IMPACT OF CYPERMETHRIN TOXICITY ON FUNDAMENTAL ASPECTS OF *CARASSIUS AURATUS*.

Nutan Kamble[1], Harsha Padwal[2], Vinod Narayane[3].
Department of Zoology, B. K. Birla College of Arts Science and Commerce, Kalyan
Maharashtra, M.S. India.

Abstract:
Pollutants like pesticides, heavy metals, detergents and effluents from various industries, agricultural fields, households etc induce alterations in the water quality when discharged untreated and indiscriminately. This causes changes in the behavior, physiology and biochemistry of aquatic inhabitants. Among these inhabitants fishes are of main concern because of their ecological and economic importance. Among all these pollutants pesticide pollution is a matter of global concern and largely of developing countries. Pesticides like cypermethrin are pyrethroids derivatives found in many commercial products including the insecticides used at households, pet sprays and shampoos. Some pyrethroids are also used in lice treatments applied directly on head and in mosquito repellents which can be applied on clothes. Hence such pyrethroids can be found abundantly in the aquatic environment. The current investigation aims at toxicity studies of Cypermethrin for which a common gold fish *Carassius auratus* is chosen as animal model. *Carassius auratus* weighing 20 Gms were purchased from the local vendor and acclimatized to laboratory conditions for 7 days prior to the experimental work. Dose lower than the LC_{50} value was used for the treatment. Three sets each containing 7 fishes were exposed to a fixed dose for 3 different durations, control set was maintained individually. Fishes from the aquaria were removed subsequently and dissected to procure tissues such as gills and muscles which were then washed clean with saline and used for biochemical study such as proteins, carbohydrates and histopathology. Changes in behavioral pattern were also observed during the study. Levels of tissue carbohydrates and proteins showed significant increase after 21 days of treatment over the values obtained after 7 and 14 days of treatment. Histopathological studies indicated necrotic changes in gill tissues and shortening of fibers in muscle tissues. Swimming pattern, schooling behavior seems to have altered in treated fishes when compared with the control group.

Keywords: Cypermethrin, pyrethroid, *Carassius auratus*.

I. INTRODUCTION

Agriculture being the main source of food for mankind, countries like India is significantly dependent on agriculture for food[4]. Development in the science and technology has served as boon in protecting the agricultural crop from the insect pest. Agrochemicals such as pesticides, insecticides and various others are not only used in agricultural fields but are also widely used in households and industrial spaces[1]. This wide range of utility enhances their presence in nearby water bodies. Accumulation of effluents like Pesticides in water bodies alter the water quality rendering it importable as well as unfit for survival of aquatic fauna[1].

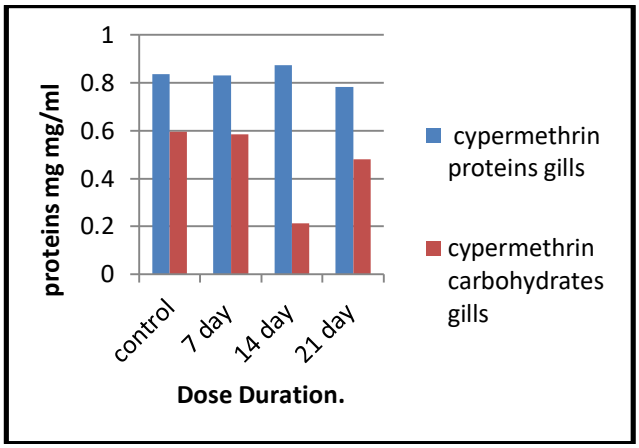
Cypermethrin is one such pesticide widely used across the globe for various agricultural, industrial as well as household purposes [3]. Aquatic inhabitants such as invertebrates, fishes, algae and mosses are greatly affected due to such pollutants [2]. Fishes in particular are of great economic concern being the cheapest source of animal proteins to humans. Supplementary proteins obtained from fishes are necessary for humans in order to counter the prevalent protein deficiency [2]. Thus it is important to analyze the water quality as well as the health of fishes as they can serve as bio indicators of pesticide toxicity [1].

Large amount of pollutants like pesticides, heavy metals, industrial effluents are whys and wherefores for huge fish kills which indicates the distortion of biological niche[2]. Fundamental biochemical parameters like opercula movement gets deviated from normal since pollutants such as pesticides affects the solubility of oxygen in water leading to inadequacy of dissolved oxygen. This insufficiency of oxygen initiates stress to the aquatic organisms particularly to fishes. Stress further leads to disruption of various biochemical metabolic processes such as carbohydrates and proteins. The present investigation aims at investigating the alterations brought about in the levels of proteins, carbohydrates and behavioral patterns of fish due to pesticidal stress.

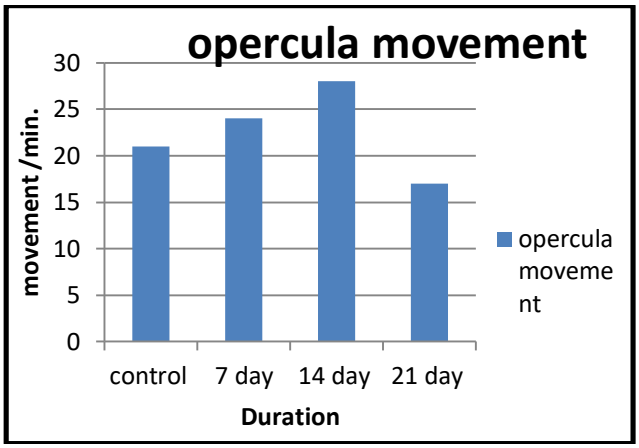
II. Materials and methods*:*

Healthy and active fishes were bought from the local fish market Kalyan and were maintained in clean aquaria of 20 liters each containing 7 fishes [4]. They were acclimatized to the laboratory conditions for 7 days prior to the experimentation work. Fishes were fed with commercially available fish food twice a day in accordance to their body weight. Experimental sets were run in triplicates. Cypermethrin an agricultural pesticide was used for toxicity studies. Dose was determined based upon the Lc_{50} value (0.125µl/L). 1/5 $^{th.}$ dose (sub lethal) of the Lc_{50} value was used for the study. After the predetermined durations of 7, 14 and 21 days fishes from the aquaria were removed and dissected with prior to the dissection, fishes were euthanized using clove oil. Gill tissues were dissected out, washed clean with saline solution and used for biochemical analysis. Total proteins were studied using Fowlin Lowry method, total carbohydrates using Anthrone method, and histopathological alterations using H E staining process [3]. Experimental as well as control sets were carefully studied and observed for fundamental changes on opercula movements and other behavioral patterns. Dissolved oxygen levels of experimental aquaria were estimated by Winkler's method. Behavioral alterations due to pesticide stress were observed and recorded [5].

III Result and discussion:



Graph 1



Graph 2.

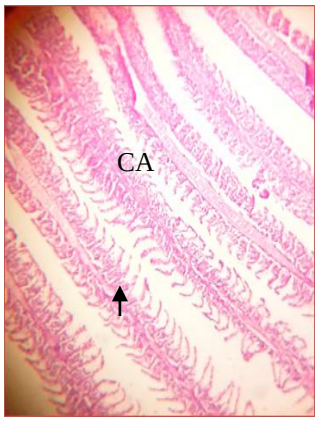


Fig. a sagittal section of gills control set x100

Fig. b sagittal section of gills control group x100

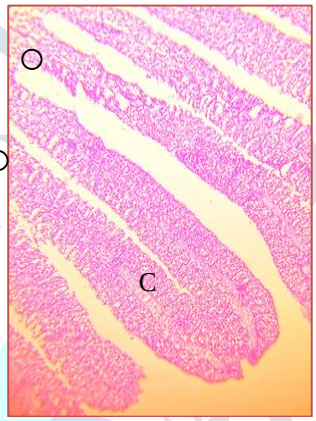


Fig. c sagittal section of gills 7 days treatment group set x100

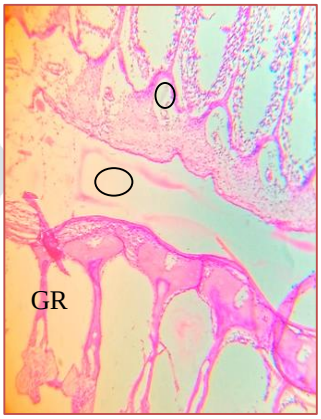


Fig. d sagittal section of gills 7 days treatment group x100

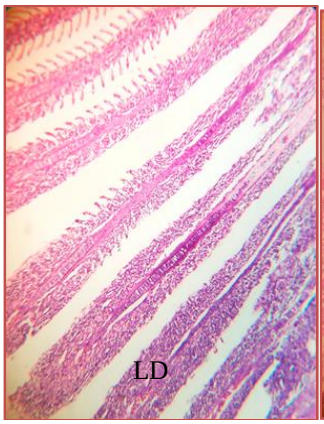


Fig. e sagittal section of gills 14 days treatment group x100

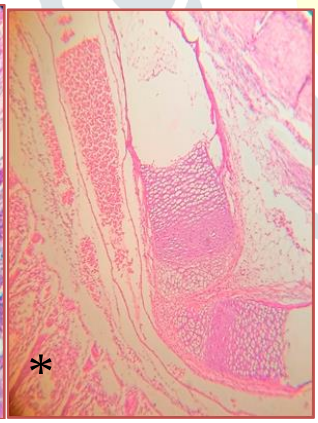


Fig. f sagittal section of gills 14 days treatment group x100

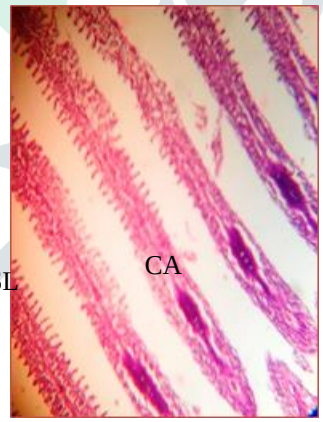


Fig. g sagittal section of gills 21 days treatment group x100

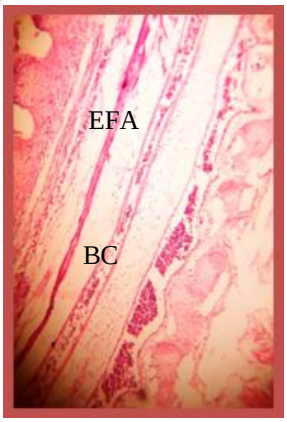


Fig. h sagittal section of gills 21 days treatment group x100

Fig. a, b – sections of gills in control set showing normal and free water channels (arrow). Fig c, d- section of gills of 7 day treatment group. Fig. e, f- section of gills of 14 days treatment group.
o- edema of gills, c-central axis. GR-gill rakers EBA-Efferent bronchiole arteriole , EBAy- Efferent bronchiole artery,* - hyperplasia LD-lamellar degeneration. BC- Blood congestion.

Behavioral observations:

Pesticides have shown different alteration in behavior of various fish species such as sluggish movements and altered swimming ability, reduced appetite, maintain their position and defend their territories[15,16] . These pesticides also have shown interruption in schooling behavior [17] due to dangling, erratic and irregular movements and disturbed swimming. On account of stress due to pesticides, fishes became immune compromised, which make them more susceptible and vulnerable to diseases, secondary infections and pathogens [18], increased movements of opercula, rapid jerk movement, equilibrium loss, body colour alterations, frequent surfacing, and elevated mucus secretion , hyper excitability and sinking to bottom were some of the major behavioral observations made in the current investigation which were prominent in all treatment groups.

Histopathological observations:

In histopathological studies of gills, the tissues obtained from control group have normal and healthy appearance as water channels are free (fig. a).and depicts normal cell arrangement with no congestion of gill lamella (fig. b). After 7 days treatment several pathological changes in the histology of gills were observed. Severe congestion of gill lamellae, fusion of sclerenchyma, edematous and distorted primary lamella, unclear Interlammelar channels, blocked Water channels are evident. Central axis is invisible (fig c.). Hyperplasia is seen at the base of gill lamella with edema (fig d). Similar histopathological alterations are seen after 14 days treatment with increased severity (fig. e). Hyperplasia at the base of the gill is found to be set in after 14 days treatment (fig. f). Whereas, after 21 days treatment the histopathological observations are indicative of slight recovery in gill tissues but at the base of the gill lamella enlargement and dilation of the efferent bronchiole artery and blood congestion is seen (fig. g, h)

Biochemical studies of gill tissue hinted at the stress induced by the cypermethrin treatment on the respiratory physiology of the fishes nearly in all sets. Biochemical estimations of proteins from the gill tissues after seven and fourteen days treatment showed insignificant decrease in the protein levels in comparison with the gill tissues procured from the control group .at Further decrease in the levels of proteins were seen in the gills tissues of 21 days treatment group. Thus it was noticed that the reduction in the protein level is gradual. The biochemical levels of total carbohydrates in gills showed almost same values in 7 days treatment group as compared to control group but in 14 days treatment group the levels were found to be insignificantly higher. In 21 days treatment group these levels were found to be restoring. These results are contradictory to those produced by the previous workers who suggested that the proteins as well as carbohydrate levels decrease from the tissues due to the treatment of cypermethrin [15].Another study reveals that there is a continuous elimination of the pesticides from the body[15].

Results obtained in the previous investigations were mainly derived from the treatment of cypermethrin using various doses over a fixed duration under an acute regimen [15], while in the current investigation; a fixed dose was delivered for three different durations. Thus unaltered levels of proteins and carbohydrates at the end of each treatment period could be result of acclimatization of the animal to the drug regimen. Results obtained from the observations of operculum movement indicated that to overcome the stress induced by the drug treatment was combated by the animal by increasing its respiratory rate at least after 7 and 14 days treatment. Lower rate of respiration was observed in 21 days treatment group. Thus it could be guessed that 21 days treatment is perhaps long enough to induce energy imbalance in the animal at the given dose. This can be further supported from the values of total carbohydrates obtained after 21 days treatment. Tough these values were found to be higher than the values obtained after 14 days treatment they were still not at par with those obtained after 7 days treatment.

A correlation between the histopathological changes and biochemical analysis can be traced. Non-significant alterations in the levels of total carbohydrates and proteins in seven days treatment group over their control values are indicative of abilities of the animal to tolerate the stress caused by the treatment whereas the significant drop in the level of total carbohydrate in 14 days treatment group is an indication of higher rate of metabolism which can be further corroborated on the basis of increased rate of operculum movements. Histological studies and comparison reveals that the gills appeared less damaged after seven days treatment than 14 days treatment. While one would expect further impairment in the histology of gills after 21 days of treatment and drop in carbohydrate levels, it is evident from the results that the gills are less damaged in comparison with 14 days. This suggests that the animals may have developed the tolerance to the pesticide over the treatment period, which is also evident from the increased levels of carbohydrates in 21 days treatment group. The rate of operculum movement tough has not shown mark of recovery.

IV.Conclusion:

From the results it can be concluded that cypermethrin has cytotoxic effects on the gills, which can be confirmed from the histopathological and biochemical studies. Irregular swimming movements, constant surfacing and excess secretion of the mucus are also suggestive of the metabolic stress caused by the cypermethrin treatment. Animal seems to have great ability to adapt to the changing chemical environment as it shows the major effects in 14 days treatment group and moderate recovery despite the continuation of the treatment in 21 days treatment group this is perhaps due to continuous elimination of the pesticide from the body of animal..

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