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Histopathological alterations in gills of freshwater bivalve, *Lamellidens marginalis* (Lamarck) after acute exposure to Thiamethoxam and Triazophos

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ABSTRACT

Present study was carried out to study gills histopathology in the freshwater bivalve, *Lamellidens marginalis* after acute exposure to Thiamethoxam and Triazophos. Several histopathological changes were observed in the gills of bivalves after exposure to the pesticides. Histopathological changes observed microscopically showed damages in the gills of bivalves exposed to Thiamethoxam and Triazophos, while gills of bivalves of control groups exhibited normal architecture. Increasing degrees of damage in the gills was observed in the bivalves exposed to Triazophos as compared to Thiamethoxam.

Key words: Histopathology, acute, gills, *Lamellidens marginalis*, Thiamethoxam, Triazophos.

INTRODUCTION

Contamination of aquatic systems has become a serious ecological problem all over the world from the last few decades. Contaminants like heavy metals, pesticides and persistent organic pollutants (PAHs, PCBs, etc) are the most common anthropogenic pollutants that enter the aquatic systems and increased alarmingly. Due their toxicity, genotoxicity, persistence, bioaccumulation and biomagnification in the food chain (Sunjog *et al.* 2016) they attract increasing attention in environmental studies. The occurrence of contaminants has the potential to affect the quality of aquatic ecosystem. Pesticides are widely used in agriculture for pest control (Monteiro *et al.* 2006). The aquatic ecosystem is facing the threat of biodiversity loss due to indiscriminate use of pesticides (Rahman *et al.* 2002) in order to improve the agricultural productivity to match the explosive population growth rate is a global phenomenon. Evaluation of the impacts of contaminants on the wellbeing of aquatic organisms and ecosystems is important to prevent harmful impacts of contamination on their structure and function.

Biomarkers are biological indicators from an exposure to a stressor responding in various ways (van der Oost *et al.* 2003).

They have been used extensively as a tool for detection of exposure and to provide the connection between external levels of contaminant exposure, internal levels of tissue contamination and early adverse effects of pollution in organisms (van der Oost *et al.* 2003, Hook *et al.* 2014). Study of biomarkers is necessary to conduct ecological risk assessments.

Histopathological changes are considered as fast and efficient for detection of acute and chronic adverse effects and therefore have been widely used as biomarkers in the assessment of the health of organisms exposed to contaminants. Histopathological methods can be used as an early warning system for the survival of species and ecosystem protection (Fatima *et al.* 2014). The advantage of using histopathological biomarkers in environmental monitoring allows examining specific target organs which perform significant functions and they are a reflection of the overall health of the entire population in the studied ecosystem (Braunbeck, 1993).

Bivalves have been used as bioindicators for the assessment of the quality of aquatic ecosystems worldwide. They are widespread, sedentary, filter feeders, easy to collect and accumulate toxicants according to bioavailable levels in the environment (Bervoets *et al.* 2005).

Bivalve molluscs reflect immediate responses to toxic substances present in surrounding water by change in histological arrangement (Kumar *et al.* 2011; Kamble *et al.* 2012). Morphological changes in gills are use to evaluate acute or chronic exposure to chemical present in water and sediment (Winkaler *et al.* 2001; Tkatcheva *et al.* 2004).

Several researchers studied histopathological changes in gills of the freshwater bivalve after exposure to pesticides (Lomte and Waykar, 1998; Phirke, 2008; Patil, 2010; Patnaik *et al.* 2011; Kumar *et al.* 2012c). The present study aimed to investigate histopathological alterations in gills of freshwater bivalve, *Lamellidens marginalis* (L) after acute exposure to thiamethoxam and triazophos.

MATERIAL METHODS

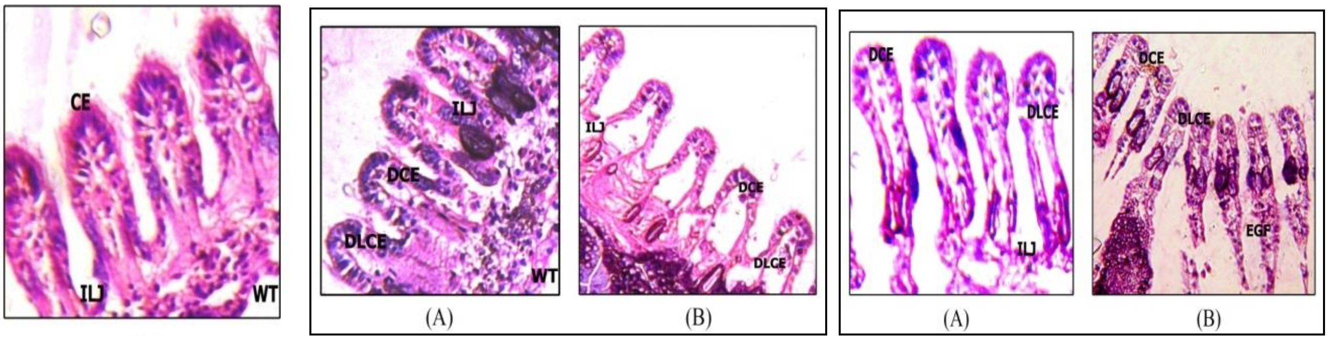
Fresh water bivalves, *Lamellidens marginalis* were collected from Hatnur dam situated on Tapi River near about 35 km away from Bhusawal city. They were cleaned and washed in a tap water and acclimatized to laboratory conditions for 5 to 6 days. During acclimatization period water in the troughs was changed every day. After the acclimatization, healthy medium sized bivalves were selected from the troughs and used for experiments. To study the effect of pesticides at cellular level, fresh water bivalves, *Lamellidens marginalis*, were exposed to the acute dose of thiamethoxam and triazophos. The acclimatized bivalves were divided into two groups with equal numbers of animals. One group was considered as control and remaining was exposed to acute concentration (LC 50/2 value of 96 hrs) of thiamethoxam (12.895 ppm) and triazophos (3.67 ppm) for 24, 48, 72 and 96 hrs.

The bivalves in each experimental group were sacrificed and their gills were fixed in aqueous Bouin's fluid after 24 and 96 hours from acute exposure. They were washed in running tap water for about six hours so as to remove the Bouin's fluid from tissues. The washed tissues were dehydrated through grades of alcohol (from 30% to 100% alcohol) and were dealcoholized and cleared in toluene. The cleared tissues were embedded in paraffin wax (58-60^{0}C) and blocks were prepared. Blocks of the tissues were trimmed and serial sections of 6 µ thickness were cut with the help of microtome. Cut sections were spread properly on the slides and were stained with Hematoxyline-Eosin. The stained sections were examined under light microscope for histopathological effect of pesticides.

RESULTS & DISCUSSION

The obtained results (Figure 1) showed histopathological changes in structural design of gills in bivalve, *Lamellidens marginalis* after exposure to acute dose of Thiamethoxam and Triazophos as compared to the bivalves maintained as control. The damages in the architecture of gills were less severe in the bivalves exposed to thiamethoxam than that of triazophos.

In bivalves maintained as control, gill lamellae consist of large number of closely set, thin, vertical, gill filaments, contain porous like structure perforated by minute opening bound by filaments. The gill filaments connected by horizontal bars, gill filaments are composed of connective tissue. Gills covered by ciliated epithelium and supported by chitanous rods.



(i) **(ii)** **(iii)**

Figure 1: Photomicrograph of sections of freshwater bivalve, *Lamellidens marginalis*,
(**i)** control, (**ii)** after acute exposure to Thiamethoxam (A - 24hrs, B - 96hrs) and (**iii)** after acute exposure to Triazophos (A - 24hrs, B - 96hrs).
(CE - Ciliated epithelium, ILJ - Interlamellar junction, WT - Water tube, DCE - Damaged ciliated epithelium, DLCE - Delaminated ciliated epithelium, EGF - Elongated gill filament)

Effect of Thiamethoxam on gills

Histopathological change in the architecture was characterized by degeneration of epithelial cells, swelling, vacuolated and necrotic epithelium. The secondary gill lamellae were united and the bases of the gill filaments became broader. Acute exposure to thiamethoxam for 96 hrs duration, the gill filament showed irregular shape [Figure 1: ii-B]. The severity of necrotic effects was found to be increased. The ciliary lining was damaged along with epithelium. Cells were scattered in the gill lamellae.

Effect of Triazophos on gills

Respiratory epithelium was enlarged with increased abnormality in cells and hyperplasia. Disconnection of gill filament was observed. Gill lamellae showed effects such as enlarged and swollen gill lamellae with abnormal shape with broad and bent tip. Acute exposure to triazophos for 96 hrs showed that, the gill epithelium was ruptured with damaged ciliary lining [Figure 1: iii-B]. Enlargement of gill filament and bent tips of gill lamellae were also observed. Detached secondary gill lamellae were seen. The gill filaments were severely damaged.

Histopathological changes after exposure to pesticides are defensive in nature. These abnormalities which occur at the tissue and cellular level are the result of complex physiological dysfunctions, since the gills are multifunctional organs involved in respiration, osmoregulation and filter feeding (Gosling, 2003). Gills remain in close contact with the external environment and main route of toxicants penetration into the bivalves and significant potential for accumulation of heavy metals and other pollutants (Chakraborty *et al.* 2010). Gills are primarily susceptible to changes in the quality of the water and considered as the primary target of the contaminants (Fernandes and Mazon, 2003) for identifying the effects of water toxicants.

Musthafa and Amanulla (2011) reported bulging of primary and curling of secondary gill lamellae, degeneration and necrosis of epithelial cells, distortion of secondary gill lamellae, destruction of epithelial cells and irregular appearance of gill lamellae in *L. marginalis* exposed to chloropyrifos. Pandey *et al.* (2016) reported remarkable changes in gill histopathology of *L. marginalis* exposed to mercury chloride. Ahire *et al.* (2017) revealed the changes in gill epithelium like pycnotic nuclei of epithelial cells and necrosis of connective tissue, reduction in intrallamilar space of freshwater mussel *L. marginalis* after exposure to lamda-chylothrin.

CONCLUSION

Gill tissues of freshwater bivalve, *L. marginalis* showed apparent evidence that, pesticides Thiamethoxam and Triazophos accountable for changes in normal cell architecture of gills. Hence, the changes in the histopathological structure of the gill can be use as biomarkers of exposure in the aquatic environment and the freshwater bivalve *L. marginalis* can be considered as a bioindicator organism to assess the water quality.

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REFERENCES

Ahire P, Bhamre P and Desai A (2017) Effect of lamda-chylothrin on gill and digestive gland of freshwater mussel *Lamellidens marginalis*. Trends in fisheries research, 6(2): 9-13.

Bervoets L, Voets J, Covaci A, Chu SG, Qadah D, Smolders R, Schepens P and Blust R (2005) Use of transplanted zebra mussels (*Dreissena polymorpha*) to assess the bioavailability of micro contaminants in Flemish surface waters. Environ Sci Technol, 39: 1492-1505.

Braunbeck T (1993) Cytological alterations in isolated hepatocytes from rainbow trout (*Oncorhynchus mykiss*) exposed in vitro to 4-chloroaniline. Aquatic Toxicolology, 25: 83-110.

Chakraborty S, Ray M and Ray S (2010) Toxicity of sodium arsenite in the gill of an economically important mollusc of India. Fish and Shellfish Immunology. 29: 136-146.

Fatima M, Usmani N, Hossain MM (2014) Heavy metal in aquatic ecosystem emphasizing its effect on tissue bioaccumulation and histopathology: a review J Environ Sci Technol, 7 (1), pp. 1-15.

Fernandes, MN and Mazon, AF (2003) Environmental Pollution and Fish Gill Morphology. In: Val AL and Kapoor BG, Eds., Fish Adaptation, Science Publishers, Enfield, 203-231.

Gosling E (2003) Bivalve molluscs: biology, ecology and culture. Blackwell, New York. p. 443.

Hook SE, Gallagher EP, Batley GE (2014) The role of biomarkers in the assessment of aquatic ecosystem health. Integr Environ Assess Manage, 10: 327–41.

Kamble VS, Gavhane UV and Kamble AB (2012) Effect of sub lethal treatment of organophosphorous pesticide Ekalux (Quinolphos 25% Ec) on histopathology of gonad in freshwater lamellibranch mollusc *Lamellidens corrianus* during monsoon season found near sangola, Dist. Solapur. Research Link, 96 XI (1): 28-30

Kumar S, Pandey RK, Das S and Das VK (2011) Pathological changes in hepatopancreas of freshwater mussel, *Lamellidens marginalis* (Lamarck) exposed to sub-lethal concentration of dimethoate. GERF Bulletin of Biosciences, 2(2): 18-23.

Kumar S, Pandey RK, Das S and Das VK (2012c) Dimethoate alters respiratory rate and gill histopathology in freshwater mussel, *Lamellidens marginalis* (Lamarck). J Appl Biosci, 38(2): 154-158.

Lomte VS and Waykar BB (1998) Effect of carbaryl and cypermethrin on the oxygen consumption of the freshwater bivalve, *Parreysia cylindrica*. Him. J. Env. Zool., 12 (2): 157-162.

Monteiro DA, de Almeida JA, Rantin FT, Kalinin AL (2006) Oxidative stress biomarkers in the freshwater characid fish, Brycon cephalus, exposed to organophosphorus insecticide Folisuper 600 (methyl parathion).Comp Biochem Physiol C Toxicol Pharmacol, 143(2):141-9.

Musthafa MS and Amanullah (2011) Histological alternation in the gill of freshwater mussel Lamellidensmarginalis exposed to sublethal concentration of an organophosphorus insecticide, chlopyropifos. Asian J. Microbiol. Biotech Environ Sci, 13 (1): 125-128.

Pandey Abhed, Shanthanagouda, AH, Pathak Devendra and Singh Avtar (2016) Histopathological effects in gills of freshwater mussels, *Lamellidens marginalis* exposed to mercury chloride. The Bioscan, 11(4): 2277-2280, 2016

Patil AG (2010) Impact of pesticides on certain physiological aspects of freshwater bivalve, *Parreysia cylindrica*. Ph.D. Thesis submitted to North Maharashtra University, Jalgaon, India.

Patnaik BB, Hongray HJ, Mathews T and Selvanayagam M (2011) Histopathology of gill, liver, muscle and brain of *Cyprinus carpio communis* L. exposed to sub lethal concentration of lead and cadmium. Afr J Biotechnol, 10(57): 12218-12223.

Phirke, P. P. (2008): Effect of thiodan and quinalphos on some physiological parameters of the freshwater bivalve, *Parreysia corrugata.* Ph. D. Thesis submitted to Dr. Babasaheb Ambedkar Marathwada University, Aurangabad, India.

Rahman MZ, Hossain ZM, Ellah MFR and Ahmed GU (2002) Effect of diazinon 60 EC on *Anabas testudineus*, *Channa punctatus* and *Barbades gomonus* Naga. The ICLARM quarterly, 25, 8-11.

Sunjog K, Kolarević S, Kračun-Kolarević M, Višnjić-Jeftić Z, Skorić S, Gačić Z, Lenhardt M, Vasić N, Vuković-Gačić B (2016) Assessment of status of three water bodies in Serbia based on tissue metal and metalloid concentration (ICP-OES) and genotoxicity (comet assay) Environ Pollut, 213, pp. 600-607.

Tkatcheva V, Hyvarinen H, Kukkonen J, Ryzhkov LP and Holopainen IJ (2004) Toxic effects of mining effluents on fish gills in a subarctic lake system in NW Russia. Ecotoxicol Environ Saf, 57: 278-289. http://dx.doi. org/10.1016/S0147-6513, (03)00079-4.

van der Oost R, Beyer J, Vermeulen NPE (2003) Fish bioaccumulation and biomarkers in environmental risk assessment: A review. Environmental Toxicology and Pharmacology, 13: 57-149.

Winkaler EU, Silva AG, Galindo HC and Martinez CBR (2001) Biomarcadores histológicos e fisiológicos para o monitoramento da saúde de peixes de ribeirões de Londrina, Estado do Paraná. Acta Sci, 23(2): 507-514.