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ULTRASTRUCTURAL DEFORMATIONS INDUCED BY 10% EC CYPERMETHRIN ON THE SCALES OF *CYPRINUS CARPIO*

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ABSTRACT

Chronic toxicity test under sublethal dose (0.44µg/l) was performed to study the scales of fresh water fish, *Cyprinus carpio* for 45 days. Scale under control group showed regular morphology whereas the cypermethrin exposed scales were noticed with severe alterations in their microstructures. Exposure of cypermethrin for 15 days revealed depletion of calcium from dorsal surface of scale, displacement of lepidonts and curled intercirculi spaces. The level of anomalies was increased on the 45th day of exposure which represents complete deterioration of scale with damaged focus, uprooted lepidonts, perforation in the tubercles with broken circuli along with complete loss of lepidonts.

KEY WORDS: *Cyprinus carpio*, Cypermethrin, Scales, Microstructures, Lepidonts.

INTRODUCTION

Pesticides being continuously used in the agriculture and industrial sector for the welfare of human, but the indiscriminate use of these chemicals can contribute to the chemical pollution causing undesirable effects on non-target organisms (Akter *et al*., 2009). Cypermethrin (*CYP)* (cyano-(3-phenoxyphenyl)-methyl-(3-C2,2-dichloroethenyl)-2,2-dimethyl) a synthetic pyrethroid (WHO, 1989) act as neurotoxic chemical which suppresses the action of acetycholine in brain and stops the function of ATPase enzymes (Vani *et al*., 2012). It is highly toxic to fish because of its low metabolic rate and can be treated as restricted use pesticide (RUP) (Saha and Kaviraj, 2008) due to its accumulative power and its half-life which is considered to be more than 48 hours.

Sensitivity of fish against the stressed condition of environment can be helpful for determining the health status of aquatic water systems (Sharma *et al*., 2017). In India, *Cyprinus carpio* is cultivated throughout the year. Continuous contact with the pesticides makes them unfit for consumption, increases its death rate which ultimately cause extinction of population (Sabra and Mehana, 2015). Scanning electron microscopy was considered as a useful tool for studying the toxic effects of pesticides on the surface morphology of fish (Dey *et al*., 2014). Therefore, the present work analyses the effect of CYP on the scales of *Cyprinus carpio* which sheds light on the harmful nature of this chemical in aquatic ecosystem.

MATERIALS AND METHODS

Procurement of fish

Healthy fingerlings (4±0.1cm, 12±0.3 gm) were procured from government fish seed farm, Nalagarh, Himachal Pradesh. These were acclimatized in a laboratory setting for 15 days with adequate aerators and filters. Fish were fed artificial feed twice a day (gold toya). The fish were starved for 24 hours prior to the start of the experiment. The toxicant employed in this investigation was CYP (made by shivalik crop sciences Pvt. Ltd Chandigarh, India). The experiments were performed in sintex tanks having water capacity of

50 L. The experiment was divided into two groups: Group I maintained as control containing dechlorinated tap water while as Group II treated with 0.44µg/l of *CYP*. The experiment was performed in triplicates (n= 10/group) for 45 days. Fish was dissected out on 15th and 45th day of the experiment.

Scanning electron microscopic studies

The scales of both (control and treated) fish were removed from the left side of the body close to the dorsal fin. These were washed in distilled water. Scales were rubbed gently between the tips of fingers to clean the mucus and then dried on the whatmann filter paper. Dried scales were coated with gold thickness of 100Å and mounted on aluminum stubs with the help of double adhesive tape (Batoye *et al*., 2021). The scales were viewed under scanning electron microscope, JEOL, JSM-6100 at CIL, Panjab University, Chandigarh.

RESULTS

Scanning electron microscopy on the scales of control group demonstrated normal morphology of microstructures which includes three distinct fields-anterior, posterior and lateral fields (Fig. 1 a). Anterior region is somewhat broader than the posterior region of scale as shown in Fig. 1 a. Well-marked focus is present at the centre towards anterior field (Fig. 1 c). Concentric lines of growth start arising from the focus to the margins of scales also called as longitudinal grooves or circuli (Fig. 1 b). These circuli are present all over the scale.

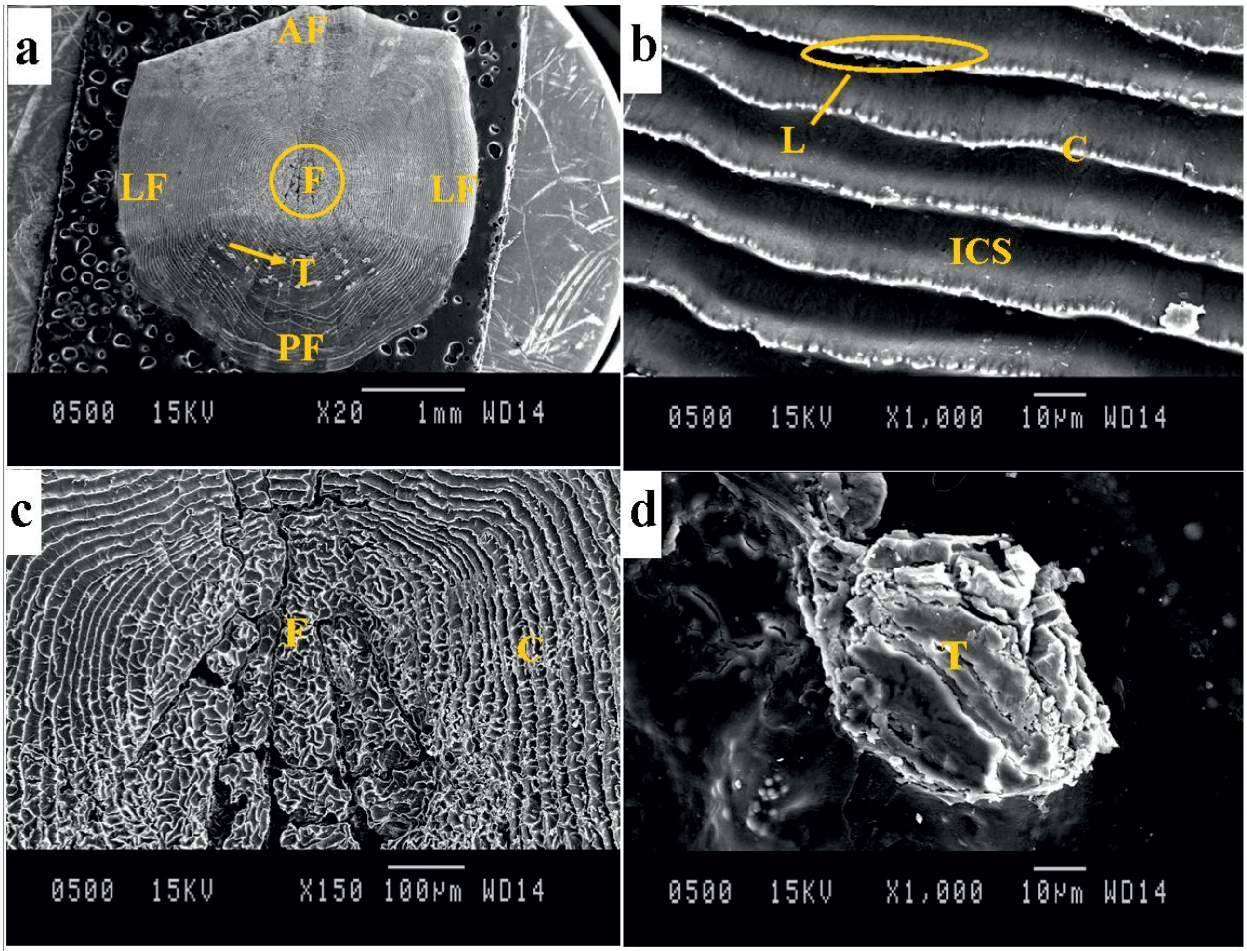

Fig 1:- Scanning electron micrographs of control scales of *Cyprinus carpio* representing (a) normal focus, anterior field, lateral field and posterior field and tubercles (b) complete row of circuli with normally placed lepidonts (c) magnified view of focus (d) magnified view of tubercle.

Abbreviations:- AF- anterior field, F- focus, LF- lateral field, PF- posterior field, T- tubercle ICS- inter circuli space, C- circuli, T- tubercle

Margins of circuli have teeth like outgrowths termed as lepidonts (Fig. 1 b). The space between two circuli margins are said to be inter-circuli spaces (Fig. 1 b). Fig. 1 d showed large, tubular swellings called tubercles in the posterior region.

On the 15th day of exposure, scales get disrupted in the anterior region due to the depletion of calcium (Fig. 2 a). Few lepidonts also get displaced from their

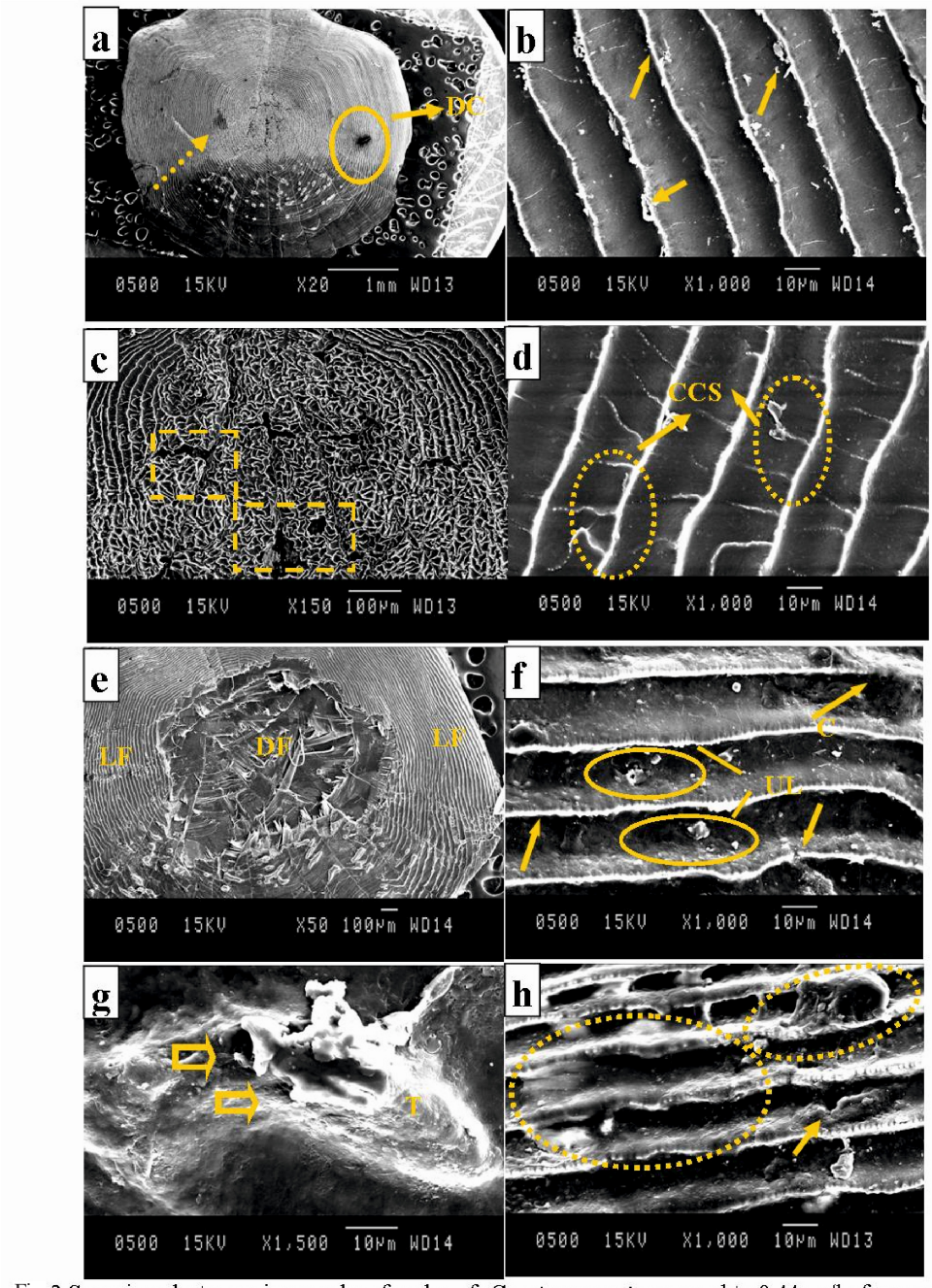

Fig. 2 Scanning electron micrographs of scales of *Cyprinus carpio* exposed to 0.44 µg/l of cypermethrin for 15 days (a-d) and 45 days (e-h):- (a) depletion of calcium (circle, arrow) (b) displaced lepidonts from different rows of circuli (arrows) (c) reabsorbed focus (dotted rectangle) (d) curling of inter circuli space (dotted oval) (e) damaged focus (f) up-rooted lepidonts (arrow, oval) (g) damaged tubercles with many perforations (block arrows) (h) breaked circuli (arrow) and complete loss of lepidonts (dotted oval).

Abbreviations:- DC- depleted calcium, DL- damage lepidonts, RF- reabsorbed focus, CCS- curled circuli space, DF- damaged focus, LF- lateral field, UL– uprooted lepidonts, T- tubercle

place (Fig. 2 b). Partial reabsorption of focus (Fig. 2 c) with curling of intercirculi space was also evident (Fig. 2 d). Degree of damage to the scales increased with the exposure days. Exposure of CYP for 45 days revealed more severe damage such as deteriorated focus (Fig. 3 a) and damaged circuli with uprooted lepidonts (Fig. 3 b). Posterior region also showed damage in the tubercles which includes pores and crevices at some places (Fig. 3 c). Breakage in the circuli was noticed with complete loss of lepidonts (Fig. 3 d).

DISCUSSION

Scales forms the intugumentary part of skin, originates from the mesodermal layer. Chemically, they are composed of collagen fibres and calcium salts (Yoshimi *et al.*, 1998). They provide protection as physical barrier. Moreover, scales can be used easily for ecotoxicological studies without sacrificing the animal (Yoshimi *et al.*, 1998; Johal and Dua, 1994). When scales come in direct contact with polluted water, they accumulate different toxicants, and become hard and brittle resulting in breakage of lepidonts. Thus, lepidonts can be used as reliable bioindicator for accessing the polluted water bodies (Khanna *et al.*, 2007). Present study has reported severe alterations in the CYP-treated fish which includes damaged circuli, uprooted and displaced lepidonts leads to loosening of fish scales from the body. Similar findings were also observed by many workers such as Jangu and Singh (2014) has performed electron microscopy on the scales of fish Catla catla. They revealed the effect of pollution in the form of calcareous damage, uprooted lepidonts and broked circuli. Fenvalerate toxicity on the scales of *Ctenopharyngodon idella* was reported by Batoye *et al.* (2021). They observed displaced lepidonts, weathered calcareous material, damaged inter circuli space with complete loss of circuli and damaged tubercle on 60th day of exposure. Khanna *et al.* (2007) analysed the heavy load of pesticides in the river Ganga. They reported alterations in the scale of *Puntius sarana and Labeo rohita* which include disrupted and eroded lepidonts. Similar results have been investigated by different workers, Johal and Dua (1994) on *Channa punctuatus* against endosulfan, Johal and Sawhney (1997) *Channa punctuatus* against malathion, toxicity of pesticides assessed on scales (Dua and Gupta, 2005), *Channa punctatus* was studied in Tung dhab drain by Kaur and Dua (2004), and *C. idella* against heavy metal, lead (Batoye *et al.*, 2021). Various ultrastructural changes in scales of *Catla catla, Labeo rohita, Cirrihinus mrigla* was observed in three polluted sites of Chenab River (Sultan *et al.*, 2016). Also, CYP treated fish showed abnormal morphology of tubercles. Damage in the scales was attributed to the change in the metabolism of the body against CYP stress. Calcium plays an important role in various cellular activities such as nerve impulsion, conduction, blood clotting and fluid maintenance (Pravina *et al.*, 2015). Organism derives out calcium from the scales to overcome pesticide stress.

CONCLUSION

Present study concluded that CYP is highly toxic to *Cyprinus carpio* even when it is present at low concentration. Scanning electron microscopic studies revealed morphological alterations in the scale which can be used as an indication to determine the stress of pesticide on fish. Hence, it is important to use these pesticides with proper guidelines and exposure limits to protect the fish fauna.

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AUTHOR CONTRIBUTION

Diksha Kumari: writing original draft, investigation, methodology, conceptualization and data analysis.
Dr. Smriti Batoye: help in designing the research project, supervisor, writing and editing.
Sheetal Sharma: help in designing the research project, analysis, writing and editing.

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