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Scanning Electron Microscopic Studies on the Gill and Blood Tissues of *Heteropneustes fossilis* Bloch 1794 (Siluriformes: Heteropneustidae) Exposed to Triazophos with Special Reference to Recovery Period

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Abstract

Triazophos is a broad-spectrum organophosphate insecticide and acaricide with some nematicidal properties used on agricultural crops like cotton, paddy, maize and okra to control aphids and leaf hoppers. The present paper emphasizes on the histopathological effects of different sub-lethal doses of triazophos on the gill and blood tissues of *Heteropneustes fossilis* with special reference to recovery period. After 10 days of exposure, gill and blood tissues were dissected and routine histological process were done. No changes were observed in the gill and blood tissues of the control fish. Scanning electron microscopic studies of treated gill tissues revealed fusion of secondary lamellae, hyperplasia, droplet of oozing material, damaged, fused and desquamation microridges of epithelial cells, hypertrophy, damages in primary lamellae, blebbing, and aneurism. The 10-day recovery studies showed swelling and fusion of secondary lamellae, irregular arrangement, and complete fusion of microridges, erosion of epithelial cells, hyperplasia, degeneration, and cytoplasmic protrusion from secondary lamellae, aneurism, and hyperplasia. In treated blood tissues, erythrocytes were found to be swollen (spherical), clubbed and contracted, disruption of cell membrane, cytoplasmic blebbing, and formation of chains. In the 10-day recovery studies, lobopodial projections, disruption of cell membrane, cytoplasmic blebbing, formation of chains, swollen cells, and erythrocyte clubbing were observed. The extent of gill and blood tissue damage was proportionate with the increased dosage and duration of triazophos exposure, and the recovery studies highlighted damages in tissues and the recovery pattern was less when compared to triazophos treated fishes.

Keywords: Triazophos, *Heteropneustes fossilis*, Gill, Blood, Histopathology, SEM

1. Introduction

Large scale application of pesticides to agricultural areas contribute to the presence of toxic substances in the environment. Organophosphorous pesticides are finding increasing and repeated applications in control of pests in agricultural fields (Jyothi and Narayan, 1997). Triazophos (diethoxy-[(1-phenyl-1,2,4-triazol-3-yl)oxy]-sulfanylidene-phosphane), whose molecular formulae $C_{12}H_{16}N_3O_3PS$ is a broad-spectrum organophosphate insecticide and acaricide with some nematicidal properties used in large quantities worldwide on crops like cotton, paddy, maize and okra to control aphids and leaf hoppers (Worthing and Hancre, 1991; Mingjing *et al*., 2003). These chemicals eventually find their way into the water reservoirs, streams and rivers, thus producing an adverse impact on the aquatic biota particularly fishes (John and Prakash, 2003; Samuel *et al*., 2019a,b).

The Asian stinging catfish, *Heteropneustes fossilis* is a species of air sac catfish, native to Bangladesh, Pakistan, India (including the Andaman Islands), Nepal, Sri Lanka and Thailand (Talwar and Jhingran, 1992). This omnivorous species can tolerate slightly brackish water, and breeds in confined waters during the monsoon months, but can breed in ponds, derelict ponds, and ditches when sufficient rain water accumulates. This fish is of high economic importance and of great demand because of its medicinal value (Talwar and Jhingran, 1991). It is considered to be highly nourishing, palatable and tasty and well preferred because of its less spine, less fat and high digestibility and nutritive value in many parts of the Indian subcontinent (Khan *et al*., 2003), and is recommended for patients after recovery from malaria for its invigorating qualities (Bhuiyan, 1964). The present paper investigates on the histopathological aspects of gill and blood tissues of *Heteropneustes fossilis* on exposure to triazophos, using Scanning Electron Microscope (SEM).

2. Materials and Methods

Heteropneustes fossilis were collected from Chengalpattu lake, Tamil Nadu, India (12.6840° N, 79.9833° E) since the selected area was devoid of agricultural land and industries. Fishes were acclimated for a couple of weeks to laboratory conditions and kept in aquaria (30 litres) under light-dark (12:12 hours) cycle and were fed *ad libitum* with artificial pellet feed in the Department of Advanced Zoology and Biotechnology, Loyola College, Chennai, Tamil Nadu, India. During the period of acclimatization and experimentation, the water used was clear dechlorinated ground water and its physicochemical characteristics were maintained throughout the experimental study as per APHA (2012). The average weight of the fish was 35.0 ±3.0g and its average length ranged from 19-22cm. Desired degree of sublethal concentrations (5, 10 and 15%) were obtained from the stock solution of triazphos prepared using distilled water. Feeding of fishes were stopped 48 hours prior to the commencement of the experiment with a view to avoid

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any possible change *in situ* in the toxicity of the pesticide. The toxicant was added into the test tank containing ten litres of water and twenty fishes. Ten replicates were maintained. Mortality was recorded 96 hours after exposure. Fishes showing no respiratory movement and response to tactile stimuli were considered dead and removed immediately. Percent mortality was calculated and the values were transformed into probit scale. Probit analysis was carried out as per Finney (1978). Slope function and confidential limits (lower and upper) of the regression line with Chi-square test were also performed (UNEP/FAO/IAE, 1987). For recovery studies, after ten days of exposure to the toxicant, the treated fish were transferred to clean tap water and observed for a period of another ten days.

After exposure to sub lethal concentrations, the fishes were sacrificed and the tissues of gill and blood of the control and experimental groups were dissected out and fixed in neutral buffered formalin for 24 hours. For SEM studies, the gill tissues were fixed in Karnovsky fixative (2% glutaraldehyde, 4% paraform-aldehyde, 0.1M sodium cacodylate buffer, pH 7.4) and rinsed in 4% phosphate buffer. The tissues were then dehydrated in a standard acetone series from 70 to 100% for 15 minutes each and then placed on stubs and platinum coated and photographs taken with aid of a JEOL JSM 6360 SEM equipment. In the case of blood, two drops of blood were immediately fixed in 1% buffered glutaraldehyde (1% in 0.2M phosphate buffer, pH 7.2) for 10-15 minutes. Fixed blood was centrifuged at 1500rpm for 5-10 minutes. Extra fixative was removed and the erythrocyte pellet was completely washed thrice with 0.1M phosphate buffer. The pellet was then gently suspended in buffer and a small drop of the suspended erythrocytes was placed over silver tape attached to the aluminum stub. Air dried samples were sputter coated with gold (100A°) and finally viewed using the above-mentioned SEM.

3. Results

The susceptibility of *Heteropneustes fossilis* to the toxic effect of triazophos observed an increased percent mortality with increase in concentration. Mortality in controls were absent. The 96 hour LC_{50} was 2.30ppm (95% confidence limit ranged from 1.89 to 2.57ppm). Increase in exposure period drastically decreased the LC_{50} values of the toxicant. Chi-square test revealed that the value was well fit at $P<0.05$ level wherein the calculated value was 0.99 and the table value was 11.07 (Table 1).

3.1. Gill

The ultrastructure of the control gill appeared normal exhibiting features such as straight filaments carrying equally spaced lamellae and relatively long and well-developed filaments with an ordered array of lamellae. The secondary lamellae were fairly thick structures, perhaps even slightly thicker than the space between adjacent lamellae. The cell boundaries were very clearly defined and the individual cell surfaces were covered with microridges, each of which was quite long showing a whorl-like arrangement. On the leading and trailing surfaces of the filament, a number of mucous cells were visible, typically being represented by small openings which emerged at the junctions between neighbouring epithelial cells. The ridged appearance of the filament surface was also found in the crypt regions between adjacent secondary lamellae, but a marked transition occurred at the junction with individual secondary lamellae. The treated gill showed alterations like fusion of secondary lamellae, hyperplasia, droplet of oozing material, altered microridges of epithelial cells at 5%; fusion of secondary lamellae, hypertrophy, damages in primary lamellae, fusion of microridges, blebbing at 10%; and aneurism, fusion of secondary lamellae, damage and desquamation of microridges at 15% (Fig. 1). In the case of recovery studies, swelling and fusion of secondary lamellae, irregular arrangement of microridges, erosion of epithelial cells at 5%; fusion of secondary lamellae, hyperplasia, cytoplasmic protrusion from secondary lamellae, complete fusion of microridges at 10%; and degeneration of secondary lamellae, aneurism, hyperplasia, and fusion and irregular arrangement of microridges at 15% were noted (Fig. 2).

3.2. Blood

In control blood, each erythrocyte appeared elliptical with an oblong nucleus. In treated blood, erythrocytes were found to be swollen (spherical) and contracted at 5%; disruption of cell membrane, cytoplasmic blebbing at 10%; and erythrocyte clubbing, formation of chains, disruption of cell membrane, and contraction of elliptical erythrocytes at 15% (Fig. 3). In recovery studies, lobopodial projections, disruption of cell membrane at 5%; cytoplasmic blebbing, formation of chains, disruption of cell membrane at 10%; and swollen cells, formation of chains and erythrocyte clubbing at 15% were observed (Fig. 4).

4. Discussion

Acute toxicity test of the present study clearly indicated the toxicity of triazophos to the experimental fish as dramatic changes was observed in its physical behaviour which was also earlier proved by Kumar *et al*. (2000) and Maheshwari *et al*. (2001). Michael *et al*. (2004) stated that multi-photon laser scanning microscope was used to determine the multi-photon excitation spectra of several polyaromatic hydrocarbon to describe the chemical distribution among tissues of fish. Breining and Britz (2000) reported surface characterization and tissue damage detections on fish species using SEM. Histolopathological

Table 1. Probit analysis of triazophos against *Heteropneustes fossilis*

Exposure period (h)	LC_{50} (ppm)	95% confidence limit (LL – UL)	LC_{90} (ppm)	95% confidence limit (LL – UL)	Intercept ±S.E.	Slope ±S.E.	Chi square
96	2.30	1.89 – 2.57	3.18	2.82 – 4.04	1.69 ±0.98	9.13 ±2.29	0.99*

LC_{50} - lethal concentration that kills 50% of the exposed fishes, LC_{90} - lethal concentration that kills 90% of the exposed fishes, LL - lower limit (95% confidence limit), UL - upper limit (95% confidence limit), *P≤0.05, level of significance of Chi-square value

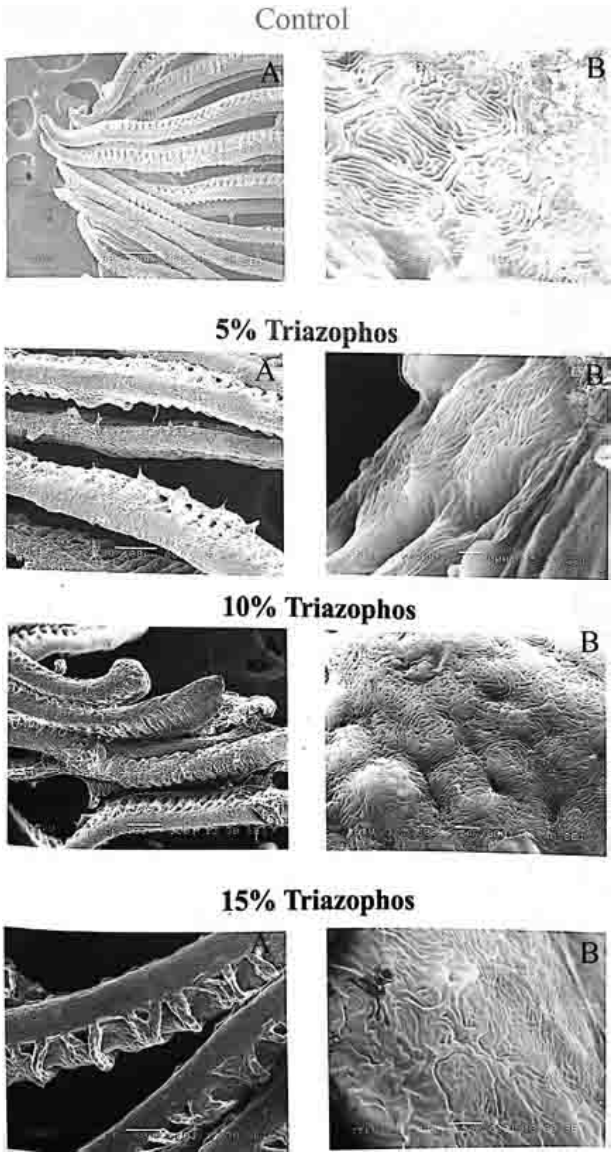

Fig. 1. *Heteropneustes fossilis* gill exposed to Triazophos – Treatment: Control Gill:
A: Gill filaments – Primary Lamellae and Secondary Lamellae; B: Microridge Epithelial Cells; **5% Treatment:** A: Fusion of Secondary Lamellae; Hyperplasia; and Droplet of Oozing Material; B: Altered Microridges of Epithelial Cells**; 10% Treatment:** A: Fusion of Secondary Lamellae; Hypertrophy; and Damages in the Primary Lamellae; B: Fusion of Microridges; and Blebbing; **15% Treatment:** A: Fusion of Secondary Lamellae; and Aneurism; B: Damages in the Microridges; and Desquamation of Microridges

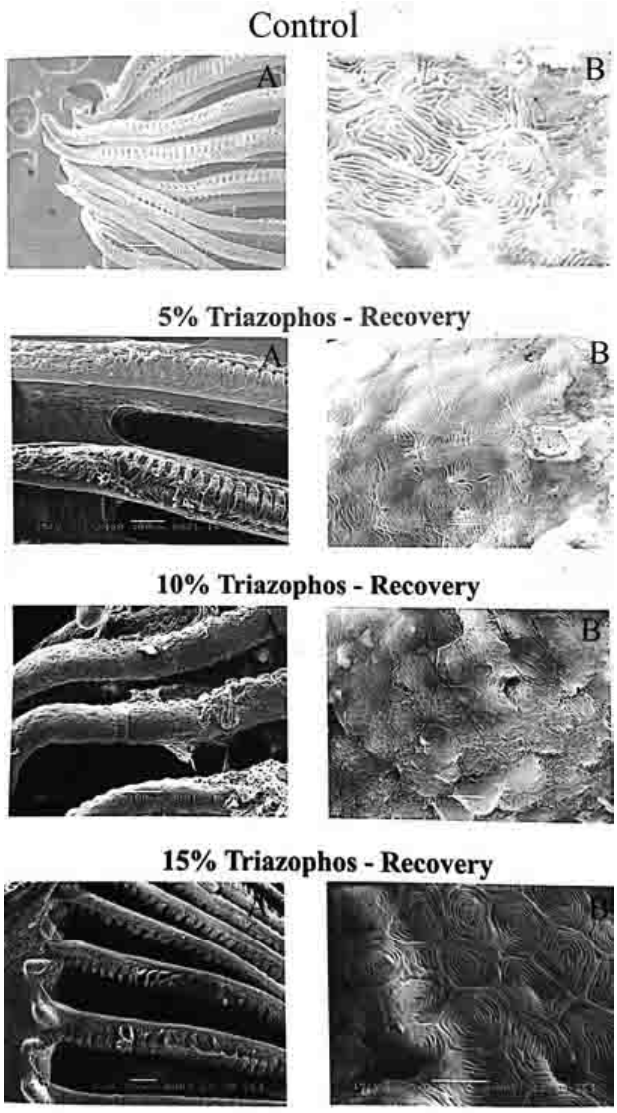

Fig. 2. *Heteropneustes fossilis* gill exposed to Triazophos – Recovery: **5% Recovery**: A: Swelling and fusion of Secondary Lamellae; B: Irregular arrangement of Microridges; and Erosion of Epithelial Cells; **10% Recovery:** A: Fusion of Secondary Lamellae; Hyperplasia; and Cytoplasmic protrusion from Secondary Lamellae; B: Complete fusion of Microridges; **15% Recovery:** A: Degeneration of Secondary Lamellae; Aneurism; and Hyperplasia; B: Fusion of Microridges; and Irregular arrangement of Microridges

alterations to *Lates calcarifer* in acute and subchronic cadmium exposure were studied by light and scanning electron microscopy (Thophon *et al*., 2003). The gills of *Heteropneustes fossilis* showed vital histological changes in the present study. Gills serve as sensitive indicators of the toxic effects of pesticide because of their direct contact with ambient conditions, large respiratory surface area, high permeability and characteristic responses of lamellar epithelial cells to toxicants (Samuel *et al*., 2008; Kaur and Jindal, 2016). Prasad (1994) through SEM studies reported alterations in the respiratory epithelium gills of *Heteropneustes fossilis* to sublethal and lethal concentrations of mercuric chloride. Gupta and Dua (2002) indicated mercury induced architectural alterations in the gill surface of *Channa punctatus* by SEM.

The blood of fishes like other vertebrates consists of cellular components (RBC, WBC and platelets) designated as formed elements suspended in plasma (Ross and Reith, 1985). The monitoring of morphological alterations in fish blood cells was a highly sensitive way to assess the effects of toxicants. In the present study, SEM observations revealed morphological damage to the erythrocytes of *Heteropneustes fossilis*. Sawhney and Johal (2000) reported erythrocyte alterations induced by malathion in *Channa punctatus* which was also corroborated in the present study. Several species of fish are susceptible to

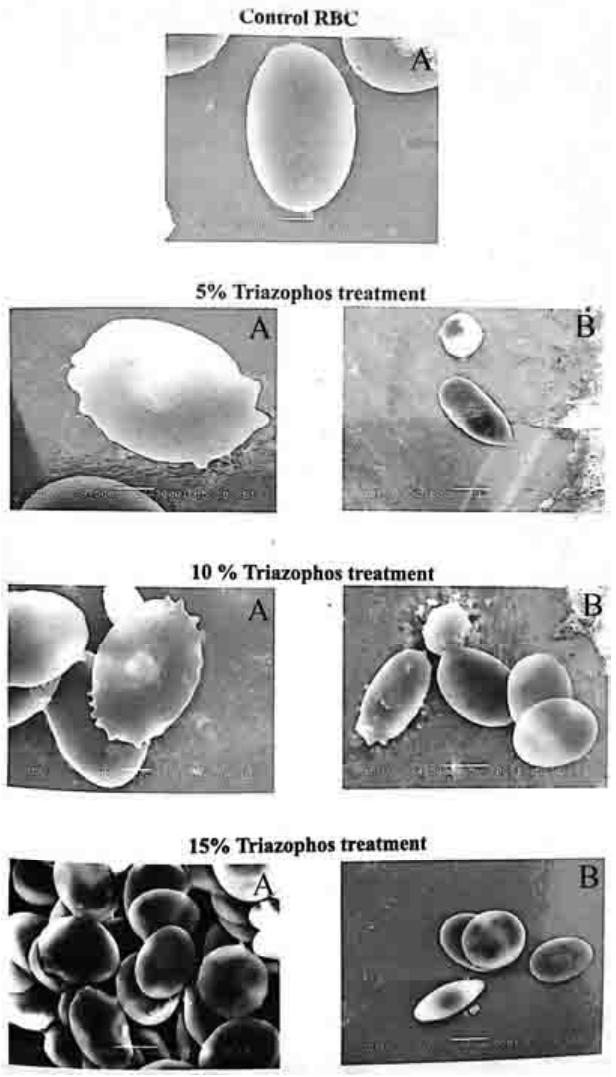

Fig. 3. *Heteropneustes fossilis* blood exposed to Triazophos – Treatment: Control; A: Erythrocyte; **5% Treatment:** A: Swollen Cells; B: Contraction of Elliptical Erythrocytes; **10% Treatment**: A: Rupture of Cell Membrane; B: Cytoplasmic Blebbing; **15% Treatment:** A: Erythrocyte Clubbing and Formation of Chain; B: Disruption of Cell Membrane; and Contraction of Elliptical Erythrocytes

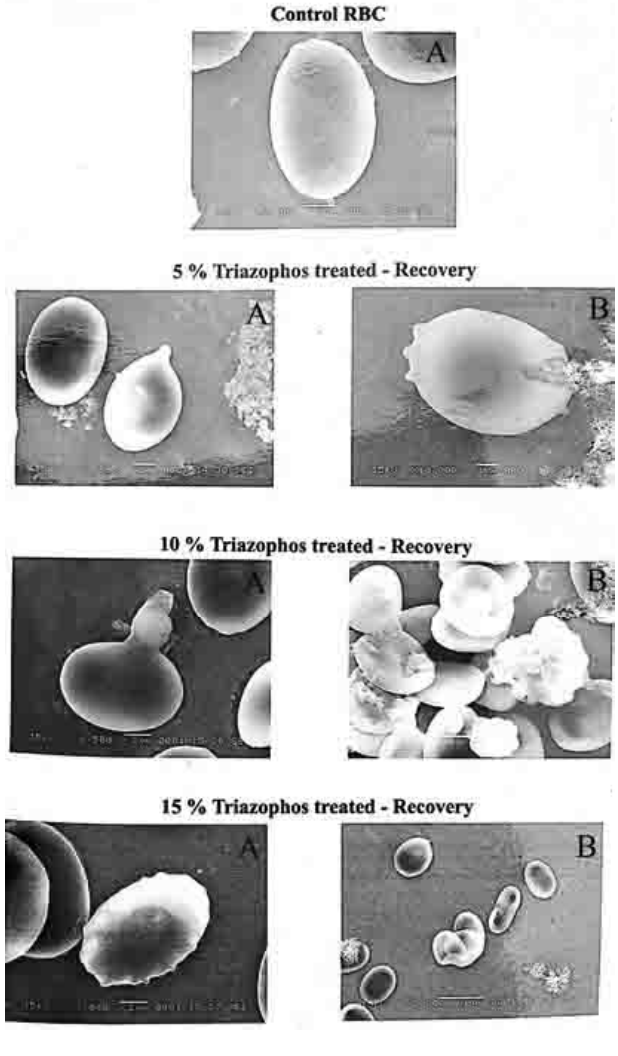

Fig. 4. *Heteropneustes fossilis* blood exposed to Triazophos – Recovery: **5% Recovery**: A: Lobopodial Projections; B: Disruption of Cell Membrane; **10% Recovery:** A: Cytoplasmic Blebbing; and Disruption of Cell Membrane: B: Formation of Chain; **15% Recovery**: A: Swollen Cells; and Erythrocyte Blebbing: B: Formation of Chain

the deleterious effects when exposed to heavy metals and effluents as reflected by haematological changes (Johansson-Sjobeck and Larsson, 1979), eosinophilia (Dawson, 1935), lymphocytosis (Gardner and Yevich, 1970) and alterations in erythrocyte morphology (Gill and Pant, 1985) which were also highlighted in the present study. In conclusion, the results clearly demonstrated that triazophos used in agriculture caused changes in the histological and ultrastructural level in the gill and blood tissues of freshwater fish *Heteropneustes fossilis* under laboratory conditions, which should certainly be taken into consideration to monitor the toxicity to fish under aquatic ecosystem, and the impressions of alterations could be considered as biomarkers in assessing the fish health and aquatic ecosystems.

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