Does glyphosate cause acute toxicity to grass carp (*Ctenopharyngodon idella*)?

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Abstract

Nowadays, glyphosate, and its commercial formulation (Roundup 41% SL, Bayer AG, Leverkusen, Germania), is the most widely used broad-spectrum herbicide in industrial agriculture for weed control. The massive use of this substance also affects aquatic ecosystems and water organisms around the world. The potential toxic effects of glyphosate on aquatic specimens should worry us. Indeed, animals such as grass carp are part of the human food chain, so exposure to glyphosate may cause environmental damage and harm human health. In this study, the effect of acute 96h exposure of the commercial formulation of glyphosate (Roundup) on blood biochemical indices of grass carp (*Ctenopharhyngoden idella*) was evaluated. Three different concentrations of glyphosate were chosen for exposure to three groups of 21 animals each. The lowest was $50 mg.L^{-1}$, the intermediate concentration was $100 mg.L^{-1}$, and the highest concentration was $150 mg.L^{-1}$. The toxicity of the pollutant was tested using lethal concentrations and determining the LC50 (lethal concentrations for 50% of fish). Histopathological damage to the gills and liver was also evaluated, as well as the survival rate of the fish. Results showed severe gill and liver damage, with necrosis, leukocyte infiltration, hyperplasia and hypertrophy, in the group exposed to the highest concentration of glyphosate ($150 mg.L^{-1}$). Many biochemical changes were also observed, confirming the toxicity hypothesized at the start of the study.

1. Introduction

In the era of industrial agriculture, agrochemicals have become a global necessity to increase the productivity of crops and fields (Vajargah et al., 2013). As a result, one of the biggest problems we face is pollution from chemicals used in the agricultural industry, which have a strong impact on the aquatic ecosystem (Dar et al. 2022; Curpan et al. 2022) (Vajargah et al. 2021). Among the most widely used pesticides (Blahova et al. 2020) of the past centuries, glyphosate certainly stands out due to its massive use in recent times. Because of its intensive use as a chemical herbicide to control weeds has become a dominant feature of industrial agriculture and, consequently, a major environmental and health concern. Environmentally because of its wider effects on biodiversity, water, and soils (Sehonova et al. 2018; Stara et al. 2019). From a health perspective because of the possible consequences of overexposure and toxic residues in food for consumption. Growing public concern about the consequences of the massive use of this herbicide in industrial agriculture has been stemmed by many governments, who consider glyphosate to be much safer than other substances with the same intent and use (Hasaneen 2012).

Glyphosate (N-phosphonomethyl Glycine) is a broad-spectrum non-selective organophosphorus herbicide. Its chemical characteristics allow it to distribute throughout the plant to kill its meristems after a few days, making it extremely effective in controlling perennial weeds (Duke 2018). Glyphosate alone is not used as a herbicide; it is always blended with different surfactants to increase its effect. The most widely used glyphosate-based herbicide today is Roundup® (Roundup 41% SL, Bayer AG, Leverkusen, Germania), which contains polyoxyethylene amine (POEA), which is a surfactant useful for improving the absorption and translocation of the active ingredient in the plant (van Bruggen et al. 2018). To date, it is possible to say that a close correlation and dependence have been established between high-yielding orchards and herbicides, as they are an essential component of plant survival and weed control. However, the herbicides that can be chosen in Iran for weed control are very limited. Currently, there are mainly two types of herbicides to choose from glyphosate and paraquat. (Meza-Joya et al. 2013; Lugowska 2018).

Because Roundup can easily reach aquatic ecosystems by runoff, drainage, leaching, it represents a hazardous and widely distributed environmental contaminant. Toxic effects involve different aspects of the aquatic organism and are not limited only to death, but also extend to alterations in their metabolism (Costa et al. 2008), growth (Fiorino et al. 2018a)h, alterations in haematological parameters (Cavas and Konen 2007), histopathological changes(Yalsuyi et al. 2021a), and of course behavioural changes (Giesy et al. 2000; Peixoto 2005; Kelly et al. 2010; Romano et al. 2012).

The massive increase in the use of glyphosate since the 1970s has led governments in several countries to take action in recent years to try to reduce or ban its use. Spain, Italy, Germany, Canada, Portugal, and the Netherlands have already reduced or banned glyphosate. The majority of glyphosate restrictions or bans worldwide were introduced after the 2015 IARC report on glyphosate found that glyphosate is a "probable human carcinogen" (Baum 2019; Klingelhöfer et al. 2021).

During these years, numerous studies have been conducted to assess the effects of this herbicide on different animal populations(Fiorino et al., 2018; Yalsuyi et al., 2021). Toxic effects of glyphosate were also studied on different classes of vertebrates. The majority of past ecotoxicology studies have confirmed that fish is a good model for assessing the toxicity of a substance in the aquatic system, due to their ability to metabolise xenobiotics, their sensitivity to contaminants, and their position in the aquatic food chain(Fazio et al., 2014; Lauriano et al., 2016; Vajargah et al., 2018; Vajargah and Hedayati., 2017; Yalsuyi et al.,2017).

Glyphosate has shown its toxic effects on many invertebrates found both on land and in water. For example, this herbicide was tested on *Daphnia magna*, and it turned out that both glyphosate and its common formulation. The commercial formulation Roundup is toxic to these aquatic organisms (Cuhra et al., 2013). In *Cherax quadricarinatus*, the freshwater crayfish (Frontera et al., 2011), exposure to this herbicide resulted in reduced somatic cell growth, muscle glycogen, lipid reserves, and muscle protein levels. (Gill et al. 2018).

The effects of glyphosate were studied by Nešković et al. on freshwater carp (*Cyprinus carpio L.*). Toxicity tests were carried out by exposing the animals to three different concentrations of glyphosate (2.5, 5 and 10 mg/L) and showed that the herbicide is mildly toxic to carp. In this regard, the research team found biochemical evidence of increased alkaline phosphatase activity in the fish liver at all concentrations. Histopathological studies showed cases of epithelial hyperplasia and sub-epithelial oedema at both intermediate and higher concentrations. In addition, there is leucocyte infiltration, hypertrophy of chloride cells, and lifting and rupture of the respiratory epithelium (Nešković et al. 1996).

Another interesting study by Cattaneo et al. tested Roundup in *Cyprinus carpio*, evaluating its effect on acetylcholinesterase enzyme activity and oxidative stress. Five different concentrations of the herbicide

(0, 0.5, 2.5, 5 and 10 mg/L) were chosen to conduct the study. The study revealed repression of acetylcholinesterase activity in the brain and muscle. Furthermore, lipid peroxidation and anti-acetylcholinesterase action stimulated by Roundup on fish was confirmed (Cattaneo et al. 2011).

The research group of Webster and Santos investigated the toxic effects of glyphosate and Roundup on brown trout (*Salmo trutta*). Again, the animals were exposed to three different concentrations (0, 0.01, 0.5 and 10 mg/L) of pollutants. The study showed that both glyphosate and Roundup cause changes in the control mechanisms for the cellular stress response, in particular apoptosis. Furthermore, both pollutants increase cell proliferation, cell turnover and lead to an up-regulation of metabolic processes (Uren Webster and Santos 2015).

The present study aimed to evaluate the toxic effects of exposure to the commercial formulation of glyphosate (Roundup 41% SL, Bayer AG, Leverkusen, Germany) in grass carp (*Ctenopharyngodon idella*). Four increasing concentrations of glyphosate were chosen for the 96-hour experiment. The mortality rate, tissue damage and blood parameters were evaluated for the experiment.

2. Materials And Methods

Preparation

Juvenile grass carp, *Ctenopharyngodon idella* (N= 150) were purchased from local farms and transported to laboratory (laboratory of Faculty of Natural Resources, University of Guilan, Guilan province, Iran) and divided into 5 tanks (300 l- 30 fish in each tank); Fish were maintained to these tanks for adaption to laboratory conditions for 2 weeks. The fish were fed by Biomar feed equivalent to 2% of biomass weight twice a day during the adaption period. Each experimental unit had independent aeration and 40% water of the tank was replaced daily. Water physicochemical parameters, the pH and temperature, dissolved oxygen (DO), NH_3 concentration, and total hardness of water ($CaCO_3$ concentration) were measured. Used equipment was: digital soil and substrate pH meter (S500 pro, Aqua Masters, Burbank, California, US), a dissolved oxygen meter for aquaculture (HI9147, HANNA Instruments, Bertoki, Slovenia), and multiparameter photometers (7100, Palintest Co., Gateshead, UK). All measurements were performed twice a day. Water parameters were as follow; pH 7.8-8.2, DO 7.9-8.6 $mg.l^{-1}$, NH_3< 0.02, temperature 24±2 °C and $CaCo_3$ 210 $mg.l^{-1}$ during adaption period. Finally light period was 16:8 h (D: N).

The LC_{50} 96h test

After the adaptation period, 84 fingerling fish were divided into 4 groups (average of weight 14.4±1.7 g) with 3 replicates (12 aquarium- 150×60×65 cm) and exposed to 4 concentrations of commercial formulation of glyphosate (Roundup 41% SL, Bayer AG, Leverkusen, Germany). Nominal concentrations of Roundup were 0, 50, 100, and 150 $ml.L^{-1,}$ and the test time was 96 hours. The fish mortality rate was calculated at 24, 48, 72, and 96 h after exposure. Fish were transferred into the aquarium or test tank 24 hours before beginning the test and they didn't feed during the toxicity test. Water physicochemical parameters were kept same to one of adaptation period (pH 7.9-8.6, DO 7.9-8.6 mg x L^{-1}, NH_3 <0.02 mg x L^{-1}, temperature 24 ± 2°C and total hardness 210 mg.l^{-1} $CaCO_3$). The LC_{50} 96h test was a static system. Finally, glyphosate concentrations were added manually and the pesticide was distributed by water circulation inside the test tank. Also, fish swimming was recorded by the camera (Canon, SX230 Hs, 5.0 -70 mm) during the LC_{50} 96h test; the camera was in front of the aquarium.

Histopathological test

The gill samples of fish were collected at 96 hours after exposing fish to Roundup (3 samples of each treatment) and they were fixed by diluted Formalin solution (Formaldehyde 10% v/v, Sigma®, Missouri, US). Formalin of the gill and liver samples was replaced 24 hours after sampling. The middle parts of the liver and second-gill arch from the fish's right side were selected for sampling, respectively. The samples were placed in a series of alcohols (50, 70, 80, and 96%) for half an hour. Immediately after that, the gill sample was washed with 1-butanol alcohol (for 2 hours) and then placed into chloroform to clarify for 1 hour. After this step, the gill samples were placed into an incubator at 37 °C for Paraffinization and softening by the solution of chloroform and paraffin (1:1). After that samples were incubated in pure paraffin at 54 °C and were prepared for tissue incisions after cooling. The tissue incisions were obtained by the automatic tissue processor machine (TP1020, Leica Microsystems Inc., Buffalo Grove, IL, US) and their thickness was 6 μm. The tissues incisions were stained by hematoxylin-eosin. Tissue damages were observed and evaluated by light microscopy (Model RH-85 UXL, UNILAB®, India). Tissue samples of treatments were collected from live fish.

Blood parameters

Blood samples were collected through the caudal vein technique. According to this technique, the sample was taken from the midline just posterior of the anal fin. Blood samples were centrifuged for 10 min at 2500 rpm and transferred to the laboratory for haematological analysis. Blood samples of all fishes in each tank were polled and finally, 12 blood samples were analyzed. Total protein, Albumin, Globulin, Triglyceride, cholesterol, and Glucose in serum were measured from each sample using an automatic biochemical analyzer (Roche Hitachi 911 Chemistry Analyzer, Tokyo, Japan) and their corresponding kits (Pars Azmoon Inc., Tehran, Iran). The samples were taken from live fish in sub-lethal concentrations treatments of Roundup at 96 h after exposure.

Data Analyses

The lethal concentration of glyphosate in intervals of 24, 48, 72, and 96 hours (LC_{50} 24h, 48h, 72h, and 96h of glyphosate) was estimated through probit test with a 95% confidence. We used the Spearman test to find the correlation between different nominal concentrations of commercial formulation of glyphosate and mortality (2-tail). Finally, the correlation between the mortality rate of fish and the concentration of Roundup was checked with the two-tailed significance Spearman tests by SPSS software (IBM SPSS Statistics 20).

The video data were analyzed by Adobe after Effects software (AAE CS6) on the Windows platform (Windows 7 Ultimate, Microsoft corporation©). The clinical signs of fish were reported by direct observation of recorded videos, count average movement of gill operculum in 1 min, and comparison colour of the object (fish) during a period by AAE CS6.

3. Results

Results of the 96 h LC50 (Lethal Concentration of 50% of the Population) test

The results of the toxicity test showed mortality in all treatment phases (except for the control group at 0 mL•l-1 glyphosate). In the group with the highest glyphosate concentration, 11 out of 21 individuals died 24 h after exposure. Another 4 died 48 hours after the first exposure, and another 4 at 72 hours. At 96h after exposure, the death of 20 out of 21 individuals present at the start of the test was counted, as shown in Table 1. The 96 h LC50 of the commercial glyphosate formulation was 75.838 mg• L-1 and its 24, 48 and 72 h LC50 were 120.343, 110.495 and 85.424 mg•L-1, respectively (Table 2). From the data obtained, it is also possible to observe in Figure 1, the correlation between the nominal concentrations of Roundup and the mortality rate of the grass carp, *Ctenopharyngodon idella* ($p<0.01$).

Results of the histopathological assay in gills

Tissue damage was observed in varying degrees in all individuals exposed to the herbicide (50, 100 and 150 mg•L^{-1}). Already in the treatment with the lowest pollutant concentration, mild lamellar fusion, basal and distal hyperplasia was observed. In addition, moderate epithelial hyperplasia was evident. At a pollutant concentration of 100 mg.L^{-1}, the damage already mentioned increased in severity, as shown in Table 3. In addition to this damage, there was a mild lamellar aneurysm, leucocyte infiltration and mild necrosis. The greatest damage to the gills was found, as hypothesized, at the highest concentration of glyphosate. The samples were characterized by severe necrosis and leukocyte infiltration, as well as moderate lamellar fusion, lamellar aneurysm, epithelial hypertrophy and basal and distal hyperplasia as shown in Figure 2.

Liver histopathology test results

Regarding tissue damage in the liver of *Ctenopharyngodon idella*, this was found to be mild at the lowest glyphosate concentration, moderate at the intermediate concentration and severe in the highest herbicide concentration treatment. At 100mg.L^{-1}, severe necrosis, cloudy swelling, and numerous macrophage aggregates were observed. As the glyphosate concentration increased, the hydropic swelling also increased. All tissue damage data are shown in Table 4 and Figure 2.

Results of blood biochemical tests

Haematological and biochemical values are shown in Table 5. The level of total protein at the 50 mg/L^{-1} concentration increased compared to the control group. The trend increased with increasing glyphosate concentration, reaching 3.68±0.21 mg/dL and in some concentrations showed a significant difference.

The albumin level was lowest in the control group, with a significant difference ($P<0.05$) compared to the other groups. The albumin level started to increase already in the group exposed to 50mg.L^{-1}, where it was 0.67±0.12 mg/dL. The other two groups, at intermediate and maximum glyphosate concentration, showed almost constant volumes, settling at 0.65±0.41 and 0.66±0.03 respectively.

However, the cholesterol level only increased slightly in the intermediate (100mg.L^{-1}) and maximum (150mg.L^{-1}) concentration groups, from 3.87±0.22 mg/dL (control group) to 4.15±0.04 and 4.16±0.04 mg/dL respectively.

The lowest amount of triglycerides was observed in the control group, and then increased in the group with the lower concentration, and then settled at approximately the same value in the two groups with intermediate and higher concentrations. No significant difference was observed between these three groups.

The minimum glucose level observed in the specimens in the 150 mg.L^{-1} glyphosate concentration group was significantly different ($P<0.05$) compared to the control group and the group with lower glyphosate concentration.

The red blood cell count (RBC) of fish in the group with the highest glyphosate concentration (150mg.L^{-1}) was the lowest ($P < 0.05$). There was a reduction in the RBC value as the concentration of the herbicide increased. The white blood cell count (WBC) followed the same decreasing trend as the RBC values. In the group with the highest concentration, however, it settled at values very similar to the group with a concentration of 100mg.L^{-1} glyphosate. The hematocrit values remained almost unchanged between the control group and the group exposed to 50mg.L^{-1} glyphosate. In the intermediate and higher concentration groups, however, values fell significantly.

4. Discussion And Conclusion

The study aimed to assess the potentially toxic effects of the commercial formulation of glyphosate, Roundup, on grass carp, a nontarget aquatic organism for this herbicide. The effects of this pollutant were assessed by analyzing survival rates, histopathological damage to the liver and gills, and blood biochemical indices. Following the results presented above, the lowest mortality rate was recorded in the control group (0%), while the highest mortality rate was recorded in the group exposed to the highest concentration of glyphosate (150mg.L^{-1}), which rose to 95.2% after 96h, with 20 out of 21 fish dying. It is therefore clear that lethal and sub-lethal concentrations of glyphosate impact the survival rate of grass carp and are therefore toxic. Glyphosate toxicity was also observed through the profound changes in liver and gill tissues, which underwent necrosis, hyperplasia, hypertrophy and leukocyte infiltration. A dose-dependent correlation between herbicide concentrations and tissue damage was confirmed. Concerning biochemical changes in the blood, we have seen a significant reduction in the red blood cell count (RBC), which can often indicate hemorrhage, hemolysis and reduced erythropoiesis (Khan et al. 2016). White blood cell counts (WBC) also declined during the exposure period, reaching their lowest point in the group with the highest glyphosate concentration. The same downward trend has also been observed in other studies conducted previously (Gholami-Seyedkolaei et al. 2013): in 2000, the research group of Kotsanis et al., seeing similar behaviour in their case study, hypothesized a correlation with the toxic effect of the herbicide in the kidney, which is the main site of hemopoiesis. Another hypothesis was made by the research group of Kavitha et al., who in 2010 hypothesized that Roundup might exhibit its toxic effect by blocking the maturation and release of white blood cells (Kavitha et al. 2010). Analyses carried out on glyphosate-treated fish also showed an increase in cholesterol. According to a 2015 study(Stoyanova 2015) in which glyphosate was exposed on common carp, the most likely hypothesis explaining this result could be the accumulation of glyphosate in the fish's liver, which changes lipid metabolism and increases cholesterol values. In addition, cholesterol is the base molecule for the construction of steroid hormones, including the stress hormone cortisol. Therefore, an increase in cortisol under stressful conditions also correlates with an increase in cholesterol levels (Kazemi 2010).

In conclusion, we can conclude from the results obtained that glyphosate, in its commercial formulation of Roundup, causes toxic effects on grass carp, altering biochemical values in the blood and causing serious damage to fish tissue. This may be a wake-up call for industrial agriculture, which makes massive use of this herbicide and can also cause serious harm to humans.

Declarations

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Author Contributions

All authors contributed to the study’s conception and design. Material preparation, data collection and analysis were performed by

- Dariush Azadikhah

- Matin Varcheh

- Ahmad Mohamadi Yalsuyi

The work and data analysis were written by Federica Impellitteri. The work was supervised by Caterina Faggio and Mohammad Forouhar.

Data Availability

The datasets in this study are available from the corresponding author on reasonable request. All data and materials are available for publication.

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Ethical Approval

Institutional guidelines for the ethical issue were followed. The experimental protocol (No. IR-GAUEC94-336-5) was authorized by the Institutional Animal Care and Ethics Committee of Gorgan University of Agricultural Sciences and Natural Resources, Golestan, Iran.

Consent to Participate

Not applicable.

Consent to Publish

All authors give consent for publication.

Conflicts of Interest

The authors declare no conflict of interest.

Code Availability

Not applicable.

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Tables

Table 1 The Mortality rate of grass carp (*Ctenopharyngodon idella*) after exposure to different concentrations of commercial formulation of glyphosate (Roundup 41% SL, Bayer AG, Leverkusen, Germany). The average weight of fish was 14.4±1.7 g.

Concentration (ppm)	Number of fish	No. of mortality			
		24h	48h	72h	96h
0	21	0	0	0	0
50	21	3	5	6	7
100	21	7	9	13	15
150	21	11	15	19	20

Note: all Concentrations of Betadine are nominal.

Table 2 Lethal concentration of commercial formulation of glyphosate (Roundup 41% SL, Bayer AG, Leverkusen, Germany) for grass carp (Ctenopharyngodon idella). The average weight of fish was

14.4±1.7 g.

Point	Concentration (mg.l^{-1})			
	24 h	48 h	72 h	96 h
LC_{10}	46.408	30.863	26.655	23.489
LC_{20}	64.034	58.199	46.829	41.459
LC_{30}	78.042	77.910	61.376	54.417
LC_{40}	100.853	94.753	73.806	65.489
LC_{50}	**120.343**	**110.495**	**85.424**	**75.838**
LC_{60}	156.778	126.237	97.042	86.187
LC_{70}	176.269	143.080	109.471	97.258
LC_{80}	199.079	162.791	124.018	110.216
LC_{90}	230.714	190.127	144.192	128.186
LC_{95}	266.838	212.701	160.853	143.027

Note: all Concentrations of Roundup are nominal

Table 3 *The correlation between the commercial formulation of glyphosate (Roundup 41% SL, Bayer AG, Leverkusen, Germany) and damages of grass carp gills (Ctenopharyngodon idella).*

Tissue damages	Concentration (mg.l^{-1})			
	0	**50**	**100**	**150**
Lamellar fusion	-	++	+++	+++
Lamellar aneurism	-	-	++	+++
Epithelial hypertrophy	-	+++	++++	+++
Leukocyte infiltration	-	-	++	++++
Basal Hyperplasia	-	++	++	+++
Distal hyperplasia	-	++	+++	+++
Necrosis	-	-	++	++++

Note: all Concentrations of Roundup are nominal. Also, None (-), Mild (++), Moderate (+++) and

Severe (++++) of tissue damages.

Table 4 The correlation between the commercial formulation of glyphosate (Roundup 41% SL, Bayer AG, Leverkusen, Germany) the damages to the liver of grass carp (*Ctenopharyngodon idella*).

Tissue damages	**Concentration ($mg.l^{-1}$)**			
	0	**50**	**100**	**150**
Necrosis	-	++	++++	++++
Hydropic swelling	-	++	+++	++++
Cloudy swelling	-	++	++++	++++
Lipidosis	-	++	+++	+++
Macrophage aggregates	-	++	++++	+++
Dilation of sinusoid	-	++	+++	+++

Note: all Concentrations of Roundup are nominal. Also, None (-), Mild (++), Moderate (+++) and Severe (++++) of tissue damages.

Table 5 Results of blood biochemical tests (mean ±SD) of adult grass carp (*Ctenopharyngodon idella*) at 96 h after exposure to different levels of commercial formulation of glyphosate (Roundup 41% SL, Bayer AG, Leverkusen, Germany).

Blood parameters	Control (0 $mg.L^{-1}$)	50 $mg.L^{-1}$	100 $mg.L^{-1}$	150 $mg.L^{-1}$
RBC (10^6 µL)	$2.31±0.06^a$	$2.30±0.8^a$	$1.56±0.09^b$	$1.29±0.72^c$
WBC (10^4 µL)	$2.27±0.53^a$	$2.23±0.69^a$	$1.81±0.12^b$	$1.79±0.32^b$
Hematocrit (%)	$45.33±0.6^a$	$44.2±0.7^a$	$37.4±0.02^b$	$32.9±0.13^c$
Glucose (mg/dL)	$4.14±0.07^a$	$4.12±0.11^a$	$3.24±0.03^b$	$2.04±0. 13^c$
Total Protein (mg/dL)	$1.88±0.31^d$	$2.94±0.12^b$	$2.96±0.13^b$	$3.68±0.21^a$
Triglyceride (mg/dL)	$1.89±0.16^d$	$2.33±0.04^a$	$2.31±0.09^a$	$2.33±0.07^a$
Cholesterol (mg/dL)	$3.87±0.22^c$	$3.89±0.31^c$	$4.16±0.04^a$	$4.15±0.04^a$
Albumin (mg/dL)	$0.52±0.05^b$	$0.67±0.12^a$	$0.65±0.41^a$	$0.66±0.03^a$

Note: different letters (a–d) in the same row indicate significant differences ($p < 0.05$).

Figures

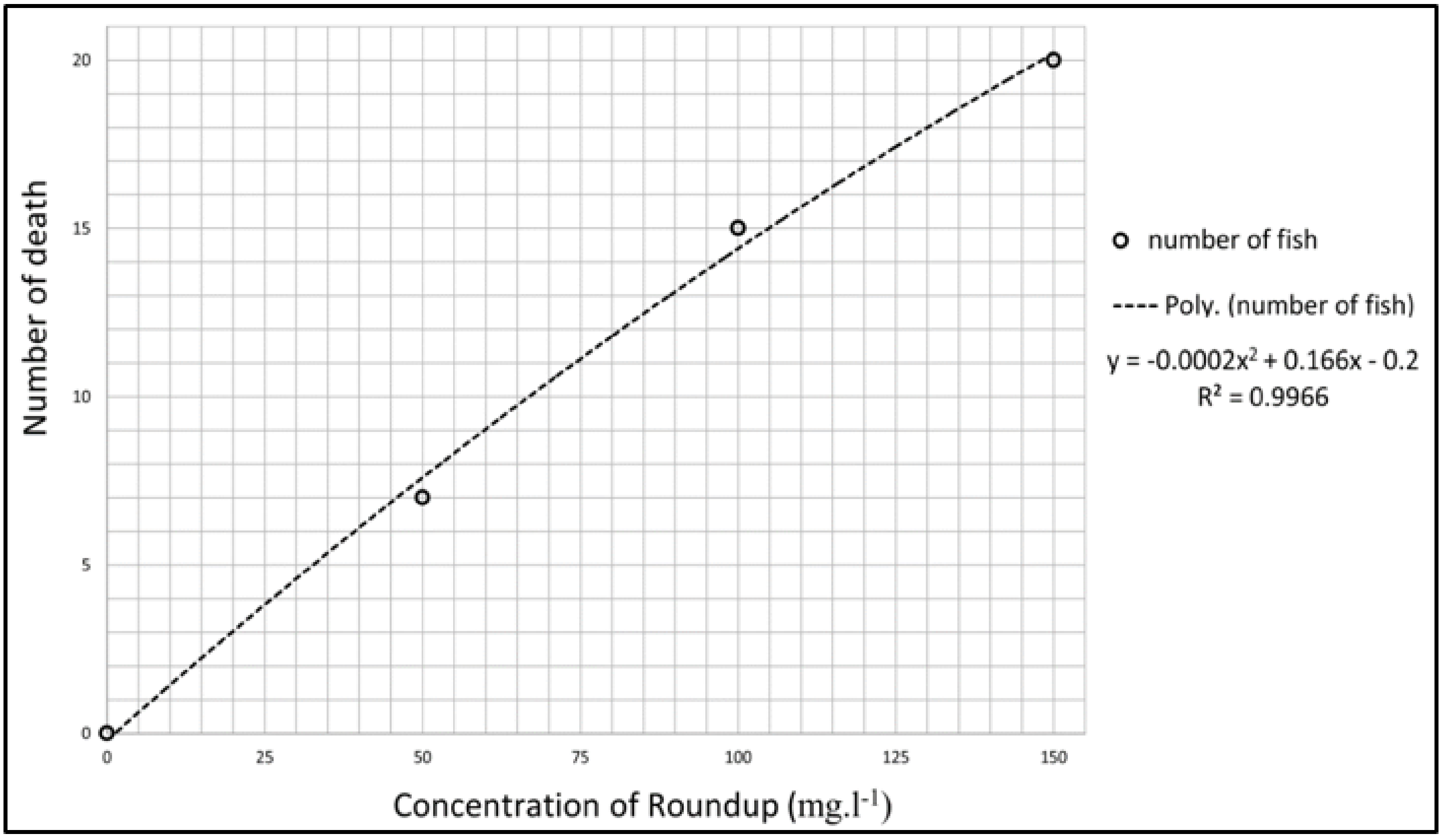

Figure 1

The correlation between nominal concentrations of commercial formulation of glyphosate (Roundup 41% SL, Bayer AG, Leverkusen, Germany) with mortality rate (number of death) of the grass carp, *Ctenopharyngodon idella* ($p<0.01$). The average weight of fish was 14.4±1.7 g.

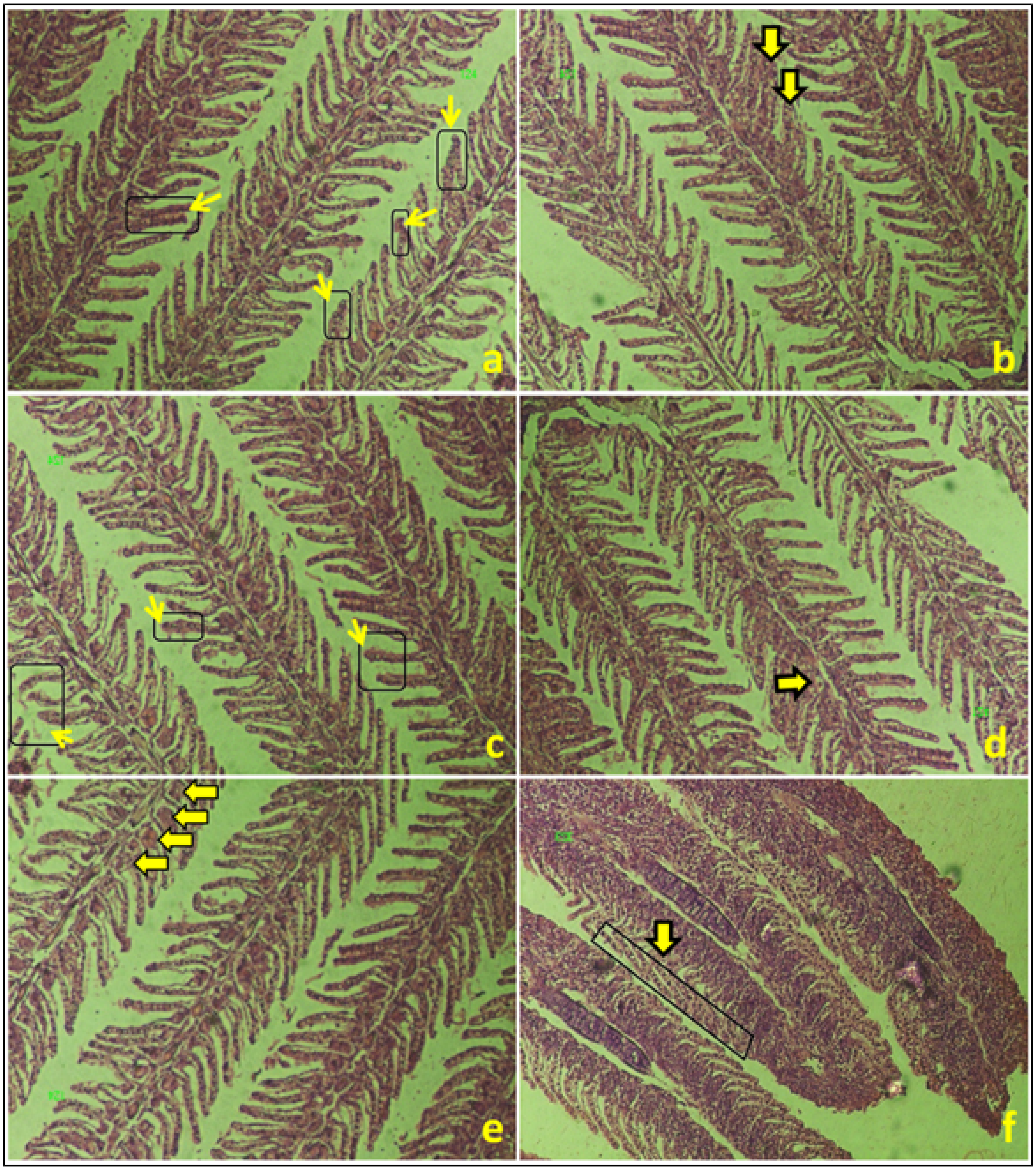

Figure 2

The grass carp (*Ctenopharyngodon idella*) histopathological changes of the gills by commercial formulation of glyphosate (Roundup 41% SL, Bayer AG, Leverkusen, Germany): a) Lamellar fusion (100 ml.l^{-1} of Roundup); b) Lamellar aneurism (150 ml.l^{-1} of Roundup); c) Distal hyperplasia (100 ml.l^{-1} of Roundup); d) Leukocyte infiltration (150 ml.l^{-1} of Roundup); e) Basal hyperplasia (100 ml.l^{-1} of Roundup); f) Epithelial hypertrophy (50 ml.l^{-1} of Roundup). All pictures are magnified ×10.

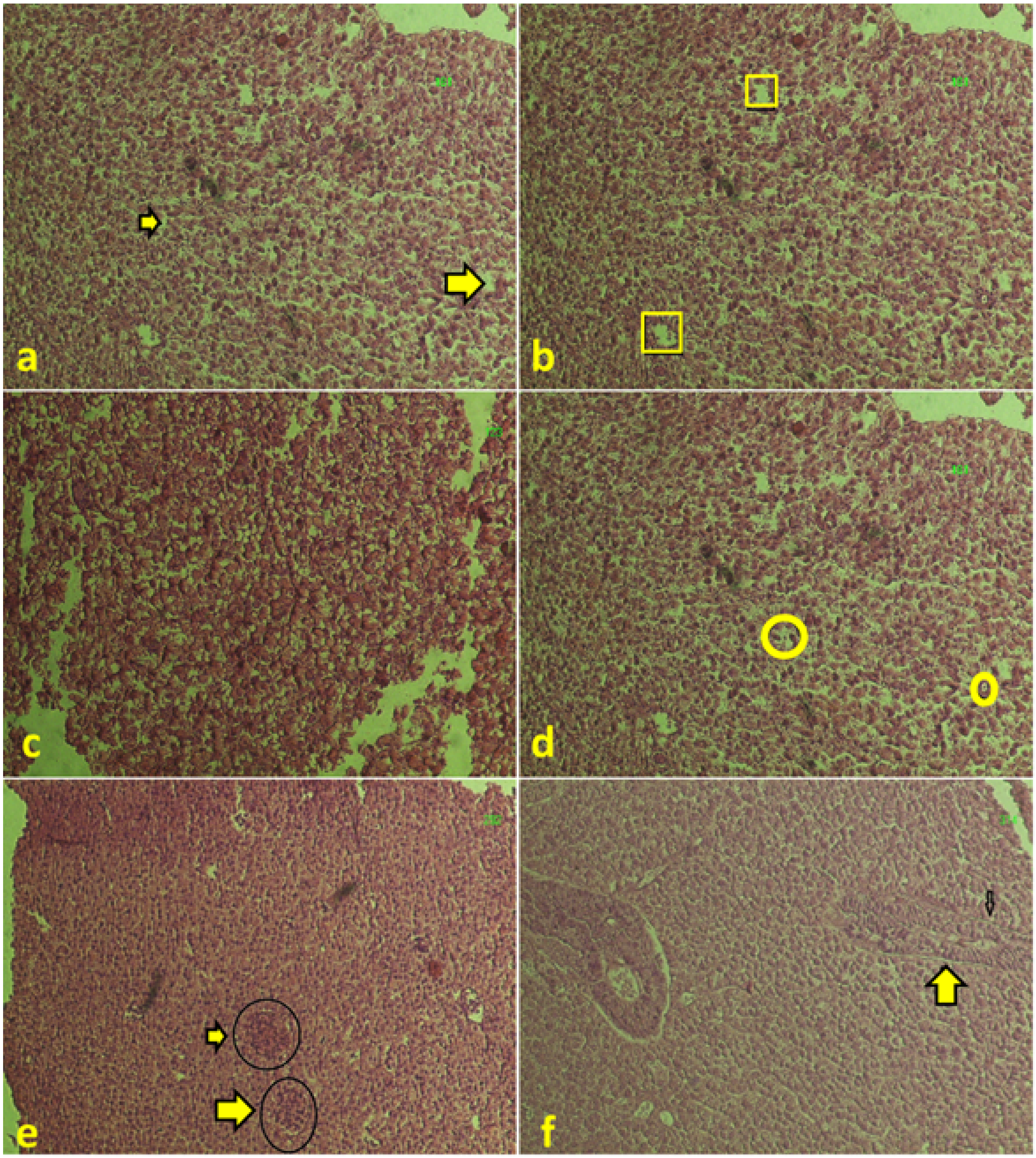

Figure 3

The grass carp (*Ctenopharyngodon idella*) histopathological changes of the liver by commercial formulation of glyphosate (Roundup 41% SL, Bayer AG, Leverkusen, Germany); a) Necrosis (50 $ml.l^{-1}$ of Roundup); b) Hydropic swelling (50 $ml.l^{-1}$ of Roundup); c) Lipidosis (100 $ml.l^{-1}$ of Roundup); d) Cloudy swelling (50 $ml.l^{-1}$ of Roundup); e) Macrophage aggregates (150 $ml.l^{-1}$ of Roundup); f) Dilation of the sinusoid (100 $ml.l^{-1}$ of Roundup). All pictures are magnified ×10.