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# Toxicity and cytotoxicity of the insecticide imidacloprid in the midgut of the predatory bug, *Podisus nigrispinus*

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ABSTRACT

The selectivity of insecticides on natural enemies in pest control are an important strategy for Integrated Pest Management. However, insecticides can have side effects on non-target organisms such as natural enemies. This study evaluated the histological and cytological changes mediated by the sublethal concentration of the imidacloprid insecticide on the midgut of non-target predator *Podisus nigrispinus* (Heteroptera: Pentatomidae), used in the biological control of pests. Imidacloprid was toxic for *P. nigrispinus* with $LC_{50} = 3.75\ mg\ L^{-1}$ and survival of 51.8%. This sublethal concentration of imidacloprid causes histological alterations in the midgut epithelium and cytotoxic features were irregular border epithelium, cytoplasmic vacuolation, and apocrine secretions in the first 6 h after exposure with the insecticide. Apoptosis in the digestive cells occurs after 12 h of exposure in the midgut. These results suggest that imidacloprid may affect the digestive physiology of *P. nigrispinus* and compromise the effective predation of this insect a biological control agent. The associated use of this insecticide with the predator in pest control should be carefully evaluated.

## 1. Introduction

*Podisus nigrispinus* Dallas (Heteroptera: Pentatomidae) is a predatory bug used as a biological control agent to control of beetles and caterpillar pest in agricultural and forest plantations (Ferreira et al., 2008; Martínez et al., 2016). Regarding *P. nigrispinus*, several studies have detailed the development, histology and ultrastructure (Martínez et al., 2014a, 2016, 2017), predator-prey interaction (Ferreira et al., 2008), and biochemical process (Fialho et al., 2012).

Predators have tolerance relative to insecticides being used in Integrated Pest Management programs (IPM) (Kim et al., 2006; Cordeiro et al., 2010; Zanuncio et al., 2011). Chemical control is the common strategy for pest insects and its use has increased in several crops worldwide (Song and Swinton, 2009; Meissle et al., 2010; Pedlowski et al., 2012); However, the selectivity of insecticides to non-target organisms is important for IPM (Metcalf, 1980; Hardin et al., 1995; Desneux et al., 2007). In this sense, the search for safe insecticides for human health and the environment has resulted in the development of specific compounds for pests and selective to non-target insects (Matsumura, 2004; Nicholson, 2007; Biondi et al., 2012).

Various insecticides cause toxic effects but not exclusively resulting in insect death (Desneux et al., 2007). These effects may be physiological and related to development, longevity, fecundity, and behavior (Desneux et al., 2004; Kim et al., 2006; He et al., 2012). Imidacloprid is used in the control of many insect pests and has moderate toxicity to vertebrates (Horowitz et al., 1998; Boina et al., 2009; Martínez et al., 2014b).

Although the site of action of imidacloprid is the nervous system, other insect organs may be secondary targets (Catae et al., 2014, 2018; Fernandes et al., 2015). Among the non-target organisms of the insecticides, the medium intestine has been reported to be one of the most affected by these chemicals (Gutiérrez et al., 2016; Catae et al., 2018; Fiaz et al., 2018a). The midgut of predatory bugs (Pentatomidae) is anatomically divided into three regions: anterior, middle and posterior,

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which perform different functions in digestion (Fialho et al., 2012, 2013).

Data on the histological and cytological effects caused by the insecticide imidacloprid on the midgut of predatory bugs are scarce. We evaluated the acute toxicity and the histological and cytological changes in the midgut of *P. nigrispinus* mediated by the imidacloprid.

## 2. Materials and methods

### 2.1. Insects

Individuals of *P. nigrispinus* and *Tenebrio molitor* L. (Coleoptera: Tenebrionidae) were obtained from the mass establishment of the laboratory of Biological Control (Universidade Federal de Viçosa, Minas Gerais, Brazil). Adults of *P. nigrispinus* were maintained at 28 ± 2 °C, 80 ± 5% RH, 12:12 h (L: D) photoperiod and fed with *Tenebrio molitor* pupae and *Eucalyptus grandis* leaves. Pupae of *T. molitor* were kept in plastic trays (60 cm long × 40 cm wide × 12 cm high) with a temperature of 25 ± 1 °C, relative humidity of 70 ± 10% and 12:12 h (L: D) photoperiod. Adults of *P. nigrispinus* and *T. molitor* pupae, without apparent amputations or malformations, were used in the bioassays.

### 2.2. Toxicity test

Imidacloprid insecticide (Evidence® WG, Bayer, São Paulo, Brazil) was used in the acute toxicity tests and diluted in 1 L of water to produce stock solution, adjusting 100 g $L^{-1}$ of insecticide to the required concentrations. The insecticidal efficacy was determined by calculating the lethal concentration values ($LC_{25}$, $LC_{50}$, $LC_{75}$ and $LC_{90}$) under laboratory conditions. Six concentrations of imidacloprid in addition to the control (distilled water) were adjusted in 1 mL stock solution (treatments and distilled water): 0.312, 0.625, 0.125, 0.25, 0.5 and 10 mg $L^{-1}$ (w/v). Pupae of *T. molitor* were soaked for 5 s at each concentration and allowed to dry in the environment. In the treatments, *T. molitor* pupae exposed to the insecticide was offered as food for an adult of *P. nigrispinus* in a glass vial (2 × 10 cm). Fifty adults of *P. nigrispinus* were used by concentration and the number of dead insects was counted after feeding with *T. molitor* pupae exposed to the insecticide up to 72 h. The lethal concentrations ($LC_{25}$, $LC_{50}$, $LC_{75}$ and $LC_{90}$) and confidence limits were determined by regression based on the probit-mortality concentration (Finney, 1964) with PROC PROBIT procedure of SAS User v. 9.0 for Windows (SAS Institute, 2002).

### 2.3. Survivorship

Newly emerged adults of *P. nigrispinus* were fed with *T. molitor* pupae exposed to four concentrations of imidacloprid ($LC_{25}$, $LC_{50}$, $LC_{75}$ and $LC_{90}$) as determined by the toxicity bioassay and control with distilled water. In survival test, insecticide exposure procedures were similar as described for the toxicity bioassays. Dead insects were quantified every six hours by 72 h. The data were submitted to survival analysis using the Kaplan-Meier estimator (Log-rank method) via the Origin Pro v 9.1 software (Originlab Corporation, 2013). Survival adults registered until the end of the experiment were treated as censored data.

### 2.4. Light microscopy

Adults of *P. nigrispinus* were exposed to the estimated lethal concentration $LC_{50}$ of imidacloprid at different time periods (30 min, 1, 3 and 6 h) and cryoanesthetized at − 4 °C. The midgut was dissected in insect saline solution (0.1 M NaCl + 0.1 M $KH_2PO_4$ + 0.1 M $Na_2HPO_4$), divided into the anterior, middle and posterior regions, and transferred to Zamboni's fixative solution (Stefanini et al., 1967) for 12 h at 5 °C. The samples were then dehydrated in a grade ethanol series (70°, 80°, 90° and 95°) and embedded in historesin (Leica Biosystem Nussloch GmbH, Wetzlar, Germany). Sections 3 μm thick were obtained, stained with hematoxylin and eosin, and analyzed under an Olympus BX-60 light microscope (Olympus Corporation, Tokyo, Japan).

### 2.5. Transmission electron microscopy

Adults of *P. nigrispinus* were exposed to the estimated lethal concentration $LC_{50}$ of imidacloprid for 6 h and cryosanesthetized at − 4 °C. The midgut of *P. nigrispinus* (divided in anterior, middle and posterior region) were dissected and transferred to 2.5% glutaraldehyde in 0.2 M sodium cacodylate buffer (pH 7.2) containing 0.2 M of sucrose for 6 h at room temperature. The samples were then post-fixed in 1% osmium tetroxide in the same buffer for 2 h, followed by washing in the buffer and dehydrating in a grade ethanol series (70°, 80°, 90° and 99°). The samples were embedded in LR white resin (London Resin Company Ltd.) and ultra-thin sections (80–90 nm thick), obtained with PowerTomes PT-X ultramicrotome glass razor (RMC Boeckeler Instruments Inc., Tucson, AZ, USA), were compared with 1% aqueous uranyl acetate and lead citrate (Reynolds, 1963) and examined on Zeiss EM 109 transmission electron microscope (Carl Zeiss, Jena, Germany).

### 2.6. Immunofluorescence

The three regions of the midgut of *P. nigrispinus* exposed by 12, 24 and 36 h to estimated lethal concentration $LC_{50}$ of imidacloprid were dissected in 0.1 M sodium phosphate buffer (PBS) and transferred to Zamboni's fixative solution for 2 h. Next, the samples were washed with PBS containing 1% Triton X-100 (PBST) and incubated with 1.5% bovine serum albumin in PBST for 2 h. The samples were incubated with anti-cleaved caspase 3 antibody (Cell Signaling Technology, Danvers, MA, USA) at 1:500 in PBS for three days at − 4 °C. After incubation, the samples were washed in PBS and incubated with anti-rabbit IgG secondary antibody, fluorescein isotiosinate (FITC) conjugated (Sigma-Aldrich, St. Louis, MO, USA) diluted 1:500 in PBS for 24 h in the dark at − 4 °C. Midgut or *P. nigrispinus* were washed, and the cell nuclei were stained with TO-PRO-3 propidium iodide (Life Technologies, Carlsbad, CA, USA) for 1 h. Midgut were mounted on 50% sucrose glass slides and examined on Zeiss LSM510 META (Carl Zeiss, Jena, Germany) laser scanning confocal microscope.

## 3. Results

### 3.1. Toxicity and survivorship

The lethal concentrations of imidacloprid estimated by Probit ($X^2$ = 21.43; df = 5; P < 0.001) for *P. nigrispinus* fed with *T. molitor* pupae exposed to the insecticide were $LC_{25}$ = 2.90, $LC_{50}$ = 3.75, $LC_{75}$ = 4.85 and $LC_{90}$ = 6.27 mg $L^{-1}$. (Table 1, Fig. 1A). Mortality at the control was < 1%.

The survivorship of *P. nigrispinus* fed with *T. molitor* pupae exposed to imidacloprid showed differences between the concentrations (Log-

**Table 1**
Lethal concentration of the insecticide imidacloprid in *Podisus nigrispinus* for 72 h after feeding with pupae of *Tenebrio molitor*. Insecticide concentrations were applied using a topical solution on the prey. $X^2$, chi-square for lethal concentration and fiducial limits based on a logarithmic scale with significance level at $P < 0.0001$.

| Concentration (df = 5) | Estimated values (mg $L^{-1}$) | Fiducial limits | | $X^2$ |
|---|---|---|---|---|
| | | Inferior | Superior | |
| $LC_{25}$ | 2.90 | 2.02 | 3.45 | 21.43 |
| $LC_{50}$ | 3.75 | 3.05 | 4.41 | |
| $LC_{75}$ | 4.85 | 4.14 | 6.27 | |
| $LC_{90}$ | 6.27 | 5.16 | 9.69 | |