African Journal of Biotechnology Vol. 10(7), pp. 1112-1116, 14 February, 2011
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB09.1468
ISSN 1684–5315 © 2011 Academic Journals

*Full Length Research Paper*

# Histopathological study on the effect of rice herbicides on grass carp (*Ctenopharyngodan idella)*

**K. Ramah**

Research associate, Department of Agronomy, Tamil Nadu Agricultural University, Coimbatore - 641 003. E-mail: kramah@rediffmail.com.

Accepted 30 December, 2009

**A pot culture experiment followed by field experiments were conducted at Annamalai University Experimental Farm, to optimize the age of fingerlings to be released along with rice herbicides and to study the effect of rice herbicides on fish histopathology. Fingerlings of size 4 - 5 cm, when released 12 days after herbicide application was observed to be safe with 100% survival. Grass carp (*Ctenopharyngodan idella)* fingerlings were exposed to rice herbicides butachlor 1.5 kg ha$^{-1}$, oxyfluorfen 0.25 kg ha$^{-1}$ and thiobencarb 1.5 kg ha$^{-1}$, 12 days after their application in the respective fields. To observe the impact of herbicides on the histopathology of the fish, the fingerlings were collected from the field trenches treated with herbicides at 15, 30 and 45 days after releasing into the field. The changes observed in the gill tissues are cartilagenous hyperplasia of gill rays, proliferation of lamellar epithelium and vacuolation of cytoplasm of lining epithelium, focal loss of lamellar epithelium and congestion of blood spaces. The changes in muscle tissues are swelling and necrosis of muscle fibres. The changes in liver tissues are congestion of sinosoids, congestion of central vein and proliferation of the bile ductular epithelium.**

**Key words:** Histopathology, grass carp, rice-fish farming, herbicidal impact.

## INTRODUCTION

Integrated fish farming has received considerable attention in recent years in many developing countries of Asia, Africa and America. Rice-cum fish culture was beneficial in increasing land productivity as well as eliminating weeds, mollusks and pests (RekhaGhosh et al., 1994). Growth of fish in rice field in an organized way is almost non-existent in India; the reasons being that increasing use of inorganic fertilizers and pesticides in rice fields which have deleterious effects on fish.

Weeds are one of the principal causes of low rice production, reducing the yield by 60 - 70% (RekhaGhosh et al., 1994). Herbicide alone or in combination with other components like fish provides better weed control and helps to realize the highest yield potential of a crop. A successful herbicide should not only control the weeds effectively but also be safer to the soil flora and fauna.

Increasing the use of biological methods for aquatic weed control was being suggested all over the world and many different organisms were assessed (Zon and Van, 1978). Herbivorous feeding habits of many fish species in intensive rice-cum-fish culture offered the opportunity for biological weed control under controlled water supply (Vinke and Micha, 1985). Fish species like tilapia, silver carp, grass carp etc., were herbivorous fishes that feed on aquatic weeds (Dahama, 1996). Grass carp can be very effective for controlling most aquatic weeds. The most effective way to use grass carp is to initially control plants with an herbicide and then use grass carp to prevent regrowth of weeds. The grass carp because of its plant eating diet have a great potential for the control of aquatic weeds. The grass carp is primarily a 'grazer', it tends to feed on the surface and in shallow water. Grass carp prefers submersed plants and the soft tips of young tender plants. Additional factors which influence the feeding behavior of grass carp include their size, age, gender and population density and the species, abundance and location of plants within a body of water.

Fish species are most sensitive to aquatic pollutants during their early life stages (Jiraungkoorskul et al., 2002). The indiscriminate use of herbicides, careless handling, accidental spillage or discharge of untreated effluents into natural waterways have harmful effects on fish population and other forms of aquatic life and may contribute long term effects in the environment.

Herbicides cause changes in the quality of water in and near sprayed areas. After herbicide applications, decrease in dissolved oxygen in the water, along with an increase in temperature, poses threat to the survival of cold water fish species. Fish breathe by movement of water, dissolved oxygen and any water contaminants present, in and out through their gills. Most herbicides have an irritating effect on lung tissue when inhaled.

## MATERIALS AND METHODS

### Pot culture experiment

The experiment for fixing the optimum size and time of fingerlings to be released into the rice fields after the application of pre-emergence herbicides was conducted in the pot culture yard of Annamalai University Experimental Farm. The cement pots of size 45 x 30 x 28 cm were filled with field soil to a height of about 5 cm and water to a height of about 20 cm. Fish fingerlings of three different sizes viz., 2 - 3, 3 - 4 and 4 - 5 cm were released into the pots at 4, 8 and 12 days after the application of butachlor 1.5 kg $ha^{-1}$, oxyfluorfen 0.25 kg $ha^{-1}$ and thiobencarb 1.5 kg $ha^{-1}$. The experiment was conducted in a completely randomized block design with five replications. Observations were made on mortality and survival of fish fingerlings on every alternate day.

### Histopathological study

To observe the impact of herbicides on the histopathology of the fish, the fingerlings were collected from the field trenches treated with herbicides (butachlor, oxyfluorfen and thiobencarb) at 15, 30 and 45 days after releasing into the field. Gill, liver, muscle and brain tissues were dissected from each fingerling from treated (herbicide) as well as control plots. The tissue dissection should be followed by fixation immediately after tissue removal and as soon as possible after death of the organism. The fixative used for this experiment was Bouin's zenker fluid. The tissues were then washed in running water for 12 - 18 hr and processed following the standard technique (Gurr, 1959). The tissues were dehydrated in ascending grades of alcoholic series of ethanol. Following dehydration, the tissue was cleared using clearing reagent xylene and impregnated in paraffin wax (58 - 60°C). Impregnation is the complete removal of the cleaning agent. Following impregnation, the tissues were embedded in melted paraffin by properly orienting it. When the paraffin solidifies, it provides a firm medium for keeping intact all parts of the tissue when sections are cut. Following embedment, tissues are sectioned into very thin slices of 6 to 8 µm thickness from the paraffin block using a microtome equipped with a very sharp stainless steel blade (Luna, 1968) and these were depa-raffinized in xylene and after passing through descending grades of alcoholic series and hydration in water, tissues were mounted on microscopic slides. Then the slides were stained in Heidenhain's iron haemotoxylin and counter stained with aqueous eosin. Stained sections were mounted in microscope and observed.

## RESULTS AND DISCUSSION

### Pot culture experiment

The results on the effect of three different herbicides on the mortality of fish fingerlings of three different sizes are presented in Table 1. Fingerlings of size 4 - 5 cm, when released 12 days after herbicide application were observed to be safe with 100% survival. This could be due to the fact that within 12 days which was the longest time gap tried, these herbicides might have dissipated considerably and with increasing size, the physiological tolerance of the fingerlings towards the herbicides and their ability to metabolize them increased, imparting higher survival rates. The half life of butachlor in transplanted rice fields was observed to be 3 to 4 days (Kathiresan, 2001). In addition to similar rapid degradation, rice herbicides like butachlor and thiobencarb were observed to be only of moderate toxicity to fishes with LC50 at 0.5 - 10 ppm (Ooi and Lo, 1992). Further reports by WHO, 1988 indicate that the acute oral and dermal toxicities of thiocarbamate (thiobencarb) compounds are generally low. These are in line with the present study which revealed that 100% survival of fishes of size 4-5 cm is possible if released even at 8 days after thiobencarb application.

### Histopathological studies

#### *Gills*

**Untreated:** Fish gill is the major organ responsible for respiratory gas exchange, osmoregulation, acid-base regulation and ammonia excretion. No recognizable changes were observed in the gills of the untreated fish. The gills comprise of rows of long, thin primary lamellar epithelium attached to a gill arch-a bit like the teeth of a comb (Figure 1a). Running across the primary filament, on both sides, are rows of tiny semi-circular foldings - the secondary lamellae. Gaseous exchange takes place by diffusion. This is facilitated by the delicate structure of the secondary lamellae. The gill epithelium is extremely thin, usually just one cell thick.

**Treated:** Light microscopic study of the gills of *Ctenopharygodan idella* (grass carp) exposed to herbicides (butachlor, oxyfluorfen and thiobencarb) showed several pathological changes. The changes observed were carti-laginous hyperplasia of gill rays (Figure 1b), proliferation of lamellar epithelium and vacuolation of cytoplasm of lining epithelium (Figure 1c), focal loss of lamellar epithelium and congestion of blood spaces (Figure 1d). This was in agreement with previous report that cell proliferation, lamellar cell hyperplasia, lamellar fusion and epithelial lifting were observed in gill filaments of *Oreochromis niloticus* when exposed for 3 months to sub-lethal concentrations of the commercial glyphosate herbicide Roundup (Jiraungkoorskul et al., 2003). On the gills of carp, *Cyprinus carpio* L. exposed to 5.0 mg/l glyphosate concentration, epithelial hyperplasia and subepithelial edema were found by Neskovic et al. (1996). The organophosphates used has been reflected it in gills, provoking hypertrophy and hyperplasy of epithelium

**Table 1.** Effect of size and time of release of fish fingerlings after herbicide application on fingerling mortality.

| Size of fingerlings | Time of release of fingerlings | Herbicide | Mortality rate of fingerlings after release (%) | | | | |
|---|---|---|---|---|---|---|---|
| | | | 2nd day | 4th day | 6th day | 8th day | 10th day |
| 2 - 3 cm | 4 days after herbicide application | Butachlor | 100 | - | - | - | - |
| | | Oxyfluorfen | 100 | - | - | - | - |
| | | Thiobencb | 80 | 20 | - | - | - |
| | 8 days after herbicide application | Butachlor | 100 | - | - | - | - |
| | | Oxyfluorfen | 100 | - | - | - | - |
| | | Thiobencarb | 60 | 40 | - | - | - |
| | 12 days after herbicide application | Butachlor | 60 | 20 | 20 | - | - |
| | | Oxyfluorfen | 100 | - | - | - | - |
| | | Thiobencarb | 40 | 40 | 20 | - | - |
| 3 - 4 cm | 4 days after herbicide application | Butachlor | 80 | 20 | - | - | - |
| | | Oxyfluorfen | 100 | - | - | - | - |
| | | Thiobencarb | 80 | 20 | - | - | - |
| | 8 days after herbicide application | Butachlor | 40 | 20 | - | - | - |
| | | Oxyfluorfen | 60 | 20 | - | - | - |
| | | Thiobencarb | 40 | - | 20 | - | - |
| | 12 days after herbicide application | Butachlor | 20 | - | - | 20 | - |
| | | Oxyfluorfen | 40 | 20 | - | - | - |
| | | Thiobencarb | 20 | - | 20 | - | - |
| 4 - 5 cm | 4 days after herbicide application | Butachlor | 40 | - | 20 | - | - |
| | | Oxyfluorfen | 40 | 20 | 20 | - | - |
| | | Thiobencarb | 20 | 20 | 40 | - | - |
| | 8 days after herbicide application | Butachlor | - | - | - | - | - |
| | | Oxyfluorfen | 20 | - | - | - | - |
| | | Thiobencarb | - | - | - | - | - |
| | 12 days after herbicide application | Butachlor | - | - | - | - | - |
| | | Oxyfluorfen | - | - | - | - | - |
| | | Thiobencarb | - | - | - | - | - |

cells and structure destruction of the lamellas (Capinpin, 1995). Kumaraguru et al. (1982) reported fusion, fusion of adjacent secondary lamellae as a result of hyperplasia in the gills of rainbow trout *Salmo gairdneri* exposed to permethrin.

## Liver

**Untreated:** The liver histology of control fish revealed the typical parenchymatous appearance (Figure 2a). At the light microscopic level, the liver was divided into ir-regularly shaped lobules separated by the hepato-pancreas and bile duct. Bile ducts accompany the hepatic artery and portal vein while coursing through the liver. The liver was made up of hepatocytes that were polygonal cells with a central spherical nucleus and a densely stained nucleolus. Central veins are found randomly throughout the hepatic parenchyma of fish. Between the cords of hepatocytes is a three-dimensional network of cylindrical blood sinusoids. The sinusoid is an irregularly dilated vessel whose caliber is larger than the diameter of regular capillaries.

**Treated:** The pathological changes observed in the liver tissues were congestion of sinusoids (Figure 2b), congestion of central vein (Figure 2c) and proliferation of bile ductular epithelium (Figure 2d). Congestion of sinusoids was the only pathological effect of organo-chlorine herbicides reported by Couch (1975) and was also recorded in our glyphosate study. On the liver of carp, *C. carpio* L. exposed to 10.0 mg/l glyphosate concentration, congestion of few sinusoids and on some places, signs of early fibrosis were recorded (Neskovic et al., 1996). *O. niloticus* exposed to sublethal concen-trations of the commercial glyphosate herbicide Roundup, showed histopathological changes in the liver viz., vacuolation of hepatocytes and nuclear pyknosis. The results indicated that long-term exposure to glyphosate at sublethal concen-trations had adverse effects on the histopathological and biochemical alterations of the fish (Jiraungkoorskul et al., 2003).

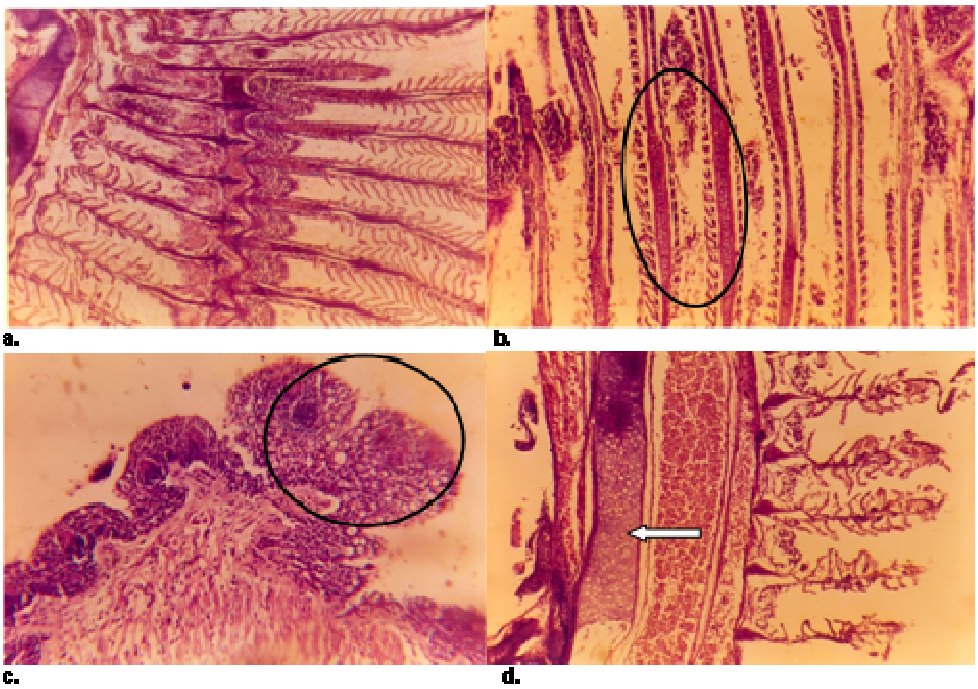


**Figure 1. a:** Gills of control fish. **b:** Cartilaginous hyperplasia of gill rays. **c:** Proliferation of lamellar epithelium and vacuolation of cytoplasm of lining epithelium. **d:** Congestion of blood spaces.

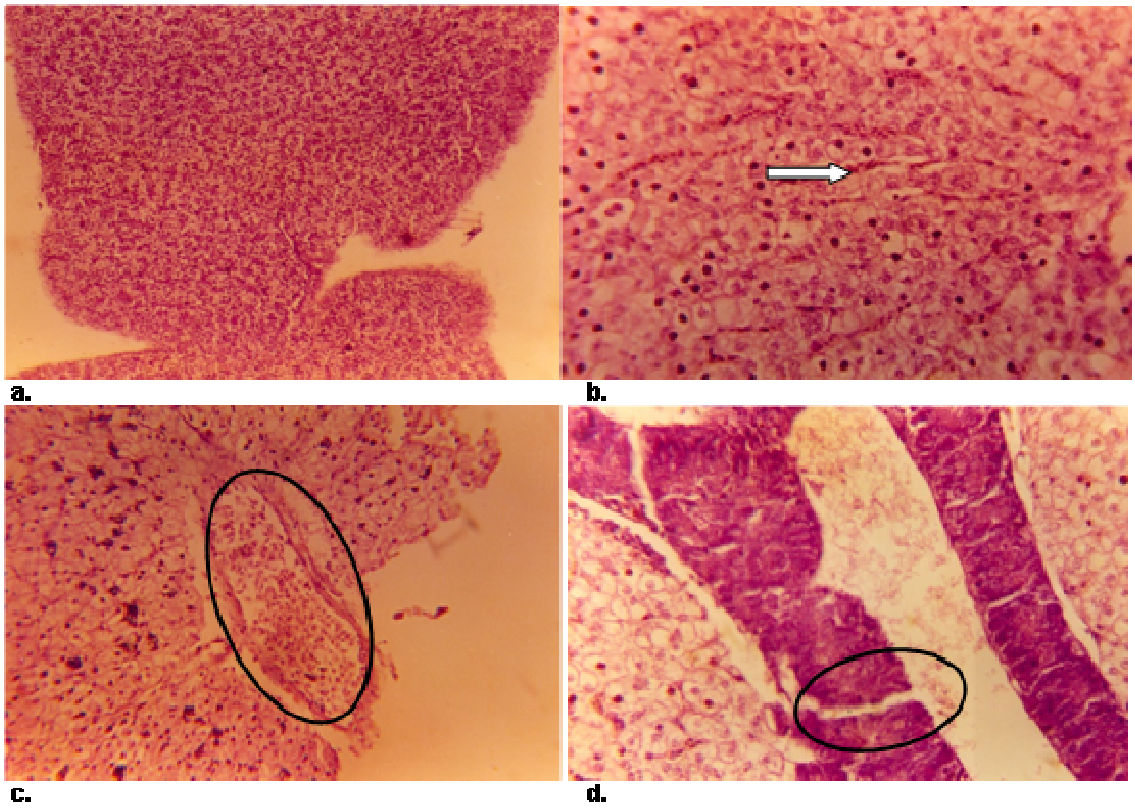


**Figure 2. a:** Liver of control fish. **b:** Congestion of sinusoids. **c:** Congestion of central vein. **d:** Proliferation of bile ductular epithelium.

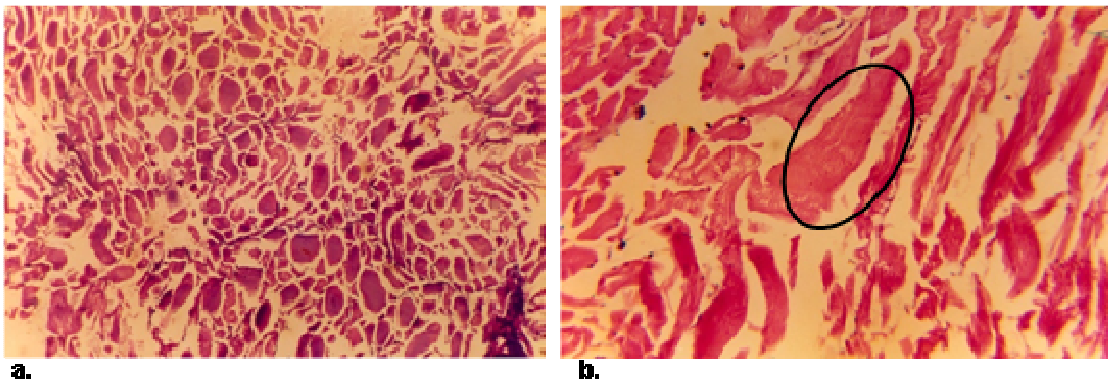


**Figure 3. a:** Muscle of control fish. **b:** Swelling and necrosis of muscle fibres (S).

### *Muscle*

**Untreated:** The muscle fibres of carp are arranged as myotomes in concentric circles and joined head to tail by connective tissue called myocommata (Figure 3a). Each fibre is surrounded by connective tissue, endomysium.

**Treated:** The pathological changes observed in the muscle tissue were swelling and necrosis of muscle fibres (Figure 3b). The magnitude of tissue distortion increased with increasing age of the fingerlings and their exposure to the herbicide treated water. Grass carp exposed to sub lethal and lethal (96 h LC 50) concentration of common pollutants like herbicide, showed a general increase in plasma glucose level, irregular fluctuation in plasma lactate level, decrease in muscle and liver glycogen contents and a general increase in hepatic glucose-6-phosphatase activity (Abo Hegab et al., 1992).

## Conclusion

Though the fishes were able to survive and put up growth, when they were released after 12 days of herbicide application, they showed histopathological changes due to the impact of herbicide. The possibility of any residual carryover effect of herbicides to human consumers through treated fishes is yet to be studied, the present study revealed that the fishes suffer some tissue deformation in gills, muscle and liver due to herbicides. As food source, fish interferes on man's life quality and so more detailed analysis of the action of what may sub lethal doses of pesticide and insecticide substances provoke in these organisms is necessary. Hence, it is concluded that using a herbicide in conjunction with fishes is unsafe from the viewpoint of fish culture enterprise and is not necessary from the viewpoint of the cropping enterprise as the herbicides themselves are delivering the goods.

## REFERENCES

Abo-Hegab S, Assem H, Kandil A (1992). Toxic effects of environmental pollutants on the carbohydrate metabolism in grass carp, Ctenopharyngodanidella. Zoologische-Jahrbucher, Abteilung-fur-Allg-emeine-zoologie-und-physiologie-der-Tiere, 96(2): 255-262.

Capinpin EC (1995). Gill lesions in Nile Tilapia associated with acute malathion poisoning. SEAFDEC Asian Aquacult. 17(3): 4-6.

Couch JA (1975). Histopathological effects of pesticides and related chemicals on the liver of fish. In: Ribelin WE, Magaki G (eds). The Pathology of Fishes. The University of Wisconsin Press, Madison, Wisconsin, USA. pp. 559-575

Dahama AK (1996). Organic farming for sustainable agriculture. Agro Botanical Publisers, India, p.128.

Gurr,E. 1959. Methods for analytical histology and histochemistry, Leonard Hill (Books) Ltd., London.

Jiraungkoorksul W, Upatham ES, Kruatrachue M, Sahaphong S, Vichasri-Grams S, Pokethitiyook P (2002). Histopathological effects of Roundup, a glyphosate herbicide, on Nile tilapia *Oreochromis niloticus*). Science Asia 28*:* 121-127.

Jiraungkoorskul W, Upatham ES, Kruatrachue M, Sahaphong S, Vichasri-Grams S, Pokethitiyook P (2003). Biochemical and histopathological effects of glyphosate herbicide on Nile tilapia (*Oreochromis niloticus*). Environ. Toxicol. 19: 260- 267.

Kathiresan RM (2001). Sustainable weed management for rice-blackgram cropping system. In: Abstracts of biennial conference of Indian Society of Weed Science, University of Agricultural Sciences, Bangalore, p. 79.

Kumaraguru AK, Beamish FWH, Ferguson P (1982). Direct and circulatory paths of Permethrin causing histopathological changes in gills of *S. gairdneri.* J. Fish Biol. 20: 87-91.

Luna LG (1968). Manual of histologic staining methods of the Armed Forces Institute of Pathology. McGraw-Hill, New York, NY.

Neskovic NK, Poleksic V, Elezovic I,Karan V, Budimir M (1996). Biochemical and Histopathological Effects of Glyphosate on Carp, *Cyprinus carpio* **L.** Bull. Environ. Contam. Toxicol. 56: 295-302

Ooi GG, Lo NP (1992). Toxicity of herbicides to Malaysian rice field fish. In: Proc. of 3rd International Conf. on plant protection in the Trop. Malaysian Plant Protectiopn Society, Kualalumpur. 3: 71-74.

RekhaGhosh B, Ghosh C, Mitra A, Jana MK, Mitra BN (1994). Techniques of rice-cum-fish culture for increasing productivity in lowlands. Indian Fmg. 44(4): 23-26.

Vinke P, Micha JC (1985). Fish culture in rice fields. Int. Rice Commn. News, (FAO/IRC), 34: 297- 314.

WHO (1988). Thiocarbamate pesticides: A general introduction. Environ. Health. Criteria. 76(49): 55.

Zon JCJ Van (1978). Status of biotic agents other than insects or pathogens as biocontrols. In: Proc. of the 4th Int. Sym. of Biol. Control of weeds, Gaineville, Florida, USA, pp: 245-250.