

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

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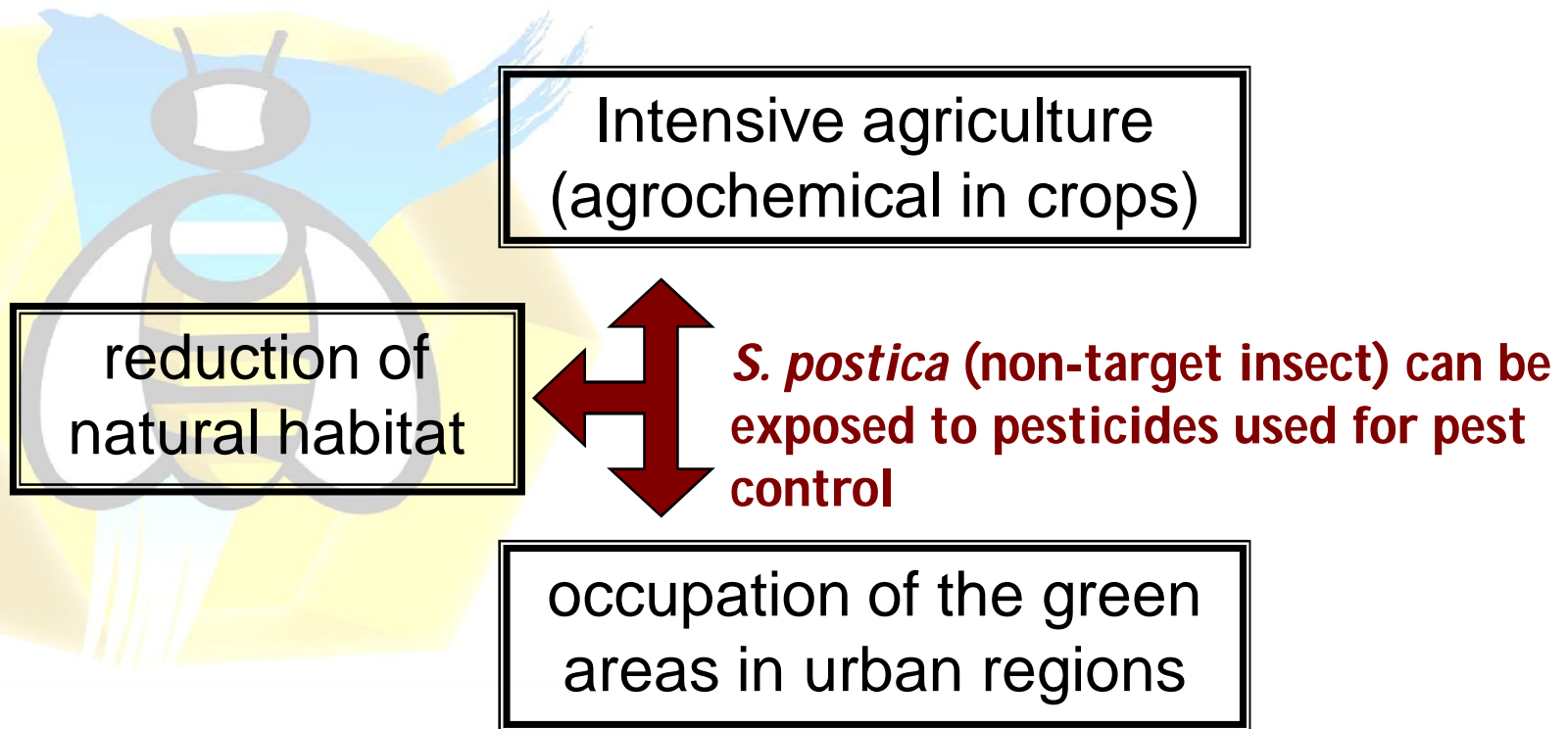
# Introduction

- 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.







## Insecticides

- Fipronil ( $\text{C}_{12}\text{H}_4\text{Cl}_2\text{F}_6\text{N}_4\text{OS}$ )

- Act in the receptor of gamma aminobutyric acid (GABA)

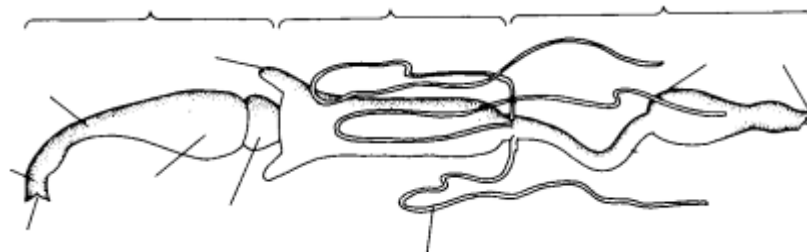
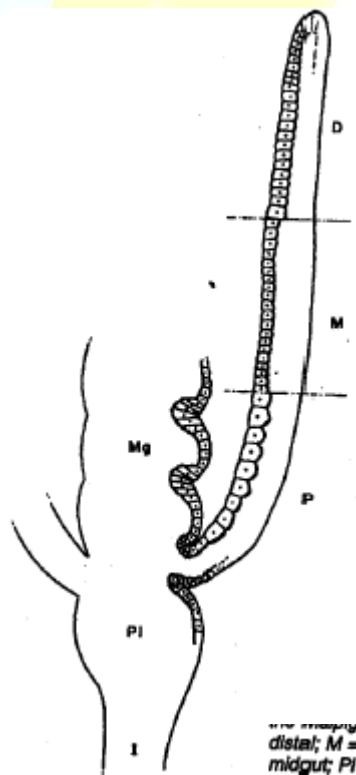
- Boric Acid ( $\text{H}_3\text{BO}_3$ )

- Acts on insect metabolism



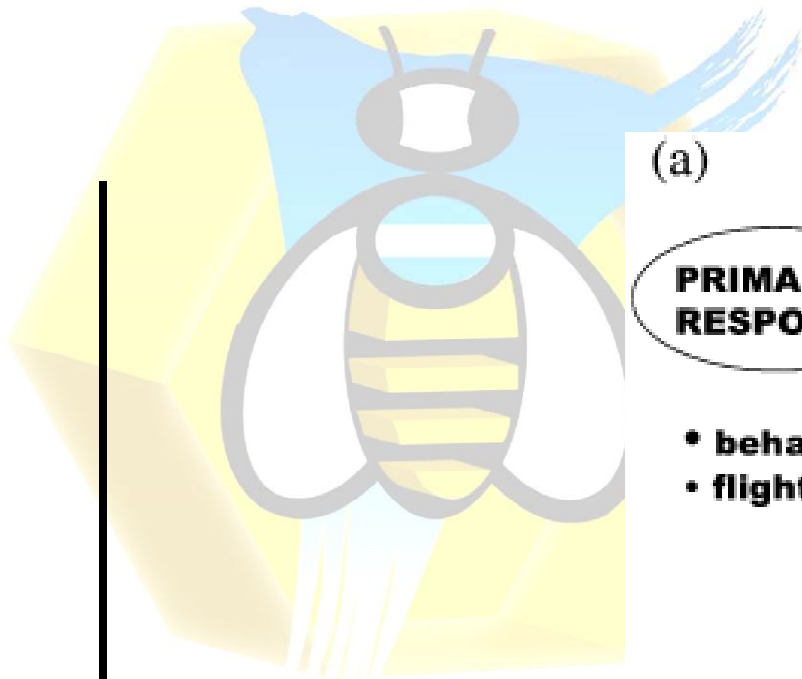
## Excretory system

### - Malpighian Tubules



Exposure to pesticides:

- Increasing of excretion rate;
- Side-effects in excretory cells.



(a)

**PRIMARY  
RESPONSE**

- behaviour
- flight

**ADAPTATION**

- induction of detoxifying systems and others proteins

**COMPENSATED  
LESIONS**

**BIOMARKERS express an EXPOSURE  
to a disturbed environment**

(b)

**PRIMARY  
RESPONSE**

- deficiency in detoxifying systems
- cholinesterase inhibition

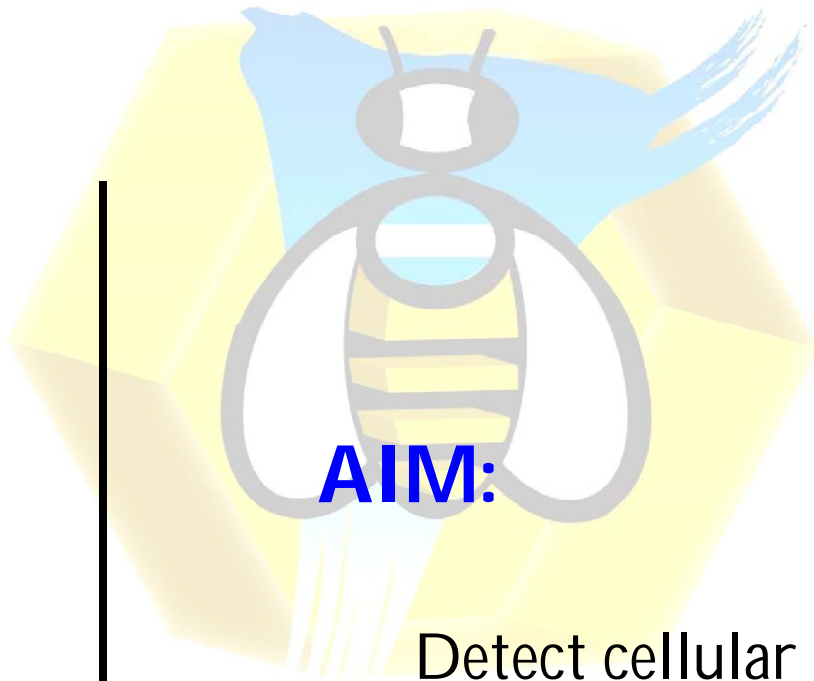
**limited  
ADAPTATION**

**Persistent stress  
Failure in detoxification**

**NON  
COMPENSATED  
LESIONS**

- Cell damage
- Physiological changes

**Some BIOMARKERS express TOXICITY**

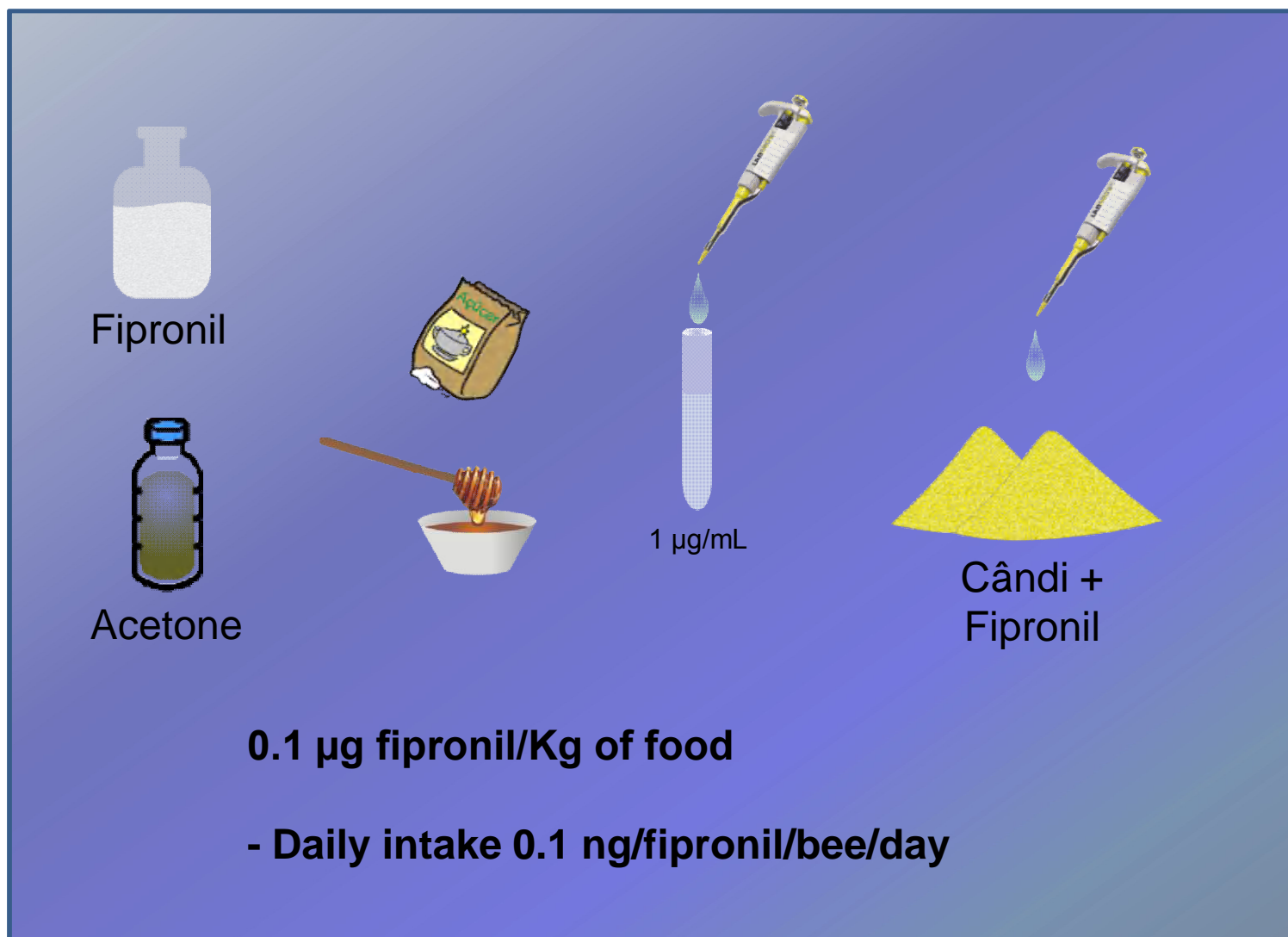


## AIM:

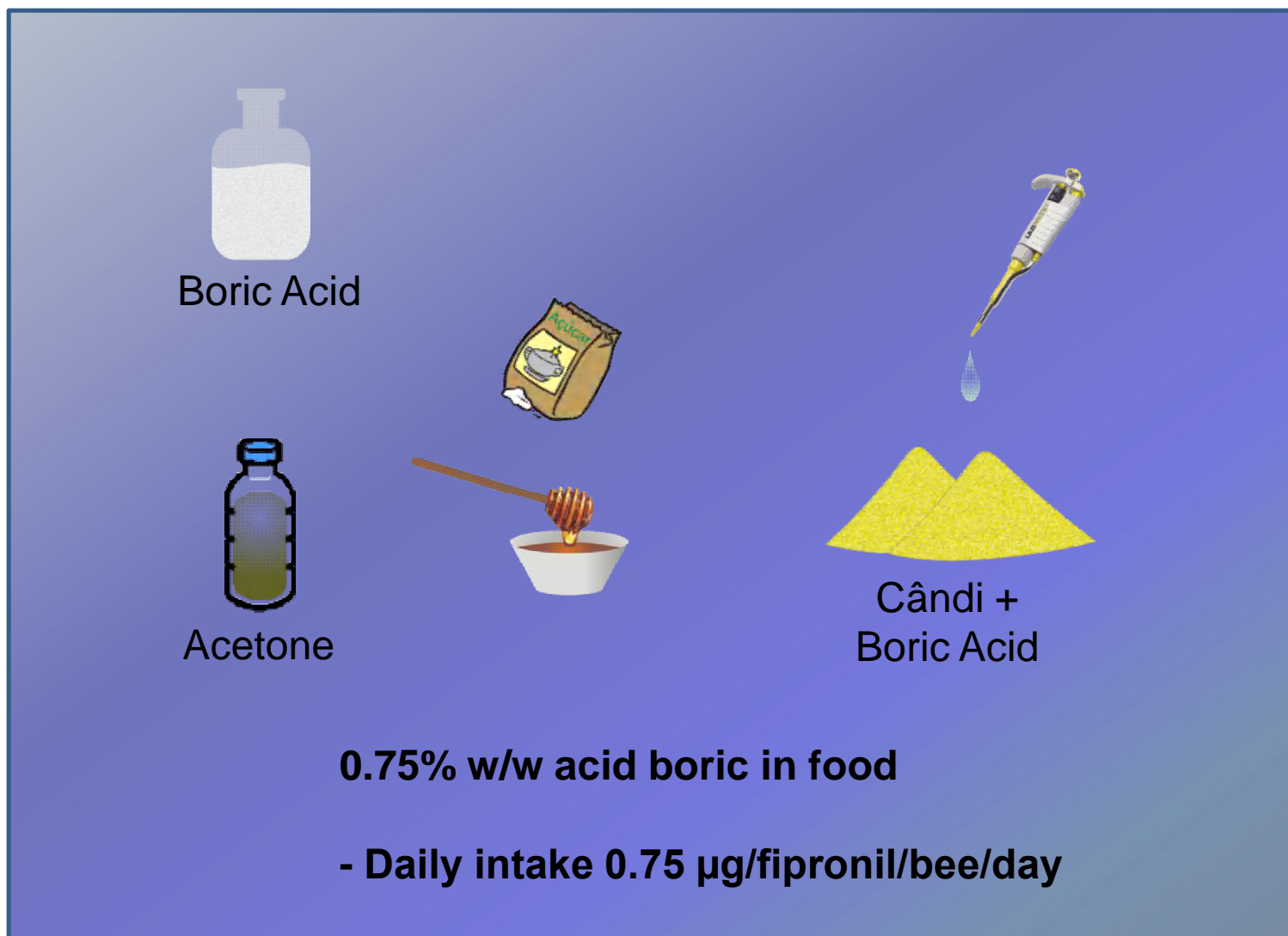
Detect cellular responses in the Malpighian tubules that indicate toxicity and/or adaptation mechanisms to stress induced by exposure of *S. postica* workers to low doses of fipronil and boric acid.



- Preparation of the contaminated diet:
  - Fipronil



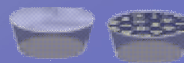
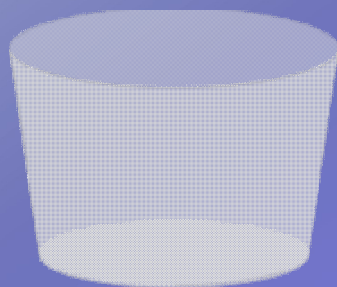
- Preparation of the contaminated diet:
  - Boric Acid



## - Toxicological bioassays



CENTRO DE ESTUDOS DE INSETOS SOCIAIS



Newly emerged bee (4 days old):

- Control Group;
- Solvent Control Group;
- Fipronil-treated group;
- Boric acid-treated group.



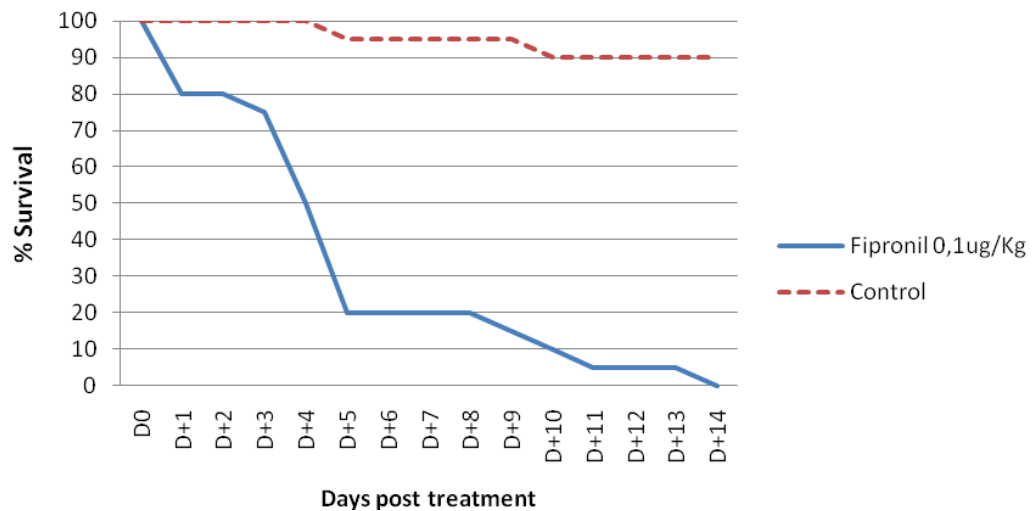
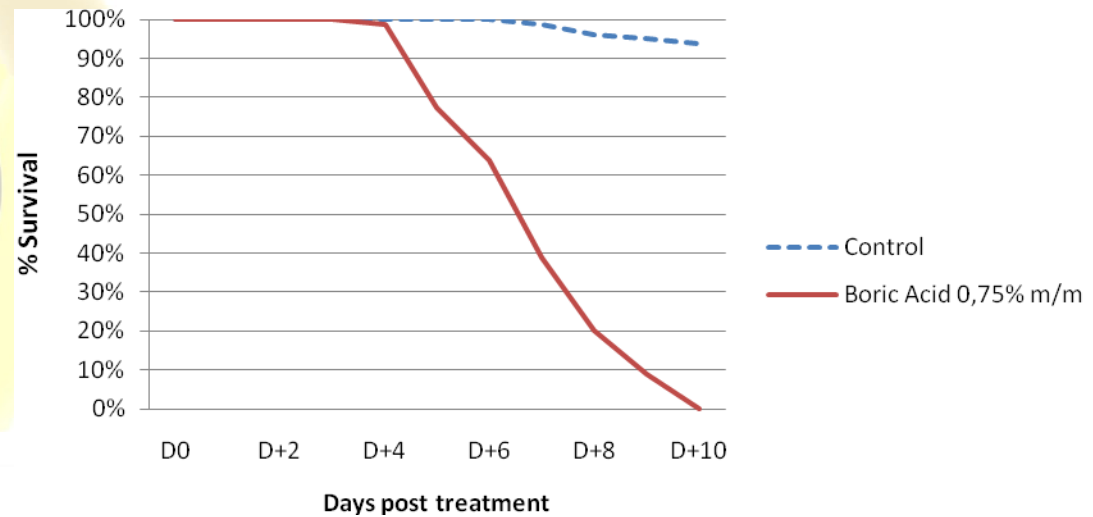


## Fixed Malpighian tubules

- Morphological and histochemical analysis;
- Ultrastructural analysis;
- Immunohistochemical detection of DNA fragmentation and the HSP70 stress protein (70-kDa Heat shock protein).

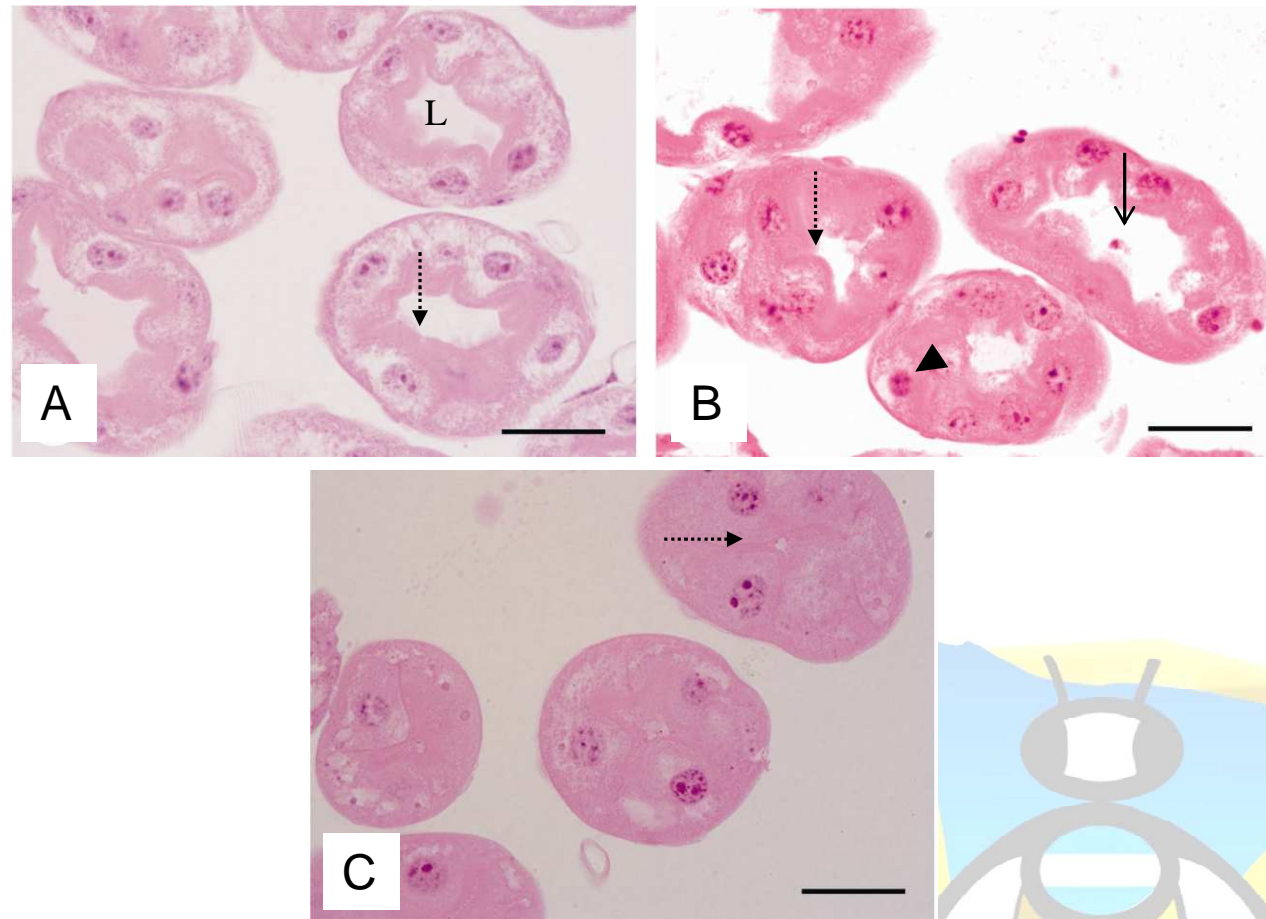
# Results

## - Bioassays



LT50 was determined using log-rank test, Graph-Pad Prism 3.0 software

# Results

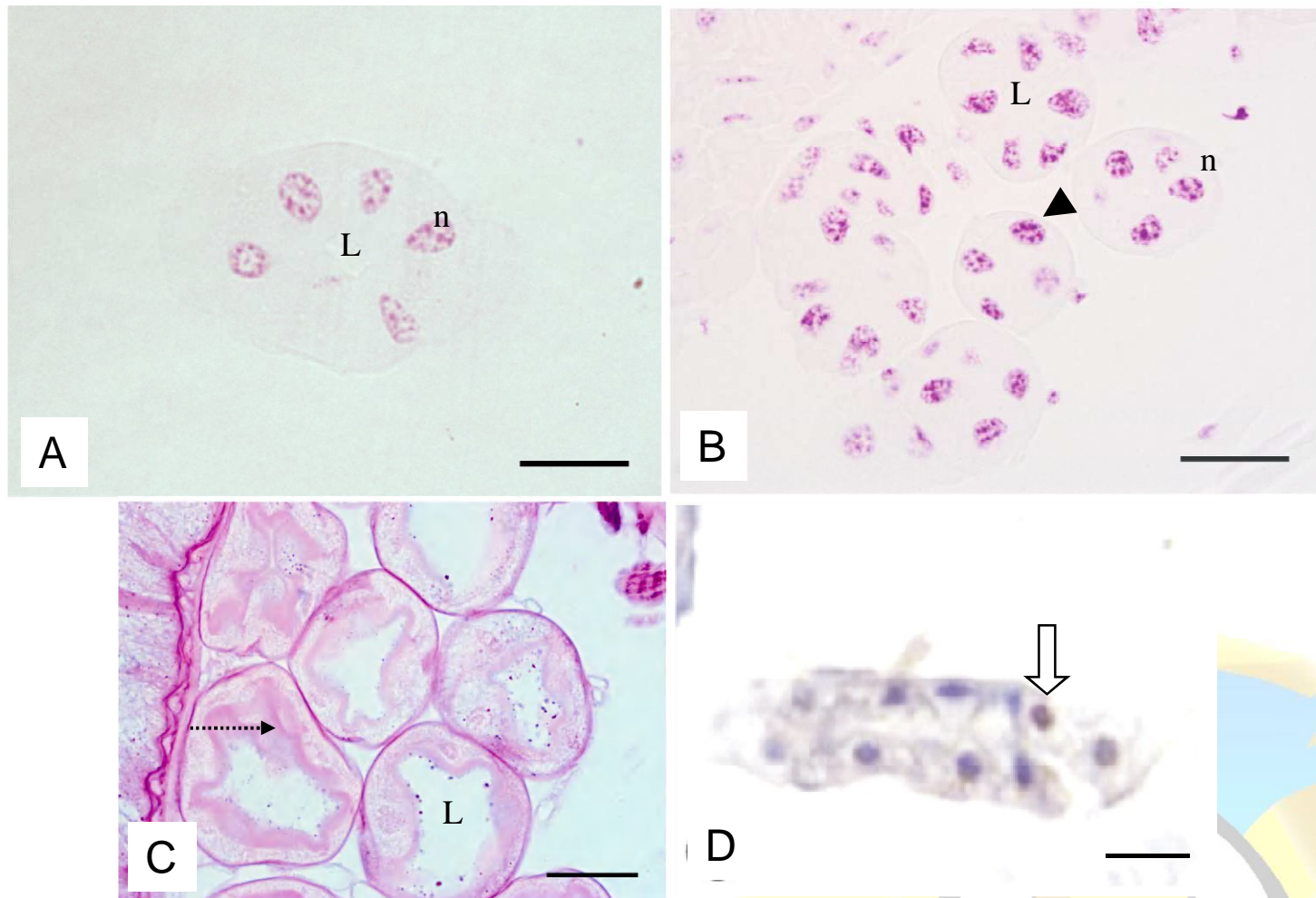


- **Histological sections of the median Malpighian tubules of *S. postica*.** Comparison of histological morphology of the Malpighian tubules stained with hematoxylin/eosin (A-C). **(A)** control group; **(B)** treated continuously with 0.75% boric acid; **(C)** treated with 0.1 ng / bee fipronil.

Brush border (dashed arrow) - evidence of removal of cellular content (\*) to the lumen (L); compacted nuclei (arrowhead). Bars: 10 μm



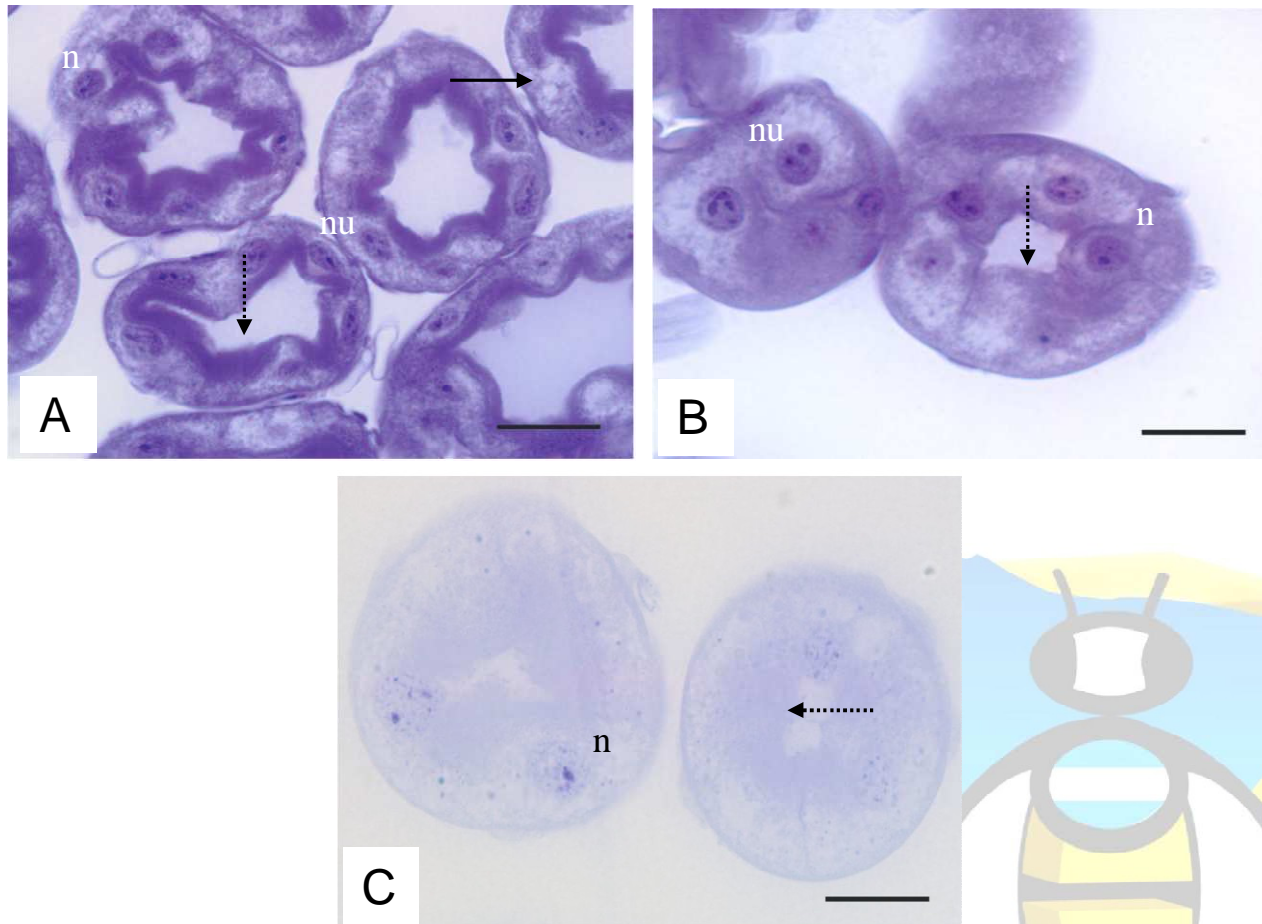
# Results



**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  $\mu$ m.

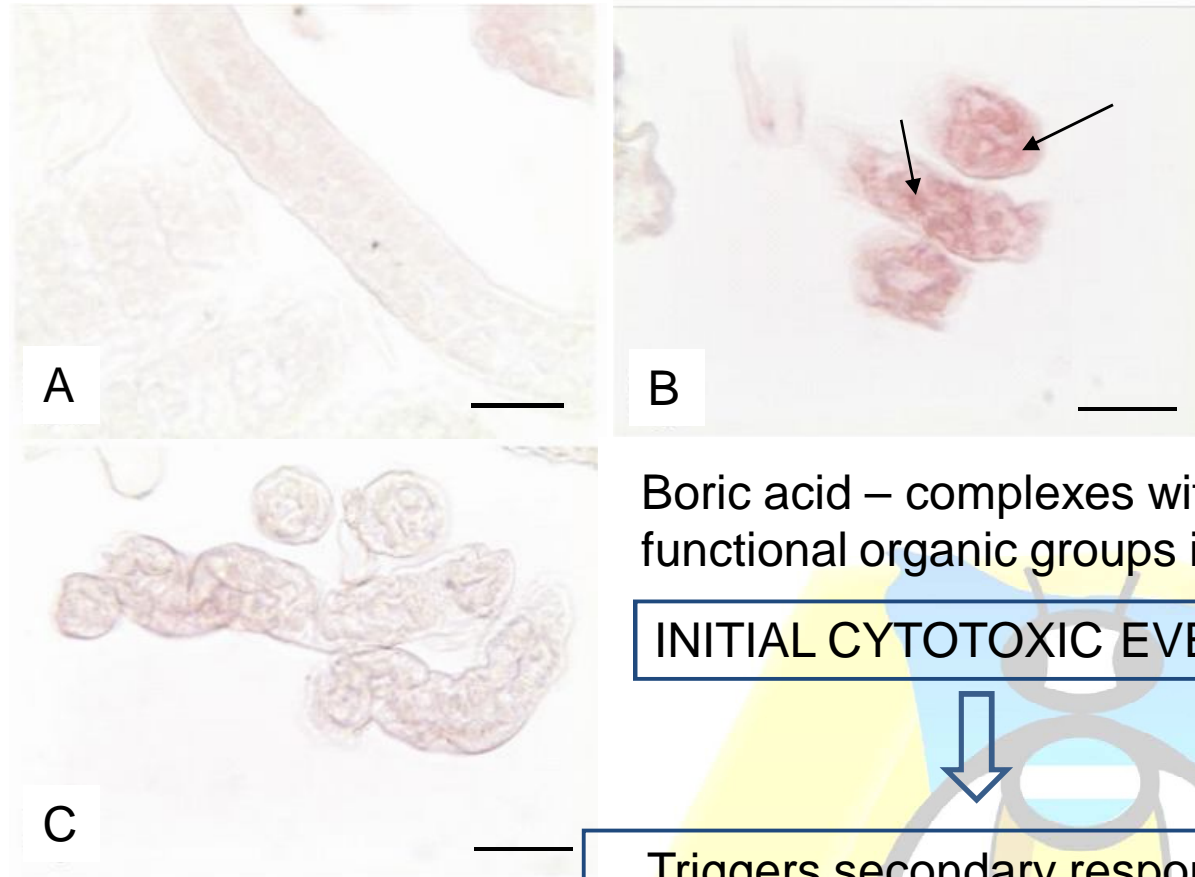
C) Histochemical test: PAS-Alcian Blue in Malpighian tubules of *S. postica*. Brush border (dashed arrow), lumen (L). Bar: 10  $\mu$ m. D) Immunodetection of DNA fragmentation, indicative of cell death - ISCDDK kit in boric acid-treated group.

# 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  $\mu$ m  
Brush border (dashed arrow); vacuolation (arrow) and nuclei (n) with nucleoli (nu).

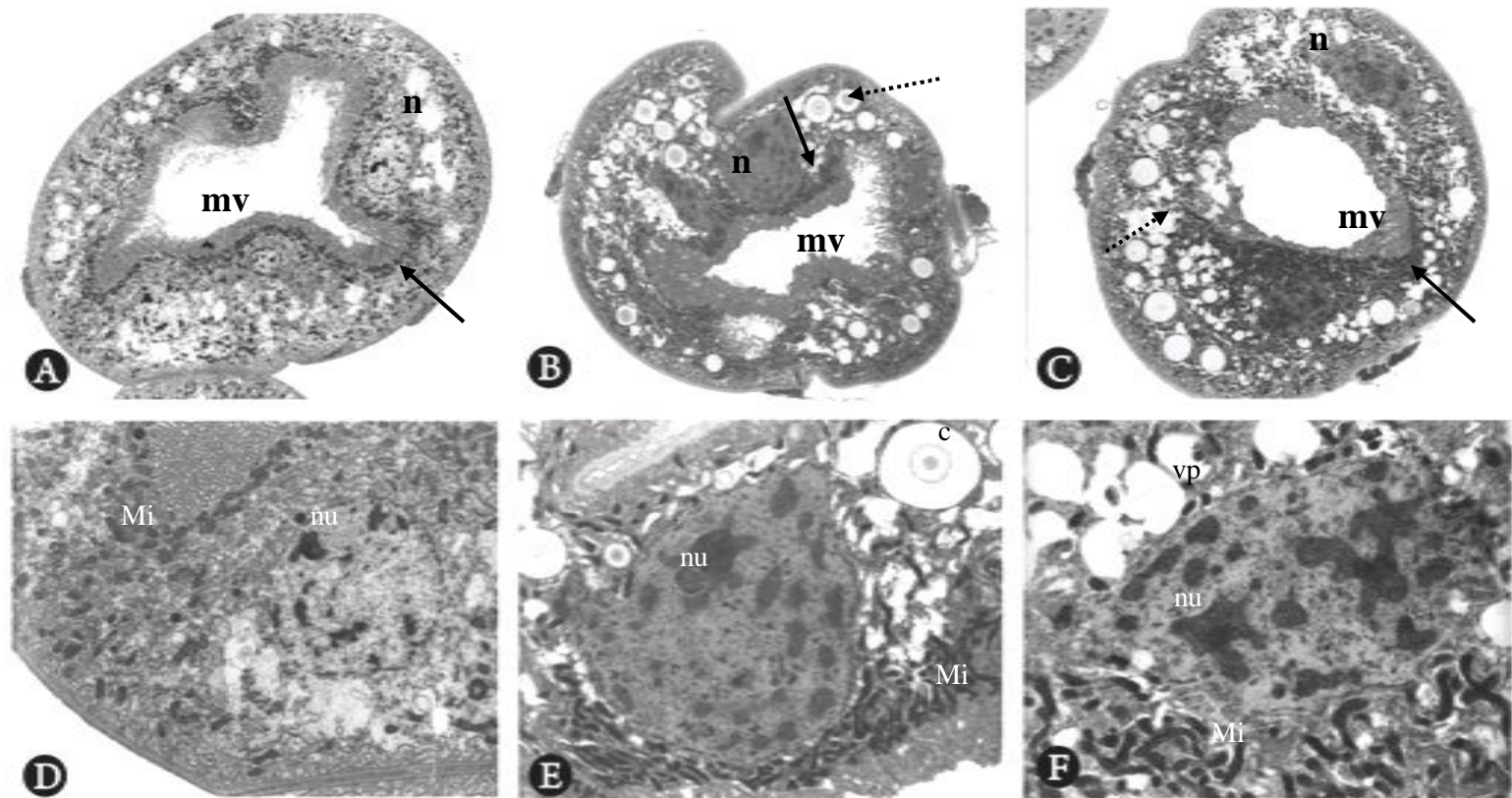
# Results



**(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.



# Results



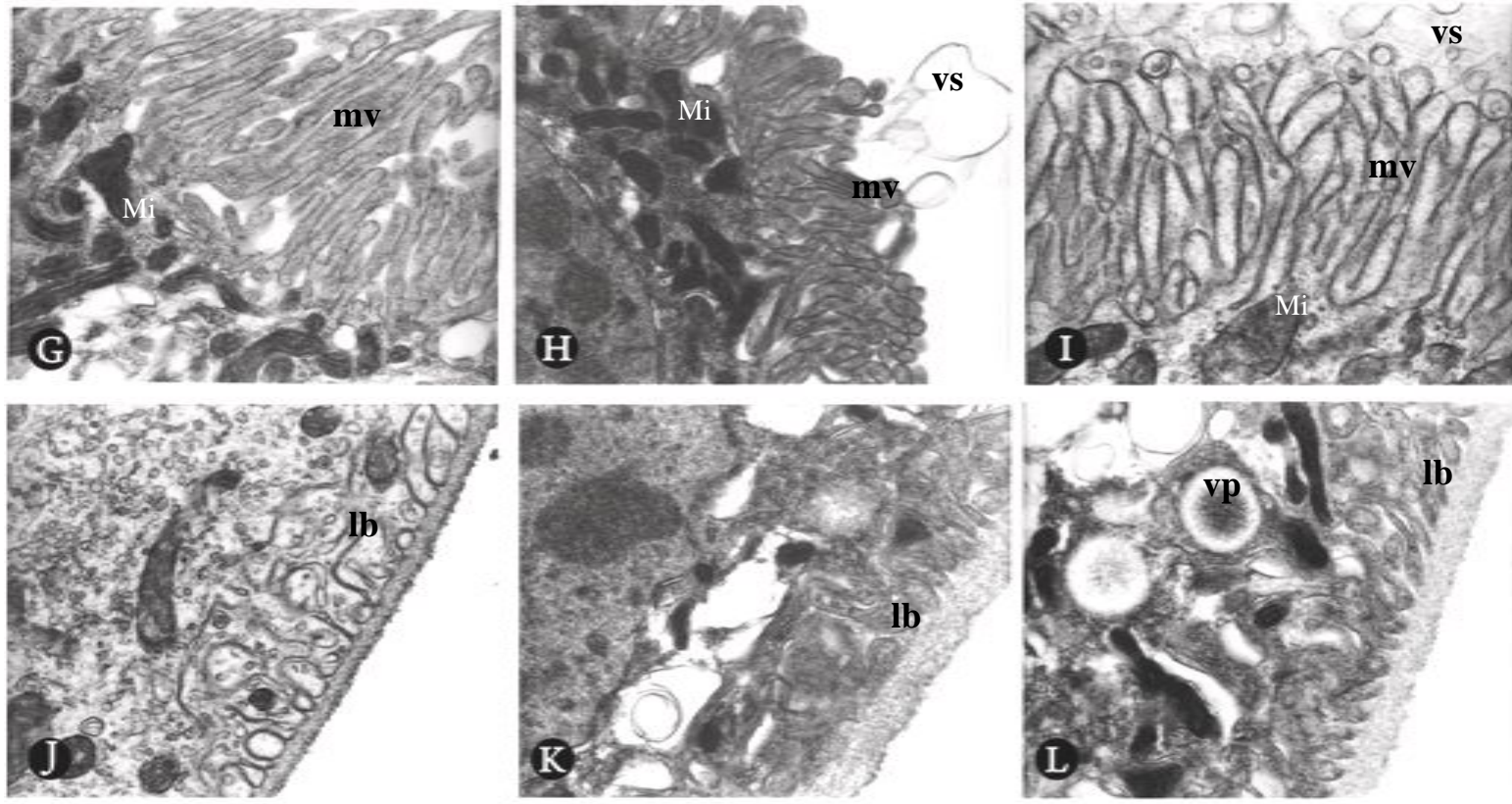
Electron transmission of the Malpighian tubules of *S. postica*.

**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

# Results



Electron transmission of the Malpighian tubules of *S. postica*.

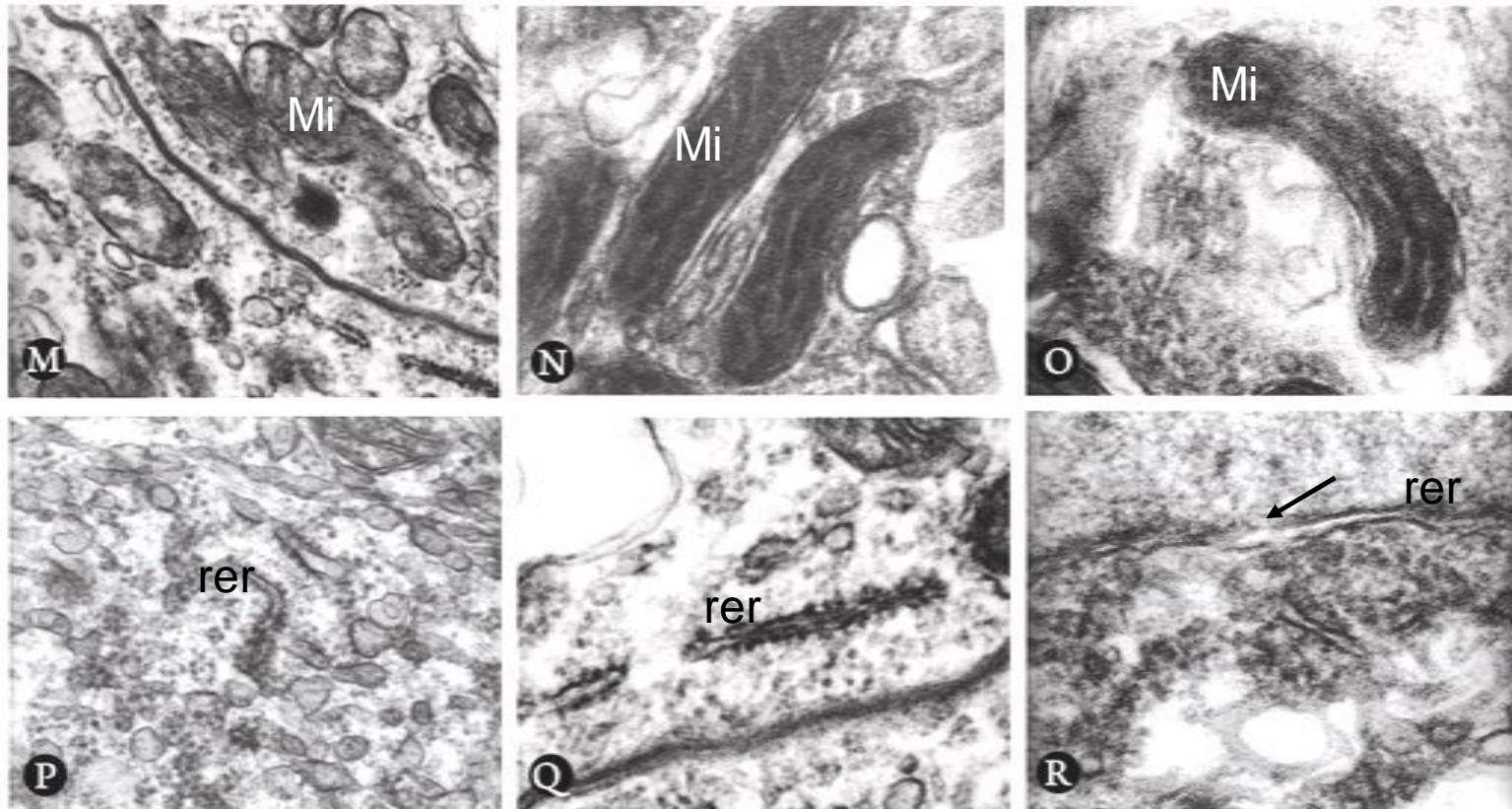
**G, I)** Control group; **H, K)** Fipronil-treated bees; **I, L)** Boric acid-treated bees.

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



# Results



Electron transmission of the Malpighian tubules of *S. postica*.

**M, P) Control group; N, Q) Fipronil-treated bees; O, R) Boric acid-treated bees.**

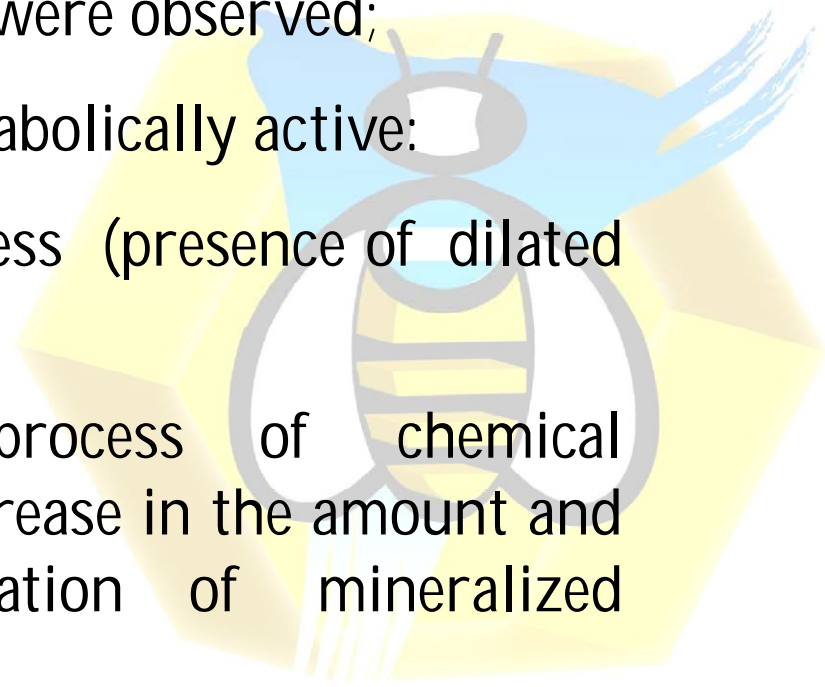
Detail of mitochondria (Mi), which are more electron dense in bee treated with the insecticides.

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



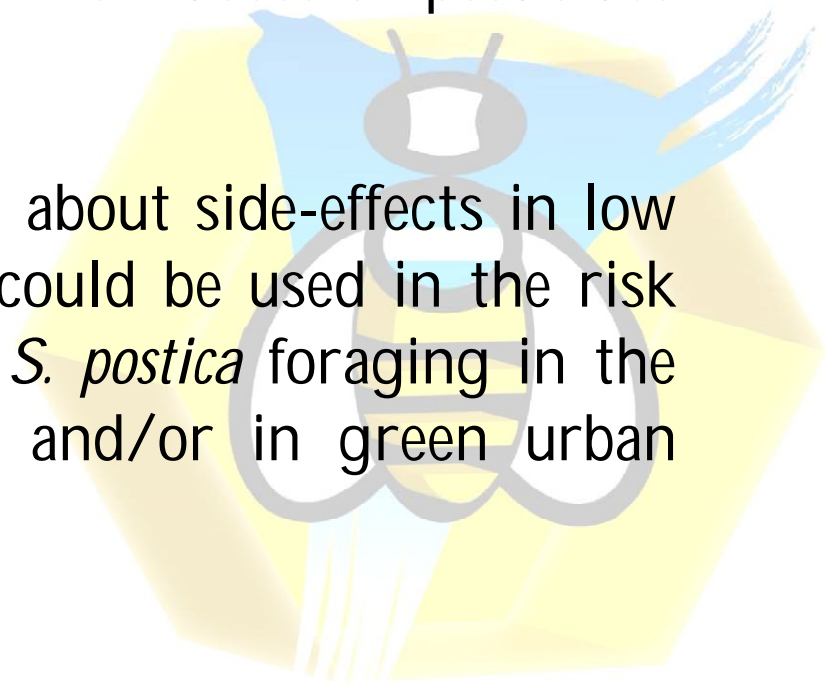
# Summary and Conclusion

- 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).



# Considerations

- 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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