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Histopathological stress responses in digestive glands of *Parreysia corrugata* exposed to pesticides

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ABSTRACT

Stress developed due to pesticide contamination of aquatic environment disturbs the structural architecture of digestive glands of the bivalves. Pesticide induced stress responses in digestive glands of freshwater bivalves, *Parreysia corrugata* after chronic exposureto quinalphos (0.108 ppm) and thiodan (0.0708 ppm) were studied. Main histopathological damages in digestive glands observed were ruptured lobules, swelling in epithelial tissue, spaces in interlobular connective tissues, broken basement membrane, detached and scattered cells. There was increase in the size of lumen in the core of lobule due to cellular death, necrosis and degeneration of the cells and thin connective tissue. Huge gaps were also observed in the connective tissues. The cytoplasm in the cells was reduced and the nucleus was degenerated. The number and size of the secretory cells was decreased. The damages in the cytoarchitacture of digestive glands were less severe in quinalphos exposed bivalves than those of thiodan exposed bivalves. Intensity of damage in the tissues of pesticide exposed bivalves was found to be increased with increase in exposure time.

Keywords: Hepatopancreas, pesticides, histopathology, bivalves

INTRODUCTION

In recent years pesticides have occupied all segments of the environment due to their use in agriculture as potent and economically useful poisons (Anees, 1978; Verma *et al.*, 1981). They are continuously added in the aquatic environment through various sources. The principal route for ecological impacts of pesticides is the contaminated water by pesticide runoff. Uptake and accumulation of xenobiotics in the tissues of aquatic organisms occur from the sediment, contaminated water column and food chain (Livingstone, 1994) causing lethal effects. The increasing use of pesticides has caused several deleterious effects on non-target organisms in the environment (Chagnon, *et al*., 2015). The aquatic contamination by pesticides used

in agriculture may constitute potential ecotoxicological risks to non-target aquatic invertebrates (Maltby *et al*., 2005). Many non-target organisms in aquatic environment have been non intentionally exposed to pesticides. These pesticides and other toxic chemicals are incorporated in the tissues of varieties of aquatic organisms and alter their structural architecture at cellular and subcellular levels. Incorporation of toxic compounds or their metabolites in lower organisms and in the vital tissues of fishes, birds and humans have been recorded to cause serious morphological alterations even at very low levels (Chakrabarthy and Konar, 1974; Mathur *et al.,* 1981). The nature of toxic effects of pesticides varies greatly as they are not specific in their action (Moore, 1969). Mollusc is one of the groups of non-target aquatic animals being exposed to various concentrations of pesticides and became victim of their toxic effects, may be due to their sedentary and filter feeding habit. Freshwater molluscs play an important role in aquatic ecosystems, providing food for many fish species and vertebrates (Maltchik, 2010). Bivalves are regarded as good bioindicators due to their widespread distribution, abundance, sedentary trait, hardiness and ability tobioaccumulate xenobiotics from water and sediments (Elder and Collins 1991). Mussels are ecologically important fauna because they are used as sensitive biomarkers of aquatic pollution (Waykar and Deshmukh, 2012). Unlu *et al.* (2005) studied histopathological effects in tissues of snail, *Lymnaea stagnalis* exposed to sublethal concentration of thiodan. Neonicotinoids are a class of neuroactive insecticides used worldwide in agricultural fields. Contamination of aquatic systems by neonicotinoids could have significant implications for commercial molluscs threatening productivity (Ewere, *et al*., 2021).

Histological changes appear as a medium-term response to sub-lethal stressors, and histology provides a rapid method to detect effects on tissues and organs (Johnson, 1993). Histopathological analysis of molluscs provides information about the general health of the animals and contaminant-specific changes in the tissues (Balamurugan and Subramanian, 2021).

The digestive glands also known as hepatopancreas are the crucial organs of bivalve as they are metabolically very active and are involved in various important functions such as, food collection, digestion, absorption and also detoxification of varieties of toxic substances upon exposure to the organic and inorganic pollutant in the water. Therefore, hepatopancreas are the most suitable organs to study their histology so as to determine the toxic effect of the pesticide. Attempts have been made to investigate histopathological effects of pesticides, quinalphos and thiodan on the digestive glands of freshwater bivalves, *Parreysia corrugata*.

MATERIAL AND METHOD

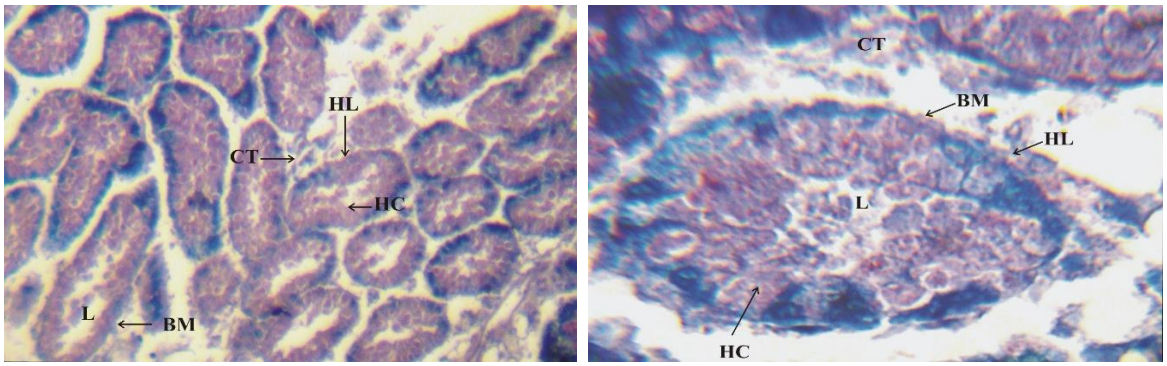
Freshwater bivalves, *Parreysia corrugata* were collected from Ambadi dam about 50 km. away from Aurangabad city. They were cleaned and washed in a tap water and acclimatized to laboratory conditions for 4 days, water in the troughs was renewed every day. Healthy medium sized bivalves were selected for experimental purpose. Three groups of acclimatized bivalves with equal numbers were kept in separate troughs for 30 days. One of the three groups was not exposed to pesticides and maintained as a control. Out of remaining two groups, one was treated by chronic concentration ($LC_{50/10}$ value of 96 hrs.) of quinalphos (0.108 ppm) and another was treated by chronic concentration of thiodan (0.0708 ppm). On 15^{th} and 30^{th} day of exposure, bivalves in control and experimental groups were sacrificed and their digestive glands were fixed in aqueous Bouin's fluid for 24 hours. The fixed tissues were washed and then dehydrated through grades of alcohol. The dehydrated tissues were cleared in toluene and blocks were prepared using paraffin wax (58 to 60 ^{0}C). Serial sections of 6 µ thickness were cut with the help of microtome. Cut sections were stained with Mallory's triple stain and examined under light microscope for histopathological effect of quinalphos and thiodan.

RESULTS AND DISCUSSION

Hepatopancreas of bivalves exposed to pesticides showed histopathological changes in their structure as compared to the bivalves of control group as shown in Fig. 1, 2 and 3.

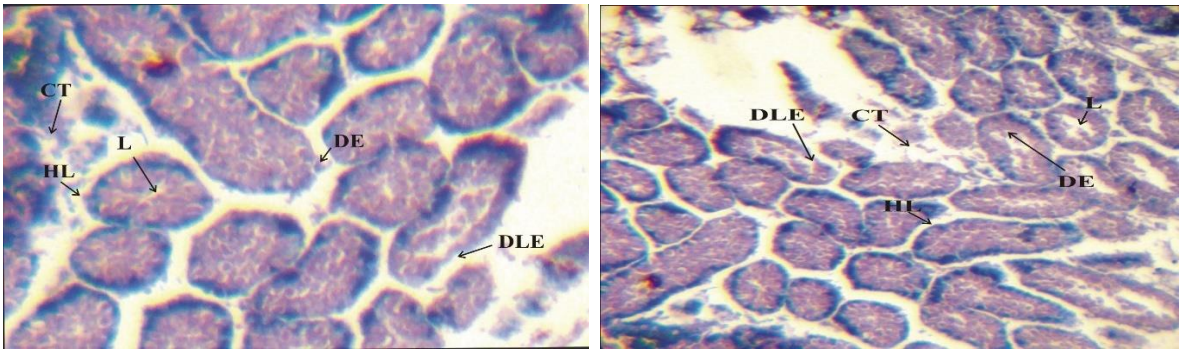
Histology of hepatopancreas of bivalves, *P. corrugata* in control group show numerous hepatic lobules (Fig. 1 A & B). Each lobule is lined by columnar cells and secretory cells resting on basement membrane. The

columnar cells are highly vacuolated with basal spherical nucleus. The secretory cells are pyramidal in shape with homogeneous cytoplasm containing large and conspicuous nucleus. In the core of hepatic lobule there is a narrow lume cell n. Interlobular space is filled by thin layer of connective tissue containing collagen fibres and amoebocyte cells, few muscle fibres are also observed. It is the storage house of metabolic reserve which is the source of energy during physiological stress. Its secretion also plays a vital role in digestion of food.



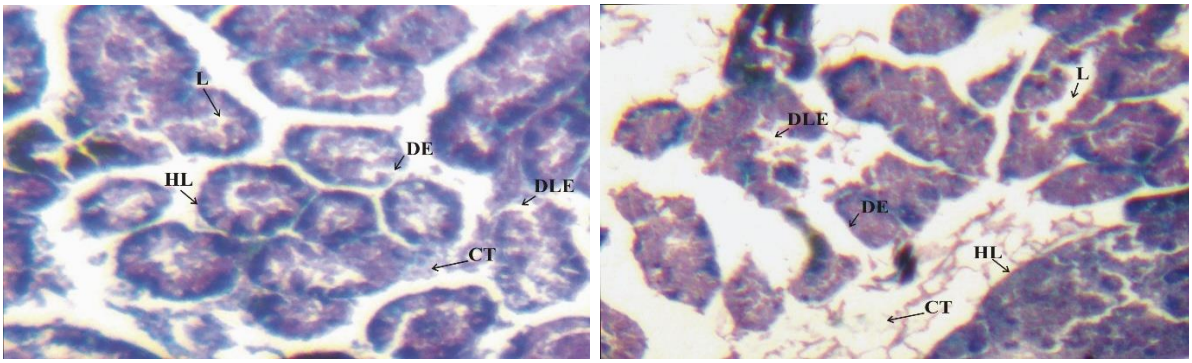
(A) **(B)**

Fig. 1. Microphotographs of Normal Digestive glands of *Parreysiacorrugata*. (A) X100, (B) X200.



(A) **(B)**

Fig. 2. (A) Microphotograph of Quinalphos exposed Digestive glands of *Parreysia corrugata* (15 days). (B) Microphotograph of Quinalphos exposed Digestive glands of *Parreysia corrugata* (30 days).



(A) **(B)**

Fig. 3. (A) Microphotograph of Thiodan exposed Digestive glands of *Parreysia corrugata* (15 days). (B) Microphotograph of Thiodan exposed Digestive glands of *Parreysiacorrugata* (30 days).

Abbreviations:

(L– Lumen, CT– Connective tissue, HL– Hepatic lobule, HC– Hepatic cells, BM– Basement membrane, DE– Damaged epithelium, DLE– Delaminated epithelium).

Effect of Quinalphos: -

Histopathological responses induced by chronic dose of quinalphos were characterized by noticeable damages in hepatopancreas (Fig. 2 A & B). 15 days exposure showed slight swelling in epithelial tissue. Spaces appeared in interlobular connective tissues. Hepatic lobules became loosely arranged. Cells were detached from the basement membrane. Severity of the effects found to be increased due to prolong exposure to quinalphos for 30 days. Epithelial cells were separated from the basement membrane and scattered in the lobules. There was increase in the size of lumen in the core of lobule. Necrosis and degeneration of the cells also observed. Connective tissue became very thin.

Effect of Thiodan: -

Chronic treatment of thiodan demonstrated pronounced effect on hepatopancreas of bivalve (Fig. 3 A & B). 15 days exposure to thiodan resulted into severe histopathological damage to hepatopancreas. The basement membrane was broken, cells separated from basement membrane and spread in the lobules. Cellular death occurred in epithelial tissue and connective tissue leading to enlargement of the lumen. Intensity of damage increased with increase in exposure period. Hepatopancreas exposed to 30 days showed huge gaps in the connective tissues, ruptured lobules, scattered epithelial cells in the connective tissue. There was loss of cell cytoplasm and degeneration of the nucleus. The number and size of the secretory cells found to be decreased.

Many investigators have reported pesticide induced histopathological abnormalities and degenerative changes in certain tissues of various molluscan species. (Prasad *et al.,* 2000; Birgul,*et al.*, 2004; Cengiz, *et al*., 2005; Unlu, *et al*., 2005; Pandit and Mundhe, 2013; Rane and Kolhe, 2019). Kumar and Pant (1984) stated the importance of histopathlogical studies in evaluating the pollution level of pesticides since their trace amount is capable of inducing considerable damage in the tissues.

Acute and chronic treatment of pesticides causes histopathological changes in digestive gland of mollusc (Thoser *et al*., 2001). Hamlet *et al*. (2014) observed an increase in the excretory vacuole, breakdown of the basement membrane, degeneration of digestive cells/tubules in the hepatopancreas of the land snail *H. aspersa* exposed to thiamethoxam. Shan, *et al*., (2020) found damages in gill and digestive tissue in the larvae of *Corbicula fluminea* after exposure to imidacloprid at concentrations as low as 0.02 mg/L. Ali, (2008) reported that the lake Manzala (Egypt) receives about 4000 million cubic meters of untreated industrial, domestic and agricultural waste water annually. El-Khayat, *et al*., (2015) studied different parameters in snail samples from lake Manzala and observed the histopathological changes in hepatopancreas of *Biomphalri aalexandrina* such as cellular necrosis followed by loss of secretory activity of the epithelial cells, atrophy, degeneration and fat vacuolation, dilated lumen and more than two hepatopancreatic tubules connected together with one larger lumen.Marked histopathological changes induced in hepatopancreas of bivalve, *Parreysia cylindrica* after acute exposure to thiamethoxam were epithelial hyperplasia, necrotic changes in basement membrane and intertubular connective tissue, rupture of epithelial layer, hypertrophy and sloughing of the epithelium. The severity of damage of hepatopancreas progressed with longer exposure (Patil, 2019). Usheva, *et al.* (2006) found erosive disturbances and heavy vacuolization of digestive cells in the epithelium of the tubules and channels, lipofuscin, necrosis and lysis of cells in connective tissue. Swelling of nerve fibers in some molluscs exposed to polluted water. Akarte (1985) suggested that, pesticides Folithion and Leybacid were effective to induce cellular destructions in hepatic lobules of *Indondia caeruleus* and *Lamellidens marginalis* during all the seasons. Unlu *et al.* (2005) observed irreversible necrotic changes in digestive glands of snail, *Lymnaea stagnalis* exposed to sublethal concentration of thiodan (35% EC.). Victor *et al.* (1990a) concluded that, the histopathological changes were due to inability of animal to digest and store food properly and hence lack of nutrients resulted in the atrophy of hepatopancreas.

CONCLUSION:

The observations noted in present study reveals that pesticides, quinalphos and thiodan developed histopathological aberrations in digestive glands of freshwater bivalves; *Parreysia corrugata* which could be resulted into physiological disfunctioning and eventually the death. Hence, bivalves can be the bioindicators for the assessment of pesticidal stress in the aquatic environment. The damages in the cytoarchitacture of digestive glands were less severe

in quinalphos exposed bivalves than those of thiodan exposed bivalves. Intensity of damage in the tissues of pesticide exposed bivalves was found to be increased with increase in exposure time.

Conflicts of Interest: The authors declare no conflict of interest.

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