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Ultrastructural alterations in scales of *Ctenopharyngodon idellus* (Cuvier & Valenciennes) induced by chlorpyrifos: a promising tool as bioindicator of pesticide pollution

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Abstract
Fish scale morphology can serve as a reliable pollution bioindicator. The effect of pesticide on scales of freshwater fish *Ctenopharyngodon idellus* was studied using scanning electron microscopy at various exposure periods (15, 30 and 60 days) at different sublethal concentrations (1.44 µg/l and 2.41 µg/l) of organophosphate, chlorpyrifos. The fish exposed to the toxicant showed significant alterations such as damaged radii, focus, circuli and severely damaged lepidonts. The intensity of scale damage was found to be toxicant concentration and exposure period dependent and also prolonged exposure to the pesticide damage the fish scales severely.

Keywords: Scale, *Ctenopharyngodon idellus*, grass carp, chlorpyrifos, toxicity.

1. Introduction
Pesticides have been in use in agriculture for thousands of years. Though the use of pesticides had a positive impact on the overall increase in food production, yet the risks associated with this include deterioration of human health, water contamination, livestock poisoning, death of beneficial insects, wildlife endangerment and increase in pesticide tolerance [1]. But at the same time, these are also responsible for pollution of our aquatic resources. These are carried into water bodies like ponds, lakes and rivers through surface runoff and alter the physico-chemical properties of water [2, 3, 4]. Fishes are particularly sensitive to wide range of pesticides, chemicals and toxic conditions. The kinds and amounts of pesticides applied to environments are almost out of control, and about one-third of organophosphates (OPs) are toxic to fishes. Chlorpyrifos is one of the most widely used organophosphate insecticides (USEPA). It has found wide application in the field of agricultural production. Effect of pesticides and other pollutants on fish can be indicated by using scales as biomarkers [5, 6], as scales are exposed to external environment, giving complete protection to skin and has larger surface area. The indiscriminate use of pesticides has given rise to many challenging problems, ultimately affects man and environment. Relatively little information is available on the impact of pesticides on fish scales. Keeping in mind the above facts, an attempt has been made to study the ultrastructural changes induced by chlorpyrifos in the scales of *Ctenopharyngodon idellus*, employing scanning electron microscopy.

2. Materials and Methods
To study the ultrastructural changes in the scales of *Ctenopharyngodon idellus* (weight: 10±2 gm, length: 10±2 cm) induced by chlorpyrifos toxicity, were collected from Nanoke Fish Seed Farm located at Nanoke village, Distt. Patiala (Punjab, India) and were acclimatized to the laboratory conditions for 15 days in glass aquarium. They were fed with palletized supplementary feed 'Gold Tokyo' once a day. Water in the aquarium was renewed daily. For acute toxicity tests, the physico-chemical characteristics of water were determined [7] and were: temperature 25±2 °C, pH 7.2±0.1, dissolved oxygen 8.0±0.3 mg/L, total alkalinity 175±10 mg/L and total hardness 18±0.5 mg/L. 96 hr LC_{50} of chlorpyrifos was determined using Probit analysis [8] and was found to be 7.24 µg/l as

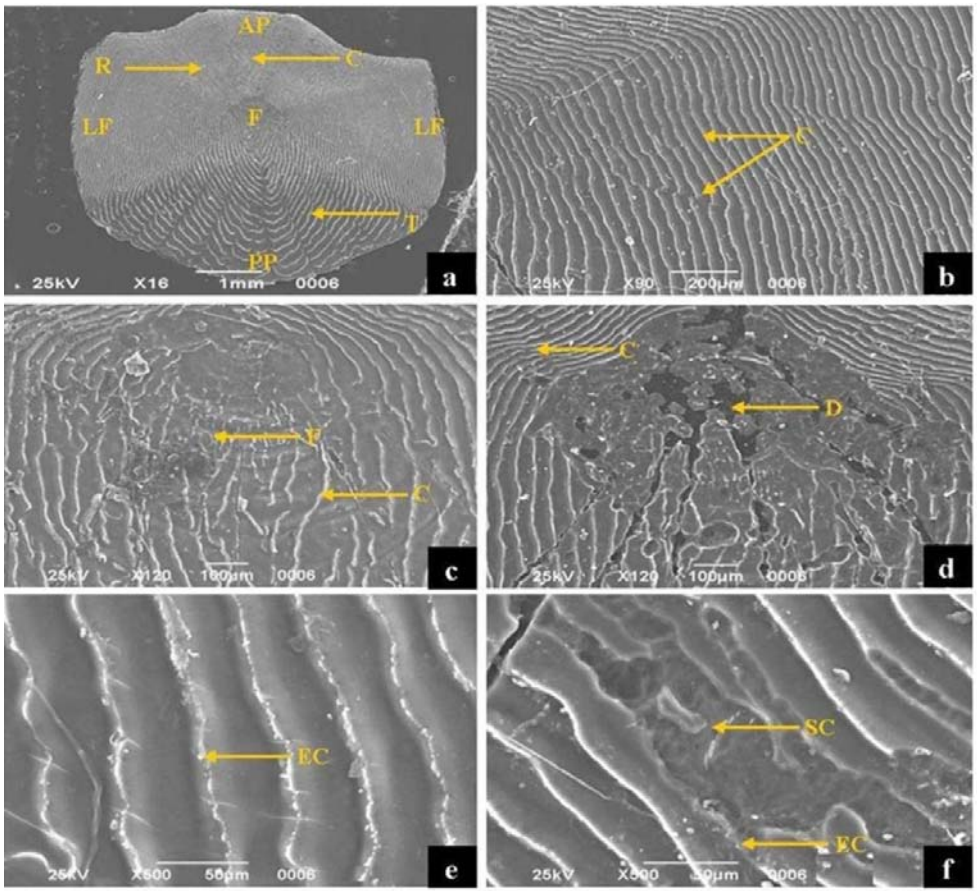
reported in our previous study [4]. For chronic toxicity tests, 30 healthy fish (10 fish in each group) were introduced into three experimental groups with one-third (2.41 µg/l) (Group-I) and one-fifth (1.44 µg/l) (Group-II) of the LC_{50} of chlorpyrifos (CPF) as sub-lethal concentration for a period of 15, 30 and 60 days along with control (toxicant free water, (Group-III).

The scales overlying the lateral line, leaving 7-8 dorsal fin rays and preferably from 2nd row, were gently removed with fine tweezers. After removal, scales were immediately washed with distilled water and were subjected to sonication for 5 min and again rinsed with distilled water. Scales were then dehydrated in 30, 50, 70 and 90% alcohol (not in absolute alcohol, as it causes curling of margins of scales). The scales were dried on filter paper and were placed between glass slides for 2-3 days. Cleaned and dried scales were then mounted on the metallic stubs by double adhesive tape with dorsal surface upward and the ventral surface sticking to the tape. Scales were sputter coated with gold (100 A°). The scales were viewed under vacuum in a JEOL, JSM-6100, scanning Electron Microscope at an accelerating voltage of 15-20 kv and low probe current at Central Instrumentation Laboratory, Panjab University, Chandigarh. Requisite animal ethical clearance from IAEC, Panjab University, Chandigarh (Ref no. IAEC/526) has been taken.

3. Results and Discussion

The ultrastructural studies made on the cycloid scale of *Ctenopharyngodon idellus* revealed that the ventral side is attached to the skin, hence it is smooth and shiny. The scale has anterior, posterior and lateral fields. The preceding scale overlaps the anterior field of scale. For SEM studies, the scale was viewed from dorsal side. Each scale was found to possess distinct focus and around it there are concentric rings called cerculi (Fig 1 a, b). These cerculi were widely spaced on anterior side, whereas posterior side had thick cerculi. Some of these were formed by the fusion of 2-3 lateral regions. On the upper side of the cerculi, it has been noticed that there were present minute, irregularly shaped tooth like processes called lepidonts. These provide anchor to the skin on the overlying posterior side of the preceding scale. In the anterior field, radii originate from focus of the scale. Posterior field has been found to be comprised of large number of swellings called tubercles.

Deep socketed lepidonts on the scale were irregularly distributed and were fewer in number on cerculi. On exposure of the fish to the chlorpyrifos for 15 d, the scale was found to be deformed. At lower concentration, the lepidonts were broken at many places or got completely separated, whereas on exposure to higher concentration reduction or total absence of lepidonts due to their partial or complete damage was seen (Fig 1 d, e, f). Damage to the lepidonts resulted in loosening of the scales from the body of the fish. Similar observations on the damage of circuli and lepidonts on the scales of malathion treated *Channa punctatus* [9], and in *Oreochromis* spp. [10] from contaminated river have been made.



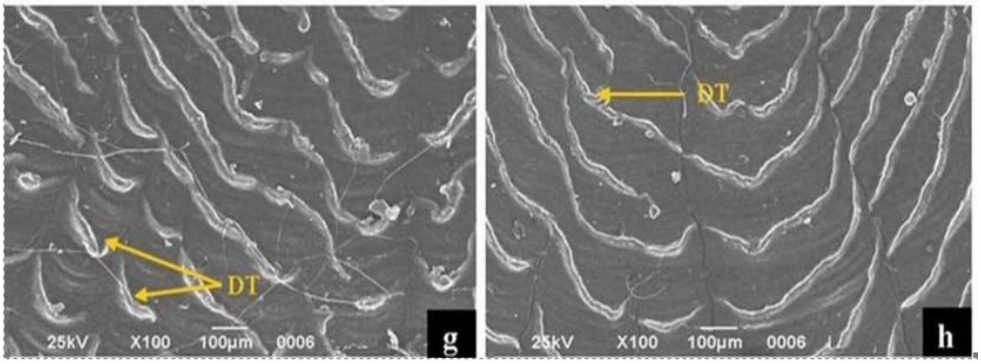


Fig 1: Scanning electron micrograph of scale of *Ctenopharyngodon idellus* of control group (a, b) and on exposure to 1.44 µg/l (c, e, g) & 2.41 (d, f, h) µg/l of chlorpyrifos for 15 days.

Abbreviations: F-Focus, C-Cerculi, R-Radii, LF-Lateral field, AP-Anterior part, PP-Posterior part, T-Tubercles, DF- Damaged Focus, DT-Damaged Tubercles, EC-Eroded Cerculi, SC– Sloughed off Cerculi.

On 30th day exposure of the fish to the toxicant, radii (R) and circuli got damaged and there was occurrence of the loosening of scales from the fish body, resulting from the disorganization of entire calcareous material (Fig 2 a-d). Similar changes in the scales have been reported in *Channa punctatus* [11]. Damage was also found to be proportional to the toxicant concentration.

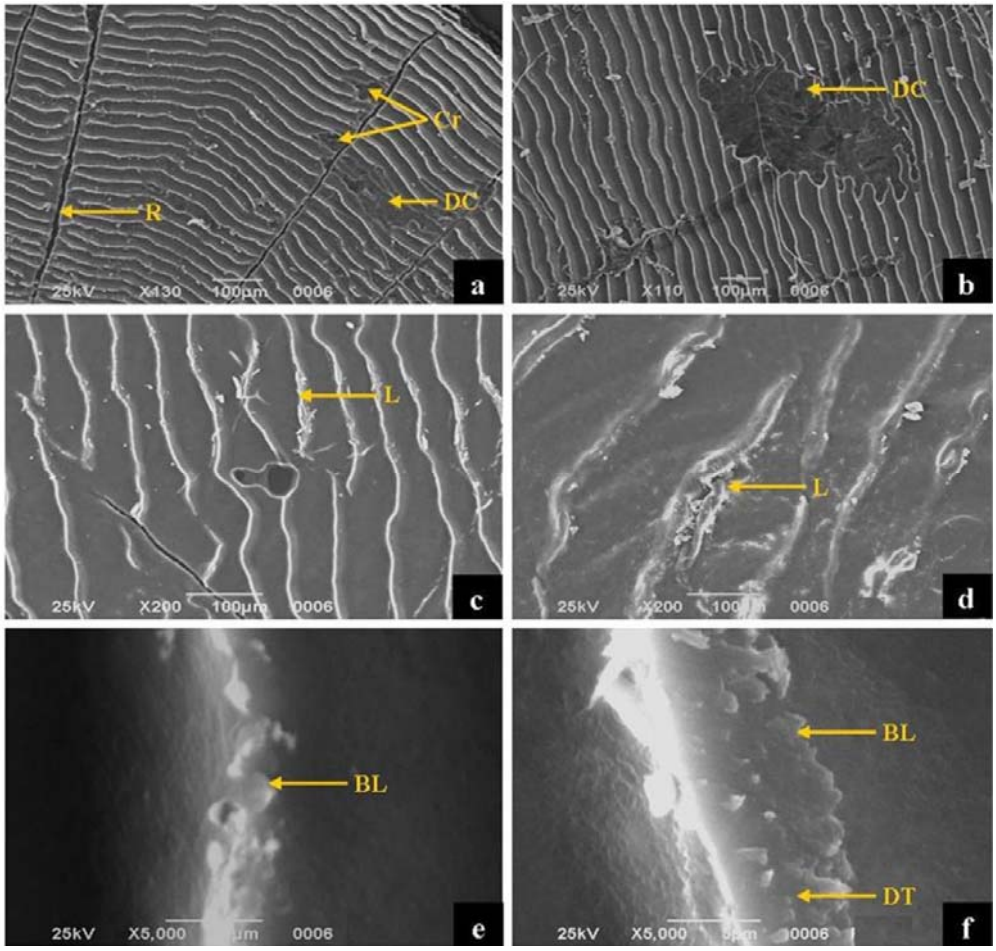


Fig 2: Scanning electron micrograph of scale of *Ctenopharyngodon idellus* on exposure to 1.44 µg/l (a, c, e) & 2.41 µg/l (b, d, f) of chlorpyrifos for 30 days.

Abbreviations: C-Cerculi, R-Radii, T-Tubercles, DC– Damaged Cerculi, DT- Damaged Tubercles, BL-Broken Lepidonts

In confirmation with earlier studies [12], during present investigation also marked cracks and breaks on the tubercles present in the posterior part of the scale exposed to CPF have been recorded (Fig 3 e, f). Further, the damage was found to be more pronounced on the focus and anterior region of the scale. Similarly, damage to lepidonts and circuli was observed in the scales of *Cyprinus carpio* on exposure to cadmium (14.5, 29, 43.5 and 58 mg L^{-1}). Further, the damage was reached up to radii and chromatophores [13]. Individual breakage in the lepidonts as well as their uprooting from the circuli with lesions & cracks has also been reported [14]. Similar findings were observed by other workers [15, 16, 17, 18].

Disorganized margins of the scale and disruption of circuli and radii at the lower toxicant concentration, whereas at higher concentration there was extreme disruption at the base of circuli along with disorganized calcareous material were also observed [19].

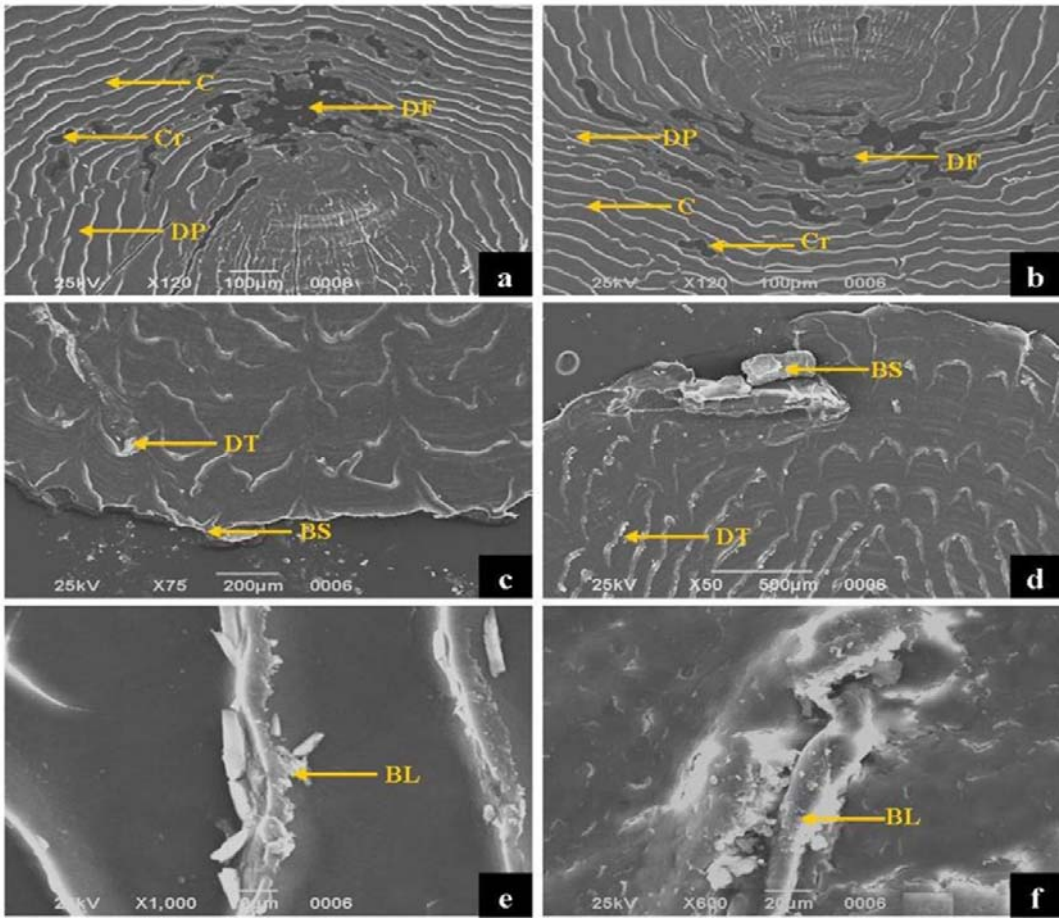


Fig 3: Scanning electron micrograph of scale of *Ctenopharyngodon idellus* on exposure to 1.44 µg/l (a, c, e) & 2.41 µg/l (b, d, f) of CPF for 60 days.

Abbreviations: F-Focus, C-Cerculi, R-Radii, LF-Lateral field, AP-Anterior part, PP-Posterior part, T-Tubercles, DF– Damaged Focus, DT-Damaged Tubercles, Cr-Cracks, BS-Broken surface, BL-Broken Lepidonts, DP-Distorted pattern of Cerculi.

4. Conclusion

CPF was found to be highly toxic to *Ctenopharyngodon idellus* even at very low concentration. Ultrastructural alterations in scale could be attributed to chlorpyrifos induced toxicity and may lead to certain diseases or cause death of the fish, provide useful information for evaluating the toxicological effects of xenobiotic on the fish and help in the diagnosis of the pollution.

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