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Alka Srivastava
Department of Zoology,
L. N. Mithila University,
Kameshwarnagar, Darbhanga,
Bihar, India

Corresponding Author:
Alka Srivastava
Department of Zoology,
L. N. Mithila University,
Kameshwarnagar, Darbhanga,
Bihar, India

Toxic effects of agrochemical on the muscles of *Channa punctatus* (Bloch)

Alka Srivastava

Abstract
Snakehead, *Channa punctatus* (Bloch), a common air breathing freshwater fish. By virtue of its omnivorous feeding habits, and easy and abundant availability and adaptability to varied environmental conditions, was selected as an ideal experimental animal for the present investigation. On exposure to sublethal concentration, marked thickening and separation of muscle bundles, haemolysis, necrosis, lesions with reduced compactness was observed. Sublethal concentration led to pronounced intramuscular oedema with minor dystrophic changes. Separation of muscle bundles and degeneration in muscle bundles with aggregations of inflammatory cells between them was an interesting observation. Also, vacuolar degeneration in muscle bundles and atrophy of muscle bundles were observed. Histological examinations revealed great variability in the intestinal lesions severity existed among most fishes and included focal deformation with necrosis of mucosal epithelial layer of some villi, enlargement of the villi due to vacuolar degeneration or cloudy swelling of the mucosal epithelial cells, lycmphocyte infiltration, dissociation and reduction of muscular bundles and serosal lysis were also detected.

Keywords: *Channa punctatus*, haemolysis, necrosis and lesions

Introduction
Pesticides have been reported to cause a variety of changes in fish, ranging from changes in colour and behaviour to damage to gonads and a decrease in the survival of the offspring. However, many of the recorded changes are not dose-dependent and are often found only in laboratory studies and not in the field. Fish species are susceptible to enzyme and hormonal disruptors. Chronic exposure to low pesticide levels can have a more profound impact on fish stocks than acute poisoning. Pesticide levels that are not high enough to kill fish are associated with subtle behavioural and physiological changes that influence both survival and reproduction. Environmental pollution caused by pesticides, especially in aquatic environments, is a serious problem. Contamination of fish by chemicals, either directly or indirectly, is a continuous health threat to the public. Human population is thus at high risk by eating these toxic fish. Karuppasamy (2002) [1] studied histopathological changes in *Channa punctatus* treated with phenyl mercuric acetate. Cengiz and Unlu (2002) [2] studied histopathological changes in gill of *Channa marulius* under CuSO4 and ZnSO4 toxicity stress.

High concentration pesticides are known to reduce the survival, growth and reproduction of fish and to have many observable effects. Due to residual pesticide effects, essential organs such as the kidneys, liver, muscles and genital organs are impaired. Severe thickening and separation of muscle bundles, haemolysis, necrosis, lesions with decreased compactness have been observed when exposed to sublethal concentrations. Sublethal concentrations resulted in pronounced intramuscular oedema with minimal dystrophic changes. Degeneration in muscle bundles with inflammatory cell aggregations. The literature shows that very little efforts has been made to study the histopathological changes caused by Carbaryl in the *Channa punctatus.* Hence the present study was undertaken to study in detail histopathological changes in the soft tissues of the freshwater fish *Channa punctatus* after acute and exposure to the Carbaryl.

Materials and Methods
Test Animal
Healthy fishes of *Channa punctatus* were collected from the local ponds and swamps and brought to laboratory for acclimatization.

The fishes were stored in glass aquaria containing dechlorinated tap water for fifteen days for acclimatization under laboratory conditions. Water was changed after every 24h.

Carbaryl was chosen for present study owing to its extensive application among the carbamates for the control of nearly 100 pests.

For conducting histological studies fishes were exposed to sublethal concentration of the test chemical. Fishes in batches were exposed to 3ppm, 0.16ppm and 0.25ppm of carbaryl for 48 hrs. Fishes were starved one day before experimentation and also not fed during exposure period. Equal number of fishes was kept in water without pesticide for the same period under lab conditions.

Tissues specimens from three fish per treatment were removed and small pieces of muscles were immediately fixed in neutral buffered formalin 10%, dehydrated in ascending grades of ethanol, embedded in soft paraffin, sectioned at 5m thickness and stained with hematoxylin and eosin (H&E)

1cm thick samples were fixed in Bouin's fixative for a period of 24hr. The tissue being pliable were larger in size during the first fixation and cut into small pieces after 1/2hr pf soaking. It was then left in the Bouin's solution for the remaining period. After complete dehydration, the samples were transferred to xylene until they appeared to be transparent. Samples were treated with xylene and then transferred to xylene-wax mixture (3:1 and 1:1) for 5 min at each stage. This was for infiltration of wax into the tissue cavities, after infiltration using xylene: wax mixture, the tissues were further infiltrated using pur, with two adjustments for 10 min. The tissues were then embedded in paraffin with ceresin (Congealing point about 600C) using L-blocks. The blocks were then sectioned to a thickness of 8m using microtome. The sections were fixed on clean dry slide using glue and dried before further staining. The slides were dipped in xylene in a decreasing series of alcohol and were stained using Weigert's - Haematoxylin stain for 5 min with acid : alcohol wash for 30 seconds. They were then passed to Van Geison's stain for 5 min and dehydrated through a series of alcohol for 10 min followed by xylene for another 10 min. After staining the permanent slides were made using DPX mountant. The photographs were taken using Digi Eye fitted Digital microscope and Computer.

Results and Discussion

Histological examinations revealed great variability in the intestinal lesions severity existed among most fishes and included focal deformation with necrosis of mucosal epithelial layer of some villi, enlargement of the villi due to vacuolar degeneration or cloudy swelling of the mucosal epithelial cells, lymphocyte infiltration, dissociation and reduction of muscular bundles and serosal lysis were also detected Fig 1 and 2. Vacuolization in submucosa & circular muscles and dilation of columnar & goblet cells of mucosal folds were observed by Sastry & Sharma (1979) [3] describe the lesion formation in villi of *Clarias batrachus* after exposure to sumithion. At higher concentration of the pesticide severe damage to the microvilli occurred. Destruction of submucosa layer as well as vacuolation on it was seen. Besides narrower mucosa layer, hemorrhage, inverted microvilli were also observed. The results are in agreement with the findings of other investigators. Destruction of columnar epithelium, submucosa fused with muscles and serosa was found in broken condition after 10 days exposure to the pesticide by Srivastava & Srivastava (1995) [4]. The muscle in the control fish have normal myotomes with equally spaced muscle bundles. As with gills, muscle tissue is also in direct contact with toxins dissolved in water. The reactions in the ultrastructure of the muscle were therefore random. Several histopathological changes have been observed. Separation of muscle bundles and intracellular oedema was an important finding. Initial stimulus can induce hyperactivity and excitability in animals, leading to subsequent muscular fatigue [5]. All these changes were clearly evident as clinical signs at the initial stage of the experiment and were subsequently reflected through histopathological changes in muscle. On exposure to sublethal concentration, marked thickening and separation of muscle bundles, haemolysis, necrosis, lesions with reduced compactness was observed.

At higher concentration of the pesticide severe damage of muscle bundles, shortening of muscle bundles, thickening of muscle bundles, severe intra muscular oedema and necrosis of muscle bundle were observed.

The histopathological alterations seen in muscles of fish in present work are in agreement with those observed by authors who have studied the effects of different pollutants on fish muscles [5, 6]. Das & Mukherjee (2000) [5] found that the muscle tissues collected from more polluted region showed severe damages than the muscle tissues collected from less polluted region such as, severe damage of muscle bundles, shortening of muscle bundles, thickening of muscle bundles, severe intra muscular oedema and necrosis of muscle bundle were observed. Das & Mukherjee (2000) [5] have stated that separation of muscle bundles and intracellular oedema were an interesting observation. The histopathological alterations in the fish muscle of both the doses are in agreement with those observation by many investigators who have studied the effect of different pollutants on fish muscle. Focal areas of myolysis were seen in the muscles of *O. spilurus* exposed to contra/insect 500/50E.

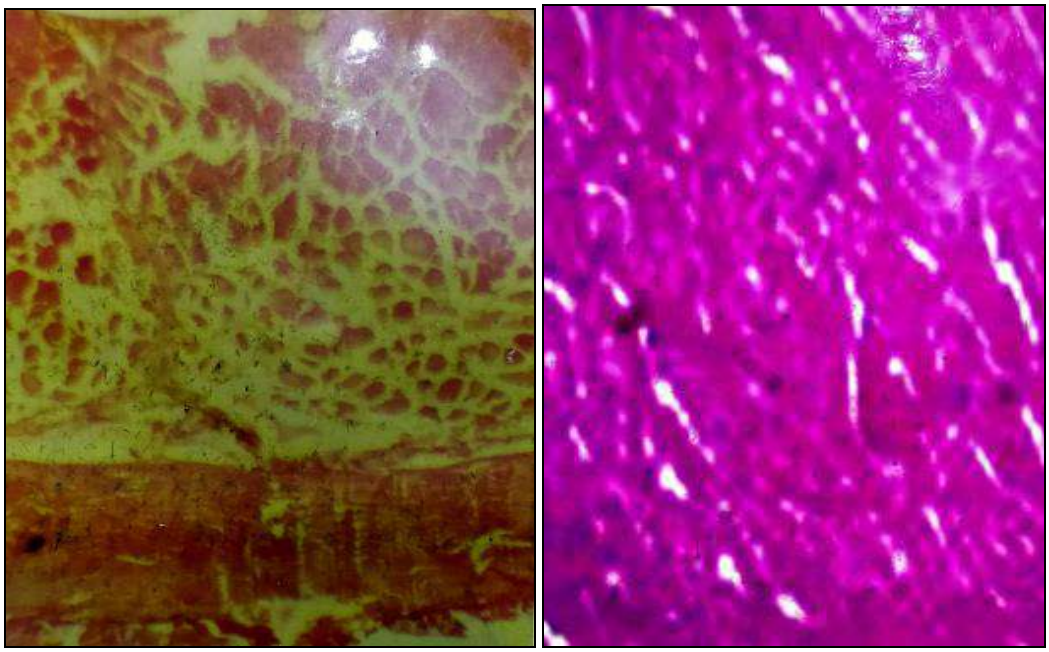

Fig 1 and 2: Shows necrosis of mucosal epithelial layer of some villi, enlargement of the villi due to vacuolar degeneration or cloudy swelling of the mucosal epithelial cells, lymphocyte infiltration.

Conclusion

All the researchers are of the opinion that the widespread use of this pesticide for both agricultural and domestic purposes could adversely affect non-target species such as fish. Histological effects of xenobiotics on fish organs have been examined by several scientists, in the light of the studies cited above, it is evident that, in the present investigation, carbaryl at lethal and sub-lethal concentrations caused substantial histological damage to the examined organs. It is concluded that more or less similar pathological changes are caused by various toxicants in the tissues of different fish, but the degree of damage differs depending on the dose of the toxicants, the period of damage varies depending on the dose of the chemical, the duration of exposure, the toxicity of the chemical and the sensitivity of the fish.

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