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RESEARCH ARTICLE

CHLORPYRIFOS TOXICITY STUDIES ON FISH SCALES OF *ANABAS TESTUDINEUS*

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Abstract

Indiscriminate use of organophosphorus compounds as pesticides is causing a threat to non-target organisms, especially in aquatic environments.The present investigation was to find the chlorpyrifos toxicity effects in scales ofthe edible freshwater fish, *Anabastestudineus.* The structural morphology, melanophore index, and decalcification effects were selected for elucidating the toxicity effects in the scales of Anabas. The fish was subjected for a period of 18 days to low dosages (12.5 ppm& 25 ppm) of chlorpyrifosand the changes were observed.The study revealed that exposureto 12.5 ppm and 25 ppm concentrations of chlorpyrifosaltered the scale morphology. The circuli of the scalesof treated fish were observed to be faint, degraded and loosely attached to the underlying skin. A remarkable change observed was the change in color of *Anabas testudineus*, it became too pale. Chlorpyrifos was found to induce the dispersion of melanophores. The study also revealed an increase in decalcification of the scaleswith the increasing concentration of chlorpyrifos. From the results of the parameters studied it is evident thatchlorpyrifos cause acute toxic effects on *Anabastestitudens* as early as 18 days of exposure.

Introduction:-

The aquatic environment is continuously being contaminated with toxic chemicals from industrial, agricultural, and domestic activities. Contamination of water with insecticides is mainly due to intensive agriculture combined with surface runoff and surface drainage. Among different insecticides, organo-phosphorous insecticides represent one of the most widely used classesof insecticidesthat frequently enter the aquatic ecosystem. Chlorpyrifos is one such insecticide that is extensively used and causes toxic effects on fish and other aquatic organisms. Fish are highly susceptible and show several alterations in physiology, and structural and behavioral patterns (Johal and Shawney,2007). Moreover, Fishbeing an important food resource for humans worldwide, it is important to assess the toxic effects of these pollutants. Too muchaccumulation of pollutants in fish may turnunhealthy for human consumption.The production of healthy fishisaffected due to the toxic effects of insecticides in the fish body. Scales being the first line of defense in fishes, any alteration in fish scales is an indicator of water pollution and fish health. Hence assessment of fish scales bears importance. Though many acute and chronic toxic effects of chlorpyrifos in different fish species are extensively studied (Padmanabha et al., 2015), this study is intended to find all the possible effects of chlorpyrifos on fish scales in*Anabas testudineus*so that the scales can be used as an indicator of fish health and water pollution.This study was to investigate the toxicity effects on scales of *Anabas testudineus*so that it can be

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used indirectly as an indicator of aquatic pollution too. Anabas is of commercial value because of its taste and nutritive value. (Afsar2012). It is also less expensive than other commercial freshwater fish.

Materials and Methods:-

Collection and maintenance of experimental animal

The fish *Anabas testudineus*were collected from a local fisherman in Thiruvalla, Pathanamthitta, Kerala, India. Animals were transported to the laboratory in large, aerated plastic bags and maintained in aquarium tanks containing well-aerated dechlorinated tap water for seven days for acclimatization. The fish were fed on a commercial pelleted diet. Both sexes were selected for the studies.

Experimental design for LC_{50}determination

Renewal toxicity test methods (APHA 1995) were done to find out the 96-hour LC_{50}concentration. Six fishes each were randomly selected from the stock and exposed to different concentrations of chlorpyrifos (12.5, 25, 37.5, 50 ppm) for 96hrs to determine the sublethal concentration of chlorpyrifos (LC_{50}) and consider the mortality rate. The treated groups were observed for 96 hours, with a parallel control. The obtained mortality was subjected to Probit Analysis (Finney, 1971) and LC_{50} was calculated.From the observation, it was calculated that the 96hr LC_{50} of chlorpyrifos was 31.25ppm.

Experimental Setup

The 96-hour LC_{50} for chlorpyrifos in *Anabas testudineus* was found to be 31.25 ppm. The fish were divided into three groups and placed in separate glass aquaria. Six fishes were used for each group per replicate. Groups 1 and 2 were exposed to sublethal concentrations of chlorpyrifos (12.5 ppm and 25 ppm) for 18 days. Group 3 was maintained in pesticide-free water to serve as a control.

Collection of scales

A few scales were removed from the dorsal region, just above the lateral line directly with the help of tweezers from both control and treated fishes. After 18 days of treatment scales from treated fishes (12.5 ppm and 25 ppm) were taken for assessment of chlorpyrifos toxicity.

Assessment of Structural variations

A stereo-zoom microscope was used to find out the structural variations in the scales. For this, the scales were collected from the dorsal region of the fish.

Estimation of Chromatophore index

The scales collected from the dorsal region were observed under the compound microscope focusing on the chromatophores present in them. A stereo-zoom microscope was used for the magnified image of scales with chromatophores.The chromatophore index was determined as the ratio of extended chromatophores to the total number of them.

Assessment of Decalcification of scales

The amount of calcium removed from the fish scales was calculated based on an already existing method (Joseph H Roe and Bernard S Khan, 1928). The calcium values were expressed as mg/g weight of scales.

Results:-

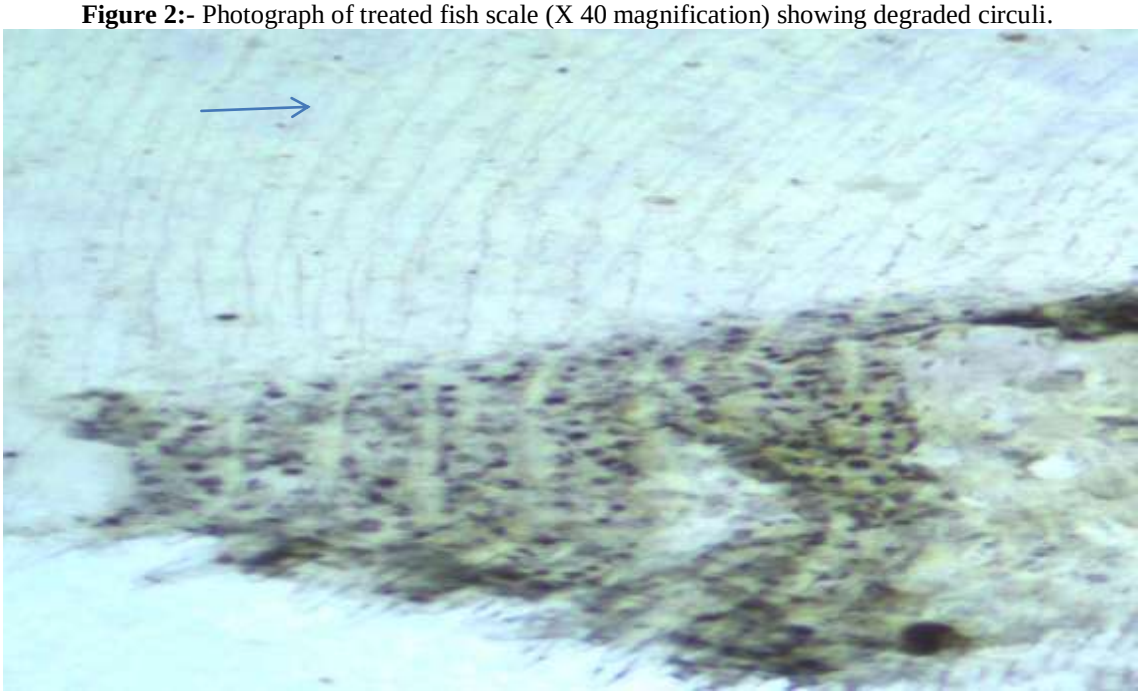
The results of the parameters studied on control and fishes exposedto sub-lethal concentrations of chlorpyrifos of 12.5 ppm and 25 ppm forshort-term exposure of 18 days were as mentioned below.

Structural Alteration of Scales

After exposure to 12.5 ppm and 25 ppm of chlorpyrifos for 18 days it was observed that the scale morphology of *Anabas testudineus* were altered. Figure 1 shows the Stereo zoom microscopic photographs of thestructural alterations in the scale. The circuli of the scales are seen degraded in the treated scales.

Figure 1:- Photograph of normal fish scale (X 40 magnification).

Figure 2:- Photograph of treated fish scale (X 40 magnification) showing degraded circuli.



Chromatophore index

The chromatophoreswereobserved to be aggregated in the control fishes.However,with increasing concentrations of chlorpyrifos, more extended chromatophores were observed. The extent of increase was statistically significant ($p<0.05$) in fish exposed to 12.5 ppm concentration and 25 ppm when compared to control (**Table1**).

Table 1:- Chromatophore Index of Control and Treated Scales.

	CONTROL	**TREATED**	
		12.5 ppm	**25 ppm**
Chromatophore Index	45.00 ± 0.05	*85.01 ±1.12	*94.54 ±0.14

Average of 6 replications * **($p<0.05$)**

Decalcification of scales

There is an increase in the amount of calcium removed from the scales of Anabas testudineuswith the increasing order of concentration of chlorpyrifos (**Table 2)**. The amount of calcium removed in treated scales was found to be 0.045 mg/10g and 0.097mg/10mg scales in 12.5 ppm and 25 ppm respectively. The change was significant at 5% level.

Table 2:- The amount of calcium removed from the scales.

	CONTROL	**TREATED**	
		12.5 ppm	**25 ppm**
Amount of calcium removed. mg/ 10g of fish scale	0.0171 ± 0.006	*0.045±0.002	*0.097±0.005

Average of six replication* **(p<0.05)**

Discussion:-

The observation for the parameters- morphology, chromatophore index, and decalcification effects of scales in *Anabas testudineus*on exposure to 18 days of chlorpyrifos has been discussed here.Chlorpyrifos, an organophosphate, is very acute and chronically toxic to aquatic organisms. It's well documented that exposure to sublethal concentrations of chlorpyrifos causes several effects on species of freshwater and marine fauna. In the present study, the chromatophore index was usedto score the degree of pigment dispersal in fish melanophores manually. There are several reports on external signals that mediate fast or slow color changes in fish chromatophores. In this study with the increasing concentration of chlorpyrifos, there is a gradual increase in the dispersion of chromatophores. This resulted in a pale color of fish morphology. The dispersion of chromatophores in this study can be compared to the results reported in the work done on guppy. In vitro methods for assessing melanin dispersion in melanophores are described, in which split fin preparations of the guppywere employed. Acetylcholine and some current neurotransmitter suspects were ineffective in dispersing the pigment (Yoko Miyashita and Ryozo Fujii.,1975).While exhibiting a melanin-aggregating action in higher concentrations, sympathomimetic monoamines in lower concentrations were found to disperse melanosomes. Such an action was more noticeable with beta-stimulating drugs, some of them being solely pigment-dispersing.

The investigation revealed that chlorpyrifos results in the decalcification of fish scales. There is a significant change in the amount of calcium removedfrom the treated fish scaleson 18 days of exposure to chlorpyrifos. The test substance chlorpyrifos used in this workinduces the breakage of bonds between the calcium ions. The present study suggested that sub-lethalamounts of chlorpyrifos can induce and adversely affect the structure and morphology of scales in *Anabas testudineus*on exposure tothe same as early as 18 days.

Conclusion:-

The study was to evaluate the toxicity effects on scales of the freshwater fish, *Anabas testudineus* upon exposure to various sub-lethal concentrations of chlorpyrifos. The parameters selected were fish scale morphology, chromatophore index, and decalcification effects. The experimental fish were divided into three groups. The fish in one group were subjected to 12.5 ppm and the other with 25 ppm concentrations of chlorpyrifos. They were observed after 18 days of treatment.

The results revealed that different concentrations of chlorpyrifos induced changes in the morphology of scales, and chromatophore index and induced adverse removal of calcium salt from the fish scales. In*Anabas testudineus*, exposure to chlorpyrifos resultedin turning the scales pale, devoid of circuli, and fragile. Thus,assessing the morphology and decalcification effect of scales in Anabas can be used as a bioindicator for the extent of accumulation of pollutants in fish.

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