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Original Article

Evaluation of Imidacloprid-induced toxicity and lipid peroxidation in the freshwater bivalve, *Lamellidens marginalis*

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Abstract: The toxicity of the neonicotinoid pesticide Imidacloprid was tested on *Lamellidens marginalis* by 96-hours LC50 test and histopathology. After acute exposure to Imidacloprid, histopathological changes were noted in the gill, mantle, and digestive gland. Exposed gills showed deformed interfilamental space, fused lamellae, disrupted chitenous rod, and vacuolated cytoplasm. In the mantle, damage was observed in the inner and outer mantle epithelium and vacuole in connective tissue. The digestive gland has ruptured the digestive tubule, and basement membrane, the lumen deteriorates, and the cytoplasm appears vacuolated. Lipid peroxidation was also observed after the exposure. These findings suggest that acute exposure to Imidacloprid caused significant histological alterations in vital organs and can affect the non-targeted freshwater bivalve *Lamellidens marginalis*.

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Introduction

Pesticides are chemical compounds used to control pests and enhance crop yields. There are various types of pesticides, including organochlorines, organophosphates, carbamates, pyrethroids, and neonicotinoids (Sparks et al., 2020). Among these, neonicotinoids, such as imidacloprid, have gained significant attention due to their widespread use and potential ecological impacts. Imidacloprid is a neonicotinoid insecticide that targets the nicotinic acetylcholine receptor (nAChR), leading to the prolonged opening of sodium channels in neurons and ultimately resulting in their depolarization and death (Mencke and Jeschke, 2002; Selvam and Srinivasan, 2019). This systemic pesticide is effective against a range of pests, including jassid, aphid, thrips, termites, and mites, and is used on crops such as cotton and ladyfinger, as well as in veterinary applications (Stanneck et al., 2012; Krämer and Mencke, 2012). However, the extensive use of imidacloprid in agriculture has resulted in spray drifts and runoff, conveying the pesticide to nearby water bodies (Morrissey et al., 2015; Klarich et al., 2017). Consequently, neonicotinoids have been commonly found in water bodies, potentially harming non-targeted organisms (Borsuah et al., 2020).

Natarajan (2016) emphasizes the vital role of water quality in supporting aquatic life. Unfortunately, the presence of pesticides in water bodies can negatively impact water quality. Pesticides are a significant contributor to water pollution and can cause direct or indirect harm to economically and ecologically important species by producing toxic stress (Köprücü et al., 2010). These substances can infiltrate water resources and accumulate in water columns, causing changes to the physical, chemical, and biological processes of water (Agrawal et al., 2010). Moreover, pesticides can lead to physiological, biochemical, and histological changes in mussels due to their bioaccumulation and biomagnification in tissues (Yancheva et al., 2017; Zhang et al., 2020). Additionally, pesticide exposure can affect gene expression (Kuchovská et al., 2021) and cause immunotoxicity, genotoxicity (Kayis et al., 2019), as well as histopathological changes by

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damaging tissue cells (Shaikh et al., 2010; Sula et al., 2020).

Bivalves, including freshwater mussels, are ecologically important since they are part of the food web (Strayer, 2008) and possess medicinal and nutritional value (Laxmilatha, 2013; Charaborty et al., 2016). Unfortunately, pesticide pollution in water bodies can negatively impact the normal metabolism, development, growth, behaviour, reproductive cycle, and life span of organisms (Amoatey and Baawain, 2019). *Lamellidens marginalis,* a benthic organism, is distributed extensively and exhibits feeding habits that increase its exposure to diverse environmental pollutants. This freshwater bivalve is frequently used as a model organism for toxicological investigations (Yusufzai et al., 2010). The objective of this investigation is to evaluate the toxicity of the neonicotinoid pesticide Imidacloprid on *L. marginalis* with respect to histopathology and lipid peroxidation.

Material and methods

Lamellidens marginalis were collected from the Darna River, Chehadi (19°55'54.02"N, 73°55' 30.42"E), and brought to the laboratory where they were acclimatized in aged tap water for 6 days in plastic troughs. The bivalves were fed spirulina algae daily to ensure proper nutrition. Overcrowding was avoided by keeping 10 bivalves in separate plastic troughs, and the water was changed every day with dechlorinated tap water. The bivalves were exposed to a 12:12 light-dark cycle during the experiment. Healthy bivalves with a shell length of 9-10 cm and weight of 70-75 g, regardless of their sex, were selected for the toxicity assay. The pesticide Imidacloprid (Bayer confider super, 30.5m/m sc) was obtained from the market.

To study the effect of Imidacloprid, *L. marginalis* were exposed to the LC_{50} concentration (40 ppm) for 96 hrs. The gill, digestive gland, and mantle tissues were fixed in Bouin's fluid, followed by treatment with alcohol (30, 50, 70, 80, 90, and 100%) for 2 hrs each. The tissues were cleared in xylene and embedded in paraffin wax. Sections of 5-8 µ were obtained using a Leica microtome RM 2235, and the sections were stained with Delafield's hematoxylin-eosin. The stained sections were observed and microphotographed using HL-22/Coslab. Statistical analysis was performed using the Microsoft Excel data analysis program.

In the TBARS assay, the end product of lipid peroxidation, MDA (malondialdehyde), was measured based on Ohkawa et al. (1989). Absorbance was recorded spectrophotometrically at 532 nm, and lipid peroxidation was expressed in nmol/mg protein. The bivalves were exposed to 0.7 ppm for studying lipid peroxidation.

Results

Acute toxicity studies: The acute toxicity test reported that the LC_{50} value of Imidacloprid was 40 ppm for 96 hrs.

Behavioural changes: Behavioural changes like secretion of mucous after 24 hrs of Imidacloprid exposure were observed during the experiment.

Histopathological observation: Normal gill lamellae of control bivalve consists of a large number of closely set, thin, vertical, gill filaments perforated by minute opening bound by filaments. The gill filament is connected by horizontal bars. Gill filaments are composed of connective tissue. The margin of the gills is covered by ciliated epithelium and supported by chitanous rods. The gill lamellae are joined together by intrallamellar junction so that space is present between gill lamellae. Lamellae are divided into water tubes. Gills are covered by three types of cilia, lateral, latero-frontal, and frontal cilia. The prominent nucleus is present in each epithelium cell (Fig. 1i).

After the exposure of 40 ppm of Imidacloprid, the gill structure showed anomalous changes. Necrosis was noticed in the gill lamellae. The damaged nucleus was observed at the base of the gill. At certain places, fused gill lamellae were observed with degenerative changes like vacuolated cytoplasm, deformed inter filamental space, and disrupted chitenous rods (Fig. 1ii).

The mantle consists of two lobes that lie inner

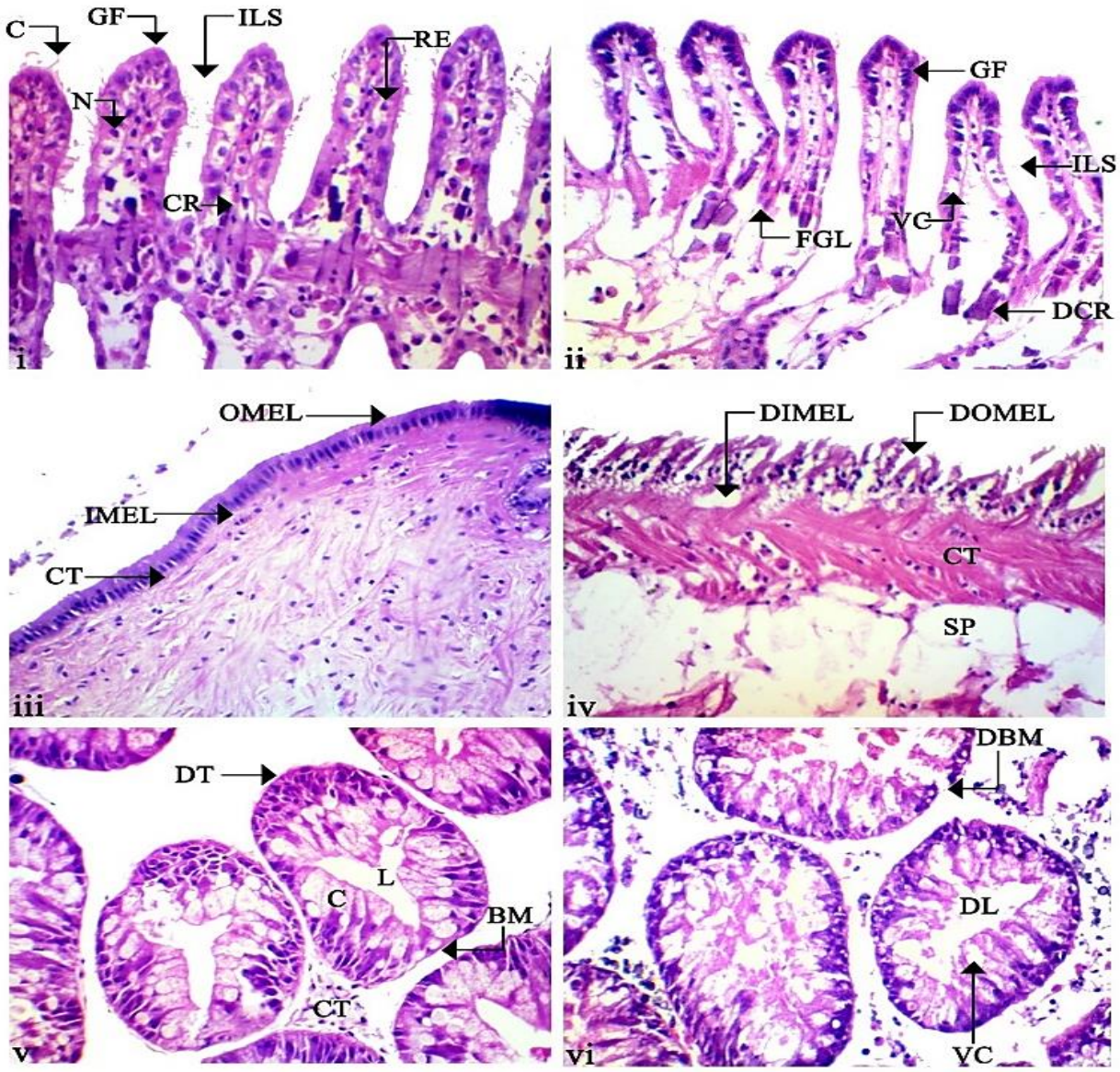

Figure 1. (i) Microphotograph of L.S (H & E) of control gill (G.F: Gill filament, R.E: Respiratory epithelium, ILS: Interlamellaer Space, N: Nucleus, and C: Cilia), (ii) Imidacloprid exposed gill (ILS: Interlamellar Space, FGL: Fused gill filament, VC: Vaculated cytoplasm, FGL: and Fused gill lamellae), (iii.) T.S of mantle of control (FM: Mantle fold, OMEL: Outer mantle epithelial layer, IMEL: Inner mantle epithelial layer, CT: Connective tissue, and SP: Space), (iv) exposed mantle (FM: Mantle fold, DOMEL: Outer mantle epithelial layer, DIMEL: Inner mantle epithelial layer, CT: Connective tissue, and SP: Space), (v) T.S of Digestive gland of control (C: Cytoplasm, L: Lumen, BM: Basement membrane and Imidacloprid exposed specimen, and (**vi)** T.S of Exposed Digestive gland, RBM: Ruptured Basement membrane, DL: Degenerated lumen, and VC: Vaculated cytoplasm).

surface of the shell and encloses the animal inside the shell. The mantle consists of connective tissue, blood vessel, nerves, and muscles. Below the columnar epithelium is a layer of connective tissue. Nuclei were prominent (Fig. 1iii). Treated mantle epithelium shows necrosis and a gap was observed in the inner mantle fold and outer mantle fold. The inner mantle epithelium layer is separated from connective tissue. Dissolved nuclei were observed in the middle of the epithelium (Fig. 1iv).

The digestive gland or hepatopancreas is the main site of all metabolic activities in *L. marginalis*. It is composed of tubules and ducts. Digestive tubules are internally lined with columnar epithelial cells resting on a thin fibrous connective tissue layer and externally covered with a basement membrane. The epithelium of digestive tubules consists of basophilic cells (secretary in function) and acidophilic cells

Table 1. Treated groups exposed to 0.07 ppm Imidacloprid ([a.] There are significant differences (P<0.05) between the control and treated groups).

TBARS	Tissue exposed	Gill	Foot	Mantle	Muscle	Hpt
(nmol/mg	Control	1.82±0.10	2.51±0.62	2.44±0.58	1.75±0.20	1.60±0.06
protein)	Treated group	15.11±0.84[a]	19.56±1.90[a]	11.44±1.17[a]	13.89±1.34[a]	8.66±0.88[a]

(digestive cells). The digestive cells are columnar in shape with a spherical nucleus situated in the basal region (Fig. 1v). After the exposure to Imidacloprid, the complete destruction of the epithelial lining and irregular placement of necrotic cells was observed along with vacuolated cytoplasm (Fig. 1vi).

TBARS (Thiobarbituric acid reactive substance assay): Levels of TBARS were evaluated in the control and experimental animals (Table 1). In the treated group, a significant (P<0.05) increment of TBARS in gill (237.47%), adductor muscle (194.28%), hpt (188.12%), mantle (175.81%), and foot (145.81%) were found. Increased lipid peroxidation in gills might be because of its importance as the primary site of absorption.

Discussion

The LC50 value for Imidacloprid after 96 hours of exposure was found to be 40 ppm. Interestingly, the LC_{50} value for *L. marginalis* was higher than that for the freshwater bivalves *U. mancus* and *C. fluminea* when exposed to Imidacloprid (Yoloğlu, 2019; Shan et al., 2020). This suggests that *L. marginalis* is more resistant and able to tolerate higher concentrations of Imidacloprid. LC_{50} values of 14.21 and 12.89 ppm were found for the freshwater bivalve *P. cylindrical* and *L. marginalis*, respectively, after exposure to the neonicotinoid thiamethoxam for acute treatment (Rane and Mahajan, 2013; Patil, 2019). There is considerable variation in the LC_{50} values of Imidacloprid reported by various investigators in both freshwater and marine water mussels and fish (Iturburu et al., 2017; Iturburu et al., 2018; Vieira et al., 2018; Alvim and dos Reis Martinez, 2019; Shan et al., 2020).

Histology is a useful tool to study the toxicological effect of environmental stressors on animals (Yavaşoğlu et al., 2016). Gills play a crucial role in respiration, food capturing, and maintaining the acid-base balance (Kumar et al., 2012; Ray et al., 2020). As the gills come directly into contact with water, they are often the first target organ of contaminants found in water (Giarratano et al., 2014), and hence, act as an indicator of environmental stress (Cappello et al., 2013). Therefore, the study of gills after exposure to Imidacloprid is crucial to assess its toxicity. The gills of *L. marginalis* showed degenerative changes in the nucleus, reduced interlamellar space, broken chitenous rods, and fused gill filaments. Similar degenerative changes in gills were observed in the freshwater clam *C. fluminea* and the marine water mussel *M.galloprovincialis* after exposure to Imidacloprid (Shan et al., 2020; Pagano et al., 2020). Moreover, exposure to thiamethoxam led to the degeneration of epithelial cells and swollen gill filaments in *L. marginalis* (Rane et al., 2019), while exposure to chloropyrifos and dimethoate led to damaged epithelial cells, irregular gill lamellae, hypertrophic nuclei, and dilated sinus filaments in *L. marginalis* (Stalin et al., 2011; Kumar et al., 2012). In *P. cylindrica*, the shape of the gill was lost due to chitenous rods, and the connective tissue core was damaged after exposure to endosulfan (Bhalchandra, 2010). In marine bivalve *P. radi*, necrosis of lamellar cells and fused filaments were observed after exposure to dimethoate (Hassan and Sheiko, 2013). Similarly, exposure of the marine water mussel *M. galloprovincialis* to the neonicotinoid Calypso 480 SC (CAL) resulted in epithelial alteration and vacuolization (Stara et al., 2020). As reported by Patil (2019), exposure to thiamethoxam at 14.21 ppm caused necrosis along with hypertrophy in *P. cylindrica*. Similarly, Kumar et al. (2011) observed epithelial cell disruption, hypertrophy, and hyperplasia in *L. marginalis* after exposure to dimethoate at 27.3 ppm. Kamble et al. (2015) reported necrosis, hyperplasia, damage to the

basement membrane, and vacuolization in *L. corrianus* during three different seasons after exposure to thiodan. In the present study, histopathological alterations were observed in the hepatopancreas of the test organism following exposure to Imidacloprid.

Even at lower concentrations, Imidacloprid is capable of inducing lipid peroxidation. Mundhe and Pandit (2014) observed an increase in lipid peroxidation in gills compared to other tissues upon exposure to monocrotophos. This finding is consistent with Lackner's (1998) assertion that increased lipid peroxidation after pesticide exposure suggests the involvement of free radical-induced oxidative cell injury, indicating the toxicity of pesticides. Similarly, Köpriicü et al. (2010) reported increased lipid peroxidation in mussels after exposure to pesticides, which further supports the notion that pesticide exposure induces oxidative stress in organisms.

Our study reveals that Imidacloprid induces significant histopathological changes in the gill, mantle, and digestive glands of *L. marginalis*, indicating its toxicity towards these vital organs. Imidacloprid, a neonicotinoid pesticide, is extensively utilized in agriculture to combat pests. Nonetheless, the indiscriminate use of this pesticide has led to environmental pollution, which could have adverse effects on non-targeted organisms. Hence, it is crucial to take into account the ecological consequences of pesticide usage when devising agricultural practices

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