مع السباحة على سطح المياه ، مع فتح أفواهها بزفير سريع ومتكرر.

بعد ذلك تم تقييم بعض القياسات المناعية والفسيولوجية خلال ثلاثين يوما من التعرض لاثنين من التركيزات تحت المميتة (١/٤، ١/٨ الجرعة المميتة للنصف خلال ٩٦ ساعة).

حيث أظهرت النتائج انخفاضا كبيرا في مضادات الأكسدة (SOD ، CAT ، GPX) وزيادة في MDA خلال فترات التعرض دلالة على اضطراب جهاز المناعة. كما أحدث المبيد تثبيطا في نشاط انزيم الأسيتيل كولين استريز (AChE) وارتفاع مستوى حمض اليوريك والكرياتينين في البلازما دلالة على الاضطرابات في وظائف الجهاز العصبي والكلوي. في حين ارتفعت أنشطة إنزيمات الكبد الآلانين ترانزفيريز (ALT) والأسبرتيت ترانزفيريز (AST) والفوسفاتيز القلوي (ALP) بشكل كبير خلال اليوم الرابع والخامس عشر من التعرض وانخفضت في نهاية فترة التجربة. كما انخفض البروتين الكلي للبلازما خلال جميع الفترات، في حين ارتفعت نسبة الدهون الكلية والجلوكوز بشكل كبير في جميع مجموعات الأسماك المعالجة في اليوم الرابع والخامس عشر من التعرض وانخفضت بشكل ملحوظ في نهاية وقت التعرض دلالة على اختلال وظائف الكبد. كذلك تناقص وزن الكبد والمناسل بالنسبة للوزن الكلي للجسم دلالة على انخفاض معدلات التغذية والتمثيل الغذائي للأسماك نتيجة سمية المبيد.

من هذه النتائج، يمكن أن نستنتج أن تعرض الأسماك المستمر لمبيد الجاسبر يعني مخاطر صحية مستمرة ويؤثر سلبا على الإنتاج. لهذا يجب أن نتخذ الاحتياطات اللازمة أثناء تطبيق المبيد وذلك باستخدام الآلات المناسبة التي تقلل من التلوث البيئي لحماية الأسماك وحفاظا على جودة المياه.