International Journal of Applied Research 2015; 1(7): 437-440

ISSN Print: 2394-7500
ISSN Online: 2394-5869
Impact Factor: 5.2
IJAR 2015; 1(7): 437-440
www.allresearchjournal.com
Received: 01-04-2015
Accepted: 02-05-2015

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Effect of herbicides on fish and histological evaluation of common carp (*Cyprinus carpio*)

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Abstract
Toxicity of the herbicides like 2, 4-D NA salt, glyphosate and paraquat were tested, fish was investigated in laboratory and toxicity tested with common carp. Median lethal concentrations were determined in acute tests. The 96 h LC_{50} value was 100, 86 and 26 mg/L were tested. The experiment was laid out in a randomized block design with four replications. Screening of different herbicides was taken up in cement pots of dimension 2' x 2.5' x 2' with water filled up to three fourth of the pot's height, holding ten water hyacinth plants and each treatment were introduced ten fish fingerlings. Three different species tested viz., common carp, mrigal and rohu fishes were reared. Fish was exposed to acute toxicity of herbicides and gills, brain, liver and kidney structure were studied. In different concentration of herbicidal changes were observed in the vital organs and was confirmed by histological analysis. Among the herbicides applied, glyphosate significantly influenced with the least fish mortality percentage of 23.0, 16.0 and 20.0 on common carp, mrigal and rohu at 4 DAS, respectively. The same herbicides were found in the lesser imparted of tissues such as gills, brain, liver and kidney of the fish was examined.

Keywords: Fish mortality, Herbicides, Water hyacinth, Histology.

1. Introduction
Herbicides are actively used in terrestrial and aquatic ecosystems to control unwanted weeds, and their use has generated serious concerns about the potential adverse effects of these chemicals on the environment and human health (Oleh *et al.*, 2009) [10]. The herbicides 2, 4-D Sodium salt is selective in nature, glyphosate and paraquat are of non-selective all are used as post emergence herbicides. It is contributed though several herbicides used in India approximately 50 % of agricultural areas including atrazine, diuron, isoproturon. The 2, 4-D Sodium salt is dichlorophenoxy-acetic acid, Phonoxy acids group, glyphosate is N-(Phosphonomethyl) Glycine, organophosphorus group and paraquat is 1, 1-dimethyl-4, 4-bipyridilium dichloride, bipyridylium group (Deivasigamani, 2013) [3]. These herbicides are used in aquatic ecosystem and are easily degraded in water system compared to other herbicides.

The herbicides 2, 4-D Sodium salts are effective for controlling submerged aquatic plants. These compounds rapidly and completely decompose in about 3 weeks (Helfrich *et al.*, 2009) [5]. Glyphosate is known to degrade rapidly in soil and natural water with DT 50 values ranging from 3-14 days in field and water. It is a non-selective herbicide that inhibits plant growth through interference with the production of essential aromatic amino acids by inhibiting the enzyme, Enolpyruvyl Shikimate Phosphate Synthase (ESPS). This enzyme is responsible for the biosynthesis of chorismate, an intermediate in phenylalanine, tyrosine, and tryptophan biosynthesis (Pedron *et al.*, 2006) [13].

The acute toxicity of glyphosate is considered to be low, according to data from the WHO (Oleh *et al.*, 2009) [10]. The toxicity and risk for humans, other mammals and birds was analyzed by Williams and colleagues (2000). Glyphosate also affected energy metabolism, free radical processes, acetylcholine esterase activity (Langiano *et al.* 2008) [8] and immune responses of histological changes in hepatocytes of *Oreochromis niloticus* Szarek *et al.* (2000) [17] and *Cyprinus carpio* Jiraungkoorskul *et al.* (2003) [6]. The herbicides treated water to be restricted from drinking, livestock watering, swimming, fish production, irrigation, and other uses until safe levels of the herbicides are reached (Helfrich *et al.*, 2009) [5]. The

herbicides paraquat and 2, 4-D Na salt which was used for controlling aquatic weeds, reduced the dissolved oxygen content and pH of water and resulted in higher mortality percentage of common carp fish (Kannan *et al.* 2002) [7].

The carp (*Cyprinus carpio* L.) is a fish species has more economic importance in India. Histopathological effects on carp were investigated in order to determine possible adverse effects of aquatic herbicides. To the best of the author knowledge, this is the first report on herbicides such as 2, 4-D Sodium salt and paraquat toxicity in common carp.

2. Materials and methods

The studies were conducted at the Department of Agronomy, Faculty of Agriculture, Annamalai University, India during 2010-11, to screen different herbicides for managing water hyacinth and to trace their impact on fish toxicology. The experiment was laid out in a randomized block design with four replications. Screening of different herbicides was taken up in cement pots of dimension 2' x 2.5' x 2' with water filled up to three fourth of the pot's height, holding ten water hyacinth plants and fishes were reared.

2.1. Chemicals

Three different herbicides were used like, 2, 4-D Na salt @100 mg/L, glyphosate @ 86 mg/L and paraquat @ 26 mg/L. It has been low to moderate toxicity in aquatic fauna (Tomlin, 2000) [18]. These herbicides were sprayed using knapsack sprayer fitted with flood jet deflector nozzle and 12 lb inch^{-2} of pressure.

2.2. Animals and toxicity

In each treatment were introduced ten fish fingerlings of 5 month old and size of 10±0.5 cm body length and 105±10g body weight (for acute tests), three different species tested viz., common carp, mrigal and rohu at the beginning of the experiments. In acute tests (10 per group) were exposed to the following herbicide concentrations 2, 4-D Na salt, glyphosate and paraquat of 100 mg l^{-1}, 86 mg l^{-1}and 26 mg l^{-1} for 4Days After Spray. The fish mortality was calculated on 4 days after spray using the formula,

$$\text{Mortality of fishes (\%)} = \frac{\text{No.of fishes died tank}^{-1}}{\text{Total No.of fishes stocked tank}^{-1}} \text{X } 100$$

2.3. Statistical analysis

The experimental data were statistically analyzed using the methods described by Panes *et al.* (1978) [11]. After subjecting the data to analysis of variance, least significant difference was worked out at 0.05 per cent probability level. The data on percentage values were transformed by angular transformation before analysis

2.4. Histological study

To study the effect of herbicidal control over non-target organism, a fish known as common carp were raised in the tanks with weed and sprayed with herbicides, the mortality of fishes have been observed and fish organs were dissected out for histopathological study. To observe the impact of herbicides on different organs of the fish, the common carp alone were collected from the herbicide treated tanks (2, 4-D Na salt, glyphosate and paraquat) after 4 DAS. Gills, liver, kidney and brain tissues were dissected from each fish from treated (herbicide) as well as control. The freshly dissected tissues were washed in running water for 12-18 hr and processed by following the standard technique (Ramah, 2011) [14]. Tissue specimens from treatments were removed and small pieces of several organs (gills, brain, liver and kidney) were immediately fixed in neutral buffered formalin 10% dehydrated in ascending grades of ethanol, embedded in soft paraffin, sectioned into very thin slices of 6 to 8 µ thickness and stained with hematoxylin and eosin (H&E), tissues are from the paraffin block using a microtome equipped with a very sharp stainless steel blade (Luna, 1968; Ramah, 2011) [9, 14]. Tissue sections were prepared according to (Bancroft *et al.*, (2002); Aliaa *et al.*, (2011) [2, 1]. The slides were stained in Heidenhain's iron hematoxylin and counter stained with aqueous eosin. Stained sections were mounted in microscope and observed.

3. RESULTS AND DISCUSSION

3.1. Fish mortality

The glyphosate showed significantly lesser lethality than 2, 4-D Na salt over fishes with the mortality percentage of 23.0, 16.0 and 20.0 and 46.0, 33.33 and 20.00 for common carp, mrigal and rohu respectively at 96 h. Untreated control fish showed no mortality percentage (Table 1).

Table 1: Effect of different herbicides spray on mortality percentage of fish at 4 Days after spray

Treatments	Mortality Percentage of Fish		
	Common crop	Mrigal	Rohu
2, 4-D Na salt @ 100mg/l	(46.00) 42.70	(33.33) 35.26	(25.00) 30.00
Glyphosate @ 86mg/l	(23.30) 28.86	(16.60) 24.05	(20.00) 26.57
Paraquat @ 26mg/l	(42.00) 40.39	(50.66) 46.73	(25.00) 30.00
Fish alone	(0.00) 0.01	(0.00) 0.01	(0.00) 0.01
$S.E_D$	3.16	2.62	0.36
CD (p=0.05)	6.32	5.25	0.73

Figures in parenthesis are original values before angular transformation

3.2. Histology study of fish organs

The results of histological changes in glyphosate treated organs and their structural features over untreated control.

3.3. Gills

Untreated: The gills of fingerlings consist of a series of folds, the gill filaments or gill lamellae, which are supported by the gill rays. The primary gill lamellae bears on either side a series of transverse, flat, leaf like structures called the secondary lamellae. The tip of the primary lamellae is broad and long with the end tapering gradually. The tip contains a large dense mass of red blood cells.

Treated: Mild congestions of blood vessels were seen in the primary lamellae, whereas a fusion of primary lamellae and marked hyperplasia of the branchial arch was evident. In the acute trials, the fish had mild secondary lamellar epithelial hyperplasia, moderate edema of the secondary lamellae rendering ballooning of the epithelia.

3.4. Brain

Untreated: The histoarchitecture of the brain tissues of fingerlings shows the normal condition of tectum and tegmentu region, these regions consist of numerous layers of neuroglial cells. The size and shape of the neuroglial cells are

in normal condition in the fish alone treatment.

Treated: Histological changes in brain at herbicides exposure showed swelling of pyramidal cells with binucleated nucli, severe necrosis of neuronal cells of cerebrum was evident, indicating loss of nissl substances. Mild vacular changes with empty spaces appeared due to increased concentration and duration of glyphosate toxicity to fingerlings. The granular layer of the tegmentum was completely disappeared and number of neuroglial cells decreased.

3.5. Kidney

Untreated: The functional unit of the kidney is nephron. It is made up of following parts: The renal corpuscle (consisting of a glomerulus and Bowman's capsule) and the renal tubules consist of a single layer of epithelial cells. The renal corpuscles are surrounded by cross sections of proximal convoluted tubules. The lumen of the distal tubule is more rounded and the apical surface of the cells was sharper. The cells forming the collecting tubules are cuboidal and smaller than those of proximal tubule.

Treated: Histopathological changes in the kidney were observed at glyphosate treatment. Renal tubules become highly expanded and their epithelial lining was separated from the tubular cells. Some renal tubules were characterized by loss of cellular integrity. Dilation, oedema and hypertrophied nuclei of renal tubules are also noticed. Glomeruli show vacuolization and disorganized blood capillaries. Necrosis and pyknotic nuclei were observed in mesenchymal tissue.

3.6. Liver

Untreated: The histological structure of control fish appeared normal. At the light microscopic level, the liver was divided into irregularly shaped lobules separated by the hepatocytes and bile duct. The liver was made up of hepatocytes that were polygonal cells with a central spherical nucleus and a densely stained nucleolus.

Treated: The glyphosate treated fish liver had slightly vacuolated cells showing evidence of fatty degeneration. Necrosis of some portions of the liver tissues was observed probably resulting from the excessive work required by the fish to get rid of the toxicant from its body during the process of detoxification. The inability of fish to regenerate new liver cells may also have led to necrosis.

4. Discussion

The data obtained for acute toxicity of herbicides, 2, 4-D Na salt, glyphosate and paraquat are in the range of LC_{50} concentrations established for common carp (100, 86 and 26 mgl^{-1} respectively. All the herbicides imparted tissue destruction in all the different types of organs compared. Regarding the gills, congestion of blood vessels in the primary lamellae and hyperplasia of branchial arch were some of the injuries suffered. Liver suffered vacuolization, focal necrosis and common lesion with different herbicides. Increased granular layer and swelling of pyramidal cells in the brain, degeneration in tubular epithelium and expanded renal tubules of the kidney are also observed with herbicide treatments (Figure). Similar results were obtained by Kathiresan and Ramah (2002); Bharat Bhusan Patnaik *et al.* (2011) and Reza Sayrafi *et al.* (2011); Gabi Dumitrescu *et al.*, (2010) [7, 11, 16, 4].

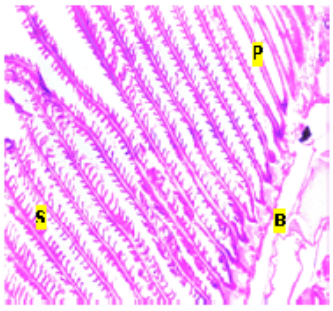


Fig 1: Gills of common carp exposed to Glyphosate, B-Basement membrane, S-Secondary lamellae, P-Primary lamellae.

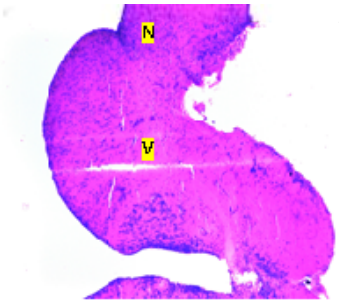
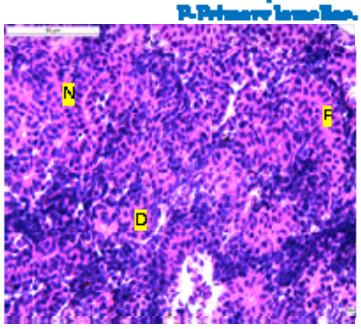


Fig 2: Brain of common carp exposed to Glyphosate, V-Vacuoles, N- Neuroglial cells,



Kidney of common carp exposed to Glyphosate, Distal tubule, P-Proximal tubule, N-Nephron

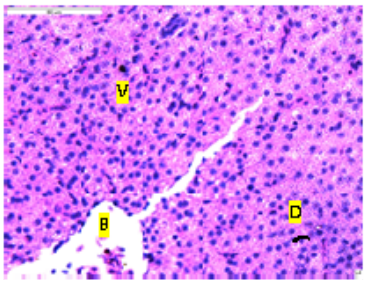


Fig 4: Liver of common carp exposed to Glyphosate, V-Vacuolization, BD- Bile duct, DG-Degeneration of Nucleus

5. Conclusion

An increase in some enzyme activities in serum and organs recorded in this study is pointing out changes in metabolic state of the fish. It was confirmed by histopathology of the organs examined. All the herbicides imparted tissue destruction in all the different types of organs compared. Among the herbicides tested, glyphosate LC_{50} values of $86mgl^{-1}$ caused the mild congestions on gills, brain, liver and

kidney tissues. Fish mortality also found to be least with the mortality percentage of 23.0, 16.0 and 20.0 on common carp, mrigal and rohu at 96 h, respectively. In addition to this, the results of the present study have determined the importance of herbicides and histopathological approaches in the evaluation of non-lethal effects of chemicals on fish and environmental safety.

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