Cellular responses in the Malpighian tubules of *Scaptotrigona postica* (Latreille, 1807) exposed to low doses of fipronil and boric acid.

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- The meliponines are social bees characterized by atrophied sting.
- They are typically found in tropical and subtropical regions of the planet.
The specie *Scaptorigona postica* (Latreille, 1807) is recognized as pollinators of some crops such as melon, onion, sunflower and coffee.
S. postica (non-target insect) can be exposed to pesticides used for pest control.

Intensive agriculture (agrochemical in crops)

Reduction of natural habitat

Occupation of the green areas in urban regions

Introduction
Insecticides

- Fipronil ($C_{12}H_4Cl_2F_6N_4OS$)
  - Act in the receptor of gamma aminobutyric acid (GABA)

- Boric Acid ($H_3BO_3$)
  - Acts on insect metabolism
Excretory system

- Malpighian Tubules

Exposure to pesticides:

- Increasing of excretion rate;
- Side-effects in excretory cells.
Introduction and others proteins

BIOMARKERS express an EXPOSURE to a disturbed environment

- behaviour
- flight
- induction of detoxifying systems

(b)

Persistent stress
Failure in detoxification

- deficiency in detoxifying systems
- cholinesterase inhibition
- Cell damage
- Physiological changes

Some BIOMARKERS express TOXICITY
AIM:

Detect cellular responses in the Malpighian tubules that indicate toxicity and/or adaptation mechanisms to stress induced by exposure of *S. postica* workers to low doses of fipronil and boric acid.
- Preparation of the contaminated diet:
  - Fipronil

- Daily intake 0.1 ng/fipronil/bee/day

0.1 µg fipronil/Kg of food

- Fipronil
- Acetone
- Preparation of the contaminated diet:
  - Boric Acid

Boric Acid

Acetone

Cândi + Boric Acid

0.75% w/w acid boric in food

- Daily intake 0.75 μg/fipronil/bee/day
- Toxicological bioassays

Newly emerged bee (4 days old):
- Control Group;
- Solvent Control Group;
- Fipronil-treated group;
- Boric acid-treated group.
Material and Methods

Fixed Malpighian tubules
- Morphological and histochemical analysis;
- Ultrastructural analysis;
- Immunohistochemical detection of DNA fragmentation and the HSP 70 stress protein (70-kDa Heat shock protein).
- Bioassays

LT 50 was determined using log-rank test, Graph-Pad Prism 3.0 software.
- **Histological sections of the median Malpighian tubules of *S. postica***. Comparison of histological morphology of the Malpighian tubules stained with hematoxylin/eosin (A-C). **(A)** control group; **(B)** treated continuously with 0.75% boric acid; **(C)** treated with 0.1 ng / bee fipronil.

Brush border (dashed arrow) - evidence of removal of cellular content (*) to the lumen (L); compacted nuclei (arrowhead). Bars: 10 μm
Histological sections of the median Malpighian tubules of *S. postica*. A-B: Comparison of Malpighian tubules stained with the Feulgen reaction of bees in the control group (A) and bees treated with 0.1 ng / bee fipronil (B). Compacted nuclei are observed (arrowhead) and lumen (L). Bars: 10 μm.

Results

Histological sections stained with Bromophenol Blue. Morphological comparison between the Malpighian tubules of bees in the control group (A); chronically treated with boric acid 0.75% (B) and treated with 0.1 ng/bee fipronil (C). Bar: 10 μm

Brush border (dashed arrow); vacuolation (arrow) and nuclei (n) with nucleoli (nu).
(A-C) Histological sections of Malpighian tubules subjected to immunolocalization of cellular stress protein, using the HSP70 monoclonal antibody (Sigma) and secondary antibody conjugated with alkaline phosphatase in the control group (C); treated continuously with 0.75% boric acid (D); bee treated with 0.1 ng/fipronil (E). Arrow indicates strong positive reaction.

Boric acid – complexes with functional organic groups in cells.

INITIAL CYTOTOXIC EVENT

Triggers secondary response = HSP70 (Buffer system)
Electron transmission of the Malpighian tubules of *S. postica*. 
A, D) Control group; B, E) Fipronil-treated bees; C, F) Boric acid-treated bees.

Microvilli (mv), mitochondria (mi) in higher concentration in the apical portion (arrow); nuclei (n); nucleoli (nu). Vacuoles/granules (dashed arrow) with concentric rings (c) in the cytoplasm. Magnification: 600x.
(F) Detail of nuclei with chromatin compaction. Magnification: 2.500x
Electron transmission of the Malpighian tubules of *S. postica*.  

**G, I)** Control group; **H, K)** Fipronil-treated bees; **I, L)** Boric acid-treated bees.

Apical portion of the Malpighian tubules showing mitochondria (Mi) associated to microvilli (mv). Note the apical microvilli with dilated vesicles of secretion (vs). Magnification: 10.500x

Basal portion of cells showing invagination depth of the basal labyrinth (lb) and vacuoles (vp) with concretions. Magnification: 10.500x
Electron transmission of the Malpighian tubules of *S. postica*.  
*M, P* Control group; *N, Q* Fipronil-treated bees; *O, R* Boric acid-treated bees.

Detail of mitochondria (Mi), which are more electron dense in bee treated with the insecticides.

Detail of rough endoplasmic reticulum (rer), with apparent release of ribosomes (arrow). Magnification: 21.500x
Summary and Conclusion

- Increased expression of HSP70 in boric acid-treated group played a role of cell protection;
- Fipronil exposure didn`t induce the HSP70 buffer system;
- In both insecticide-treated groups some cells undergoing programmed cell death, since no typical features of necrosis were observed;
- Most of cells remained metabolically active:
  - In the excretion process (presence of dilated apical microvilli)
  - And inactivation process of chemical compounds (large increase in the amount and degree of organization of mineralized granules).
In addition to toxicity tests, morphological and histochemical methods are important tools to understand sublethal effects of low doses of pesticides on bees;

The data from this research about side-effects in low doses of pesticides on bees could be used in the risk assessment of stingless bee *S. postica* foraging in the vicinity of cultivated fields and/or in green urban areas.
THANKS!

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