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RESEARCH

Histomorphology and ultrastructure of the hepatopancreas of a brachyuran crab, *Varuna litterata* (Fabricius, 1798)

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Abstract
The present study aims to describe the histomorphology and ultrastructure of the hepatopancreas of a brachyuran crab, *Varuna litterata* using light and transmission electron microscopy. The hepatopancreas is an orange-yellow-coloured bilobed organ that comprises numerous blind-ended tubules. Each tubule is divided into proximal, mid, and distal regions and lined by pseudostratified epithelium with five cell types (E-, F-, R-, B-, M-cells). Each cell has distinct cytomorphological features and their relative percentages can vary along the length of the tubules. E-cells are embryonic cells and confined to the extreme distal end. It has a high nucleus-to-cytoplasm ratio and is involved in mitotic activity. M-cells are infrequently scattered throughout the length of the tubule without any brush border and play an important role in nutrient and endocrine regulation. R-cells exhibit a distinct polar organization of the cell organelles into apical, medial, and basal areas and are responsible for nutrient absorption, storage, and detoxification processes. F-cells contain abundant rER that gives fibrillar appearances to the cytoplasm and are involved in the synthesis of digestive enzymes. B-cells have an apical endocytotic complex in the early stage and a large central vacuole in the final stage of maturation. Mature B-cells are eliminated into the tubular lumen by holocrine secretion. These are considered for intracellular digestion and excretory functions. These findings provide the basic information regarding the identification, characterization, and functions of the hepatopancreatic cell types of *V. litterata* that might be useful in further nutritional research as well as in histopathological and biomarker studies.

Keywords Decapoda · Midgut gland · Morphometric analysis · Hepatopancreatic epithelial cells · Fine structure · Digestive functions

Introduction

In decapod crustacea, the hepatopancreas is considered the primary organ involved in digestion, nutrient absorption, and storage of digested products, mainly in the form of lipid and glycogen (Vogt et al. 1989; Johnston et al. 1998; Franceschini-Vicentini et al. 2009). It is also called the midgut gland, digestive gland, gastric gland, digestive diverticula, or middle intestinal gland and is believed to be the analogous organ to the liver in higher organisms (Gibson and Barker 1979; Silva et al. 2018). It is responsible for the synthesis of digestive enzymes, excretion, and detoxification of xenobiotics (Icely and Nott 1992; Vogt 1994). This vital organ occupies a large portion of the cephalothorax cavity in the decapod crustacean's body and is connected to the pyloric stomach by a primary duct. It consists of one or more lobules formed by numerous tubules with open ends at the proximal zone and blind ends at the distal zone (Icely and Nott 1992; Sousa and Petriella 2000). Various histological, histochemical, and ultrastructural studies reveal that in most decapod crustaceans, the pseudostratified epithelium of the hepatopancreatic tubule consists of four different cell types called E-(embryonic), R-(resorptive or absorptive), F-(fibrillar) and B-(blister-like) cells (Stanier et al. 1968; Gibson and Barker 1979; Caceci et al. 1988; Sousa et al. 2005). A fifth type termed an M-(midget) cell, has also been found in the hepatopancreas of some species (Al-Mohanna et al. 1985; Icely and Nott 1992; Vogt 1994). Each cell type

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Full length article

Acute exposure to a neem based biopesticide and mahua oil cake changes haemocyte parameters in freshwater crab, *Varuna litterata* (Decapoda, Crustacea)

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ABSTRACT

Present study aims to evaluate the immunotoxic effects of two biopesticides, Nimbecidine Plus (a neem biopesticide) and mahua oil cake (MOC) on the haemocyte populations of a freshwater crab, *Varuna litterata* after acute exposure. Four-day static renewal bioassay test was performed where sixteen healthy adult male crabs were exposed to 96-h LC_{50} values of Nimbecidine Plus (0.006284 ppt) and MOC aqueous extract (7.631 ppt) separately in the laboratory condition. Control groups were maintained throughout the experimental period without any biopesticide exposure. Various haemocyte parameters such as total count (THC), differential count (DHC), haemocyte density, cytomorphological anomalies and reactive oxygen species (ROS) were measured in the biopesticides-exposed and control crabs after 24, 48, 72, and 96 h of exposure. After treatment with Nimbecidine Plus and MOC, several cytomorphological deformities (cytoplasmic and nuclear membrane disintegration, chromatin condensation, pyknosis, karyorrhexis, karyolysis, nuclear vacuolation, altered cell shape, cellular coagulation, cytoplasmic discharge, vacuolation) were observed in hyalinocytes, small granule haemocytes and large granule haemocytes with modulation of their relative percentages at different exposure times. THC, DHC, haemocyte density and ROS levels were significantly altered ($p < 0.05$) in biopesticides-exposed crabs at different exposure periods. The toxicity of both biopesticides did not persist throughout the entire exposure time. Nimbecidine Plus exhibited nonlinear toxic impacts on different haemocyte parameters at initial, mid and higher exposure periods whereas MOC showed linear toxic effects mostly at initial exposure time. In comparison to MOC, Nimbecidine Plus showed higher immunotoxic effects in *V. litterata*. Outcome of this experiment might provide useful information to understand the immune responses of *V. litterata* against biopesticide toxicity.

1. Introduction

Use of biopesticides has been increasing in the agriculture and aquaculture fields to overcome the hazardous effects of chemical pesticides [1]. In general, the plant-originated biopesticides are naturally degradable, environment friendly and at certain concentrations destroy pest organisms. Numerous plant species and plant-derived products are reported to have insecticide, nematicide, fungicide properties, antifeedant properties, repellent components, attractant abilities and insect growth inhibiting properties, which make the plant-originated biopesticides an effective pest control agent [2]. In India, neem (*Azadirachta indica*; family-Meliaceae) based biopesticides are widely used to control pests in agricultural fields as well as to control insect predators, parasites and pathogenic bacteria of fishes in aquaculture systems [3]. Azadirachtin (a tetranotriterpenoid) is the principal active component of neem, which has the pesticide property [4]. Mahua oil cake (MOC) is another plant-originated piscicide that is extensively used in the freshwater inland aquaculture systems of India [5]. It is a product from the perennial madhuka tree species (*Bassia latifolia* Roxb; Syn. *Madhuca latifolia*) belonging to the family Sapotaceae [6]. The piscicidal property of MOC is attributed to its saponin content [7]. Saponins are a diverse group of low molecular weight secondary plant metabolites and MOC contains 6–8% water-soluble triterpenoid saponin [8].

Although biopesticides are considered to be eco-friendly and good alternative sources to synthetic chemical pesticides, indiscriminate use of such biopesticides may increase the risk of contaminating water

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Cytomorphological characterization, classification and counting of haemocytes in freshwater crab, *Varuna litterata* (Crustacea: Decapoda)

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ABSTRACT: Present study aims to characterize and classify the haemocyte structural types and to determine the total and differential haemocyte counts in freshwater crab, *Varuna litterata*. Haemolymph was collected from the crabs and subjected to the light microscopy, transmission electron microscopy, and flow cytometric analysis for the cytomorphological characterization and classification. Total and differential haemocyte counts were also carried out. On the basis of various cytomorphological features, haemocytes of *V. litterata* were classified into hyalinocytes, small granule haemocytes and large granule haemocytes. Hyalinocytes are round, oval, irregular, spindle or ellipsoid shaped cells with low to high nucleo-cytoplasmic ratio and contained a number of cytoplasmic organelles and a few minute-sized granules in the cytoplasm. Small granule haemocytes are large sized ovoid or ellipsoid shaped cells with a relatively high nucleo-cytoplasmic ratio and possessed numerous small sized granules. Large granule haemocytes are comparatively large-size, circular or ovoid shaped haemocytes with a high nucleo-cytoplasmic ratio and contained numerous large round refractile granules and a few small granules. Total haemocyte count of *V. litterata* is noted as 1.021×10^6 to 3.108×10^6 with a mean value of $2.145 \pm 0.84 \times 10^6$ cells ml^{-1}. The relative percentages of hyalinocytes, small granule haemocytes and large granule haemocytes are accounted as 13.93, 55.24 and 30.83% respectively in the haemolymph. Outcome of this study might provide valuable information regarding the haemocyte profile of *V. litterata* that would be helpful to carry out further studies on haemocyte structural types to know about their specific functions and immune mechanisms.
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KEY WORDS: *Varuna litterata*, haemocyte structural types, ultrastructure, flow cytometry, total haemocyte count, differential haemocyte count.

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RESEARCH ARTICLE

Evaluation of the acute toxicity of mahua oil cake aqueous extract and its effect on the behavioral responses of the freshwater grapsid crab, *Varuna litterata* (Fabricius, 1798)

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Abstract

Mortality and behavioral alterations are monitored as the sensitive endpoints in toxicological studies and may be applied as useful biomarkers to assess piscicidal pollution in aquatic environment. Present study assesses acute toxicity of the piscicide, mahua oil cake (MOC), and its effect on the behavioral responses of the freshwater grapsid crab, *Varuna litterata*, under laboratory conditions. To determine the LC50 values, a 4-day acute static renewal toxicity test was done where 10 adult male crabs (mean length 2.870 ± 0.379 cm; mean weight 9.891 ± 3.951 g) were exposed to different concentrations (1, 5, 10, 15, 20, 25, 30, 35 ppt) of MOC aqueous extract with a control at different exposure periods. The LC50 values are 19.109 ppt for 24 h, 16.052 ppt for 48 h, 11.827 ppt for 72 h, and 7.631 ppt for 96 h. The high LC50 values indicate less sensitivity of this crab to the MOC extract than other aquatic animals. MOC extract has toxic effect on the mouthparts activity, whirling motion of water current producing activity, froth releasing activity, aggregation, balance and coordination actions, medium escaping behavior, locomotor activity, and fecal matter excretion of this crab in different exposure periods. Behavioral responses such as froth releasing activity, aggregation, and medium escaping behavior can be used as biomarkers of MOC pollution in aquatic environment.

Keywords Mahua oil cake · Grapsid crab · *Varuna litterata* · LC50 · Behavioral responses · Biomarkers

Introduction

Farmers generally use some plant derivatives, chlorinated hydrocarbons and organophosphates, as fish toxicants to eradicate predatory and weed fishes (Jhingran 1982). Due to persistent toxicity of chlorinated hydrocarbons and organophosphates in fish culture ponds (Choudhuri 1975; Apud et al. 1989), use of plant derivative piscicides is encouraged in the fisheries management (Unnithan 1997). Among the piscicides of plant origin, mahua oil cake (MOC) is most extensively used in the freshwater inland aquaculture systems of India (Lakshman 1983; Das et al. 2018) due to its availability and effectiveness. It is a product from the perennial madhuka (Bassia Koenig ex Linn.) tree species, *Bassia latifolia* Roxb (Syn. *Madhuca latifolia*; *Madhuca indica* J. F. Gmel) belonging to the family Sapotaceae (Bhatia 1970; Lakshman 1983).The cake is used as manure either alone or in mixture with other cakes, fertilizers (Sarkar 1988), non-conventional fish feed ingredient (Rath et al. 2017), and piscicide. The piscicidal property of MOC is attributed to its saponin content (Bhatia 1970). Saponins are a diverse group of low molecular weight secondary plant metabolites and MOC contains 6–8% water-soluble triterpenoid saponin (CIFRI 1985; Unnithan 1997; Vinothkumar et al. 2018). The toxicity of MOC lasts for 15–20 days in water (CIFRI 1968) and subsequently it is transformed into organic manure in the pond. It kills fishes at 200–250 ppm in 6–10 h (CIFRI 1985). Most of the toxicity studies on MOC were performed on freshwater fishes (Homechaudhuri et al. 1986; Homechaudhuri and Banerjee 1991; Unnithan 1997; Chatterjee et al. 2009; Rajput and Gaur 2015; Vinothkumar et al. 2018).

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