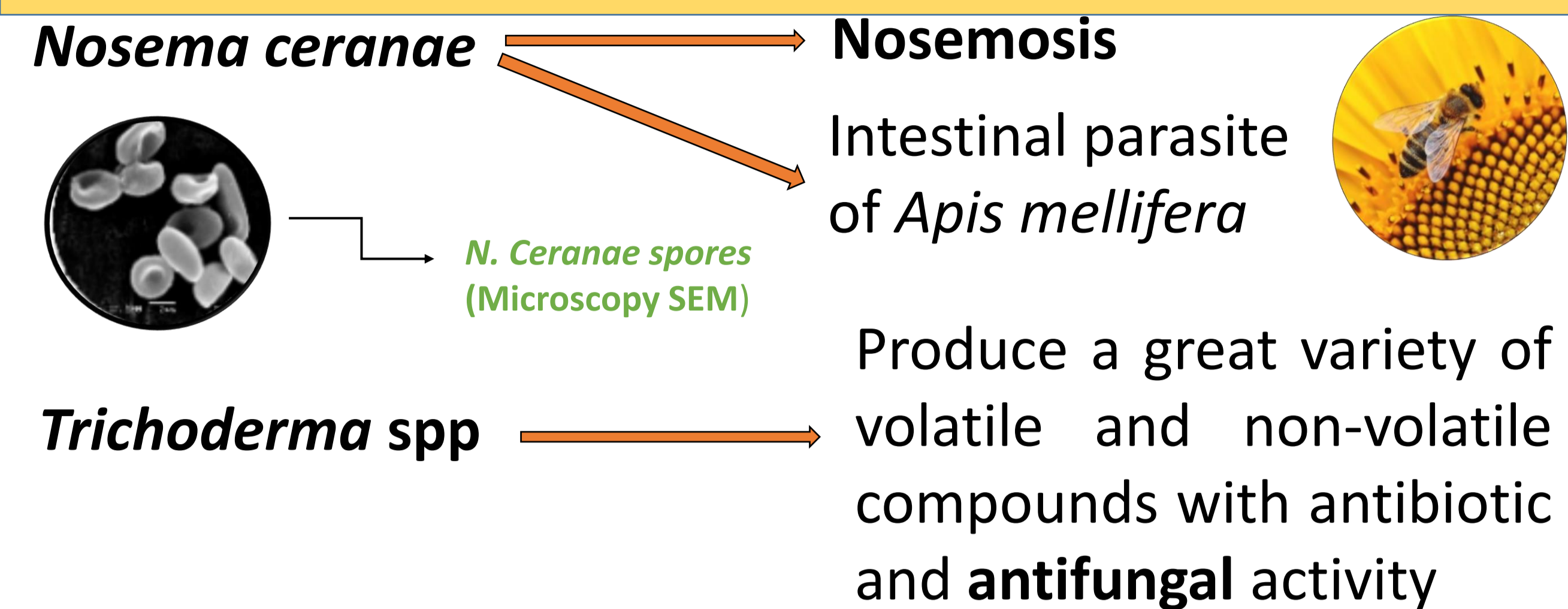


Isolation of fungal metabolites as a therapeutical alternative to control *Nosema ceranae* (Microspora, Nosematidae) in *Apis mellifera* (Hymenoptera, Apidae)

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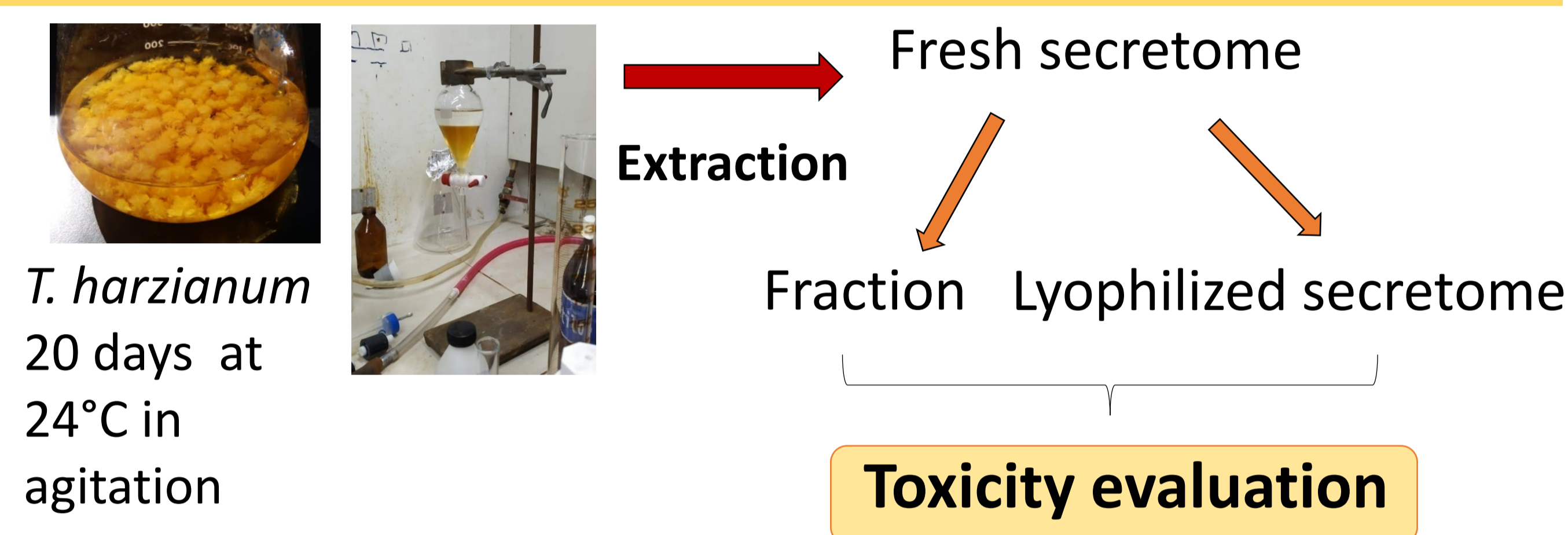
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Objective
 To investigate, under laboratory conditions, the possible antiparasitic effect of the metabolites extracted from one fungal specie of the genus *Trichoderma*, to control the disease called nosemosis, which seriously affects bee colonies worldwide.

Methodology

Isolation and extraction of metabolites



Results

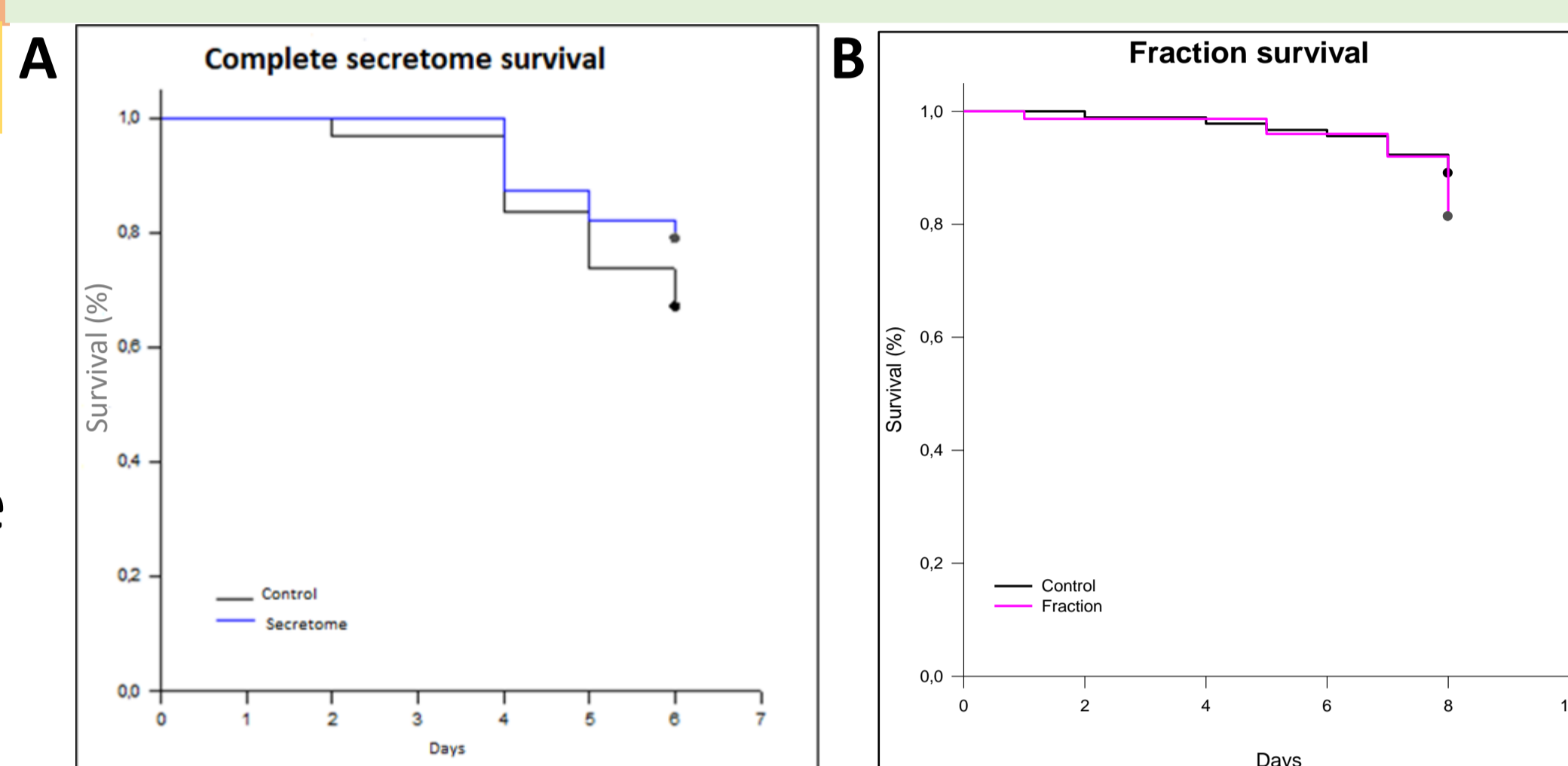


Figure 1. Survival curves of bees administered with A) the secretome or B) the isolated fraction of *Trichoderma harzianum*

No significant effects were observed on bees survival under under secretome or fraction treatment.

- Secretome (Log-Rank and Kaplan-Meier; Z = 3.339; p = 0.068).
- Fraction (Log-Rank and Kaplan-Meier; Z = 1.748; p = 0.186)

Bioactivity

Nosema ceranae spore solution was placed in direct contact with the fungal secretome and the fraction for 24 h and was observed by fluorescence microscopy with probes that hybridize the DNA.

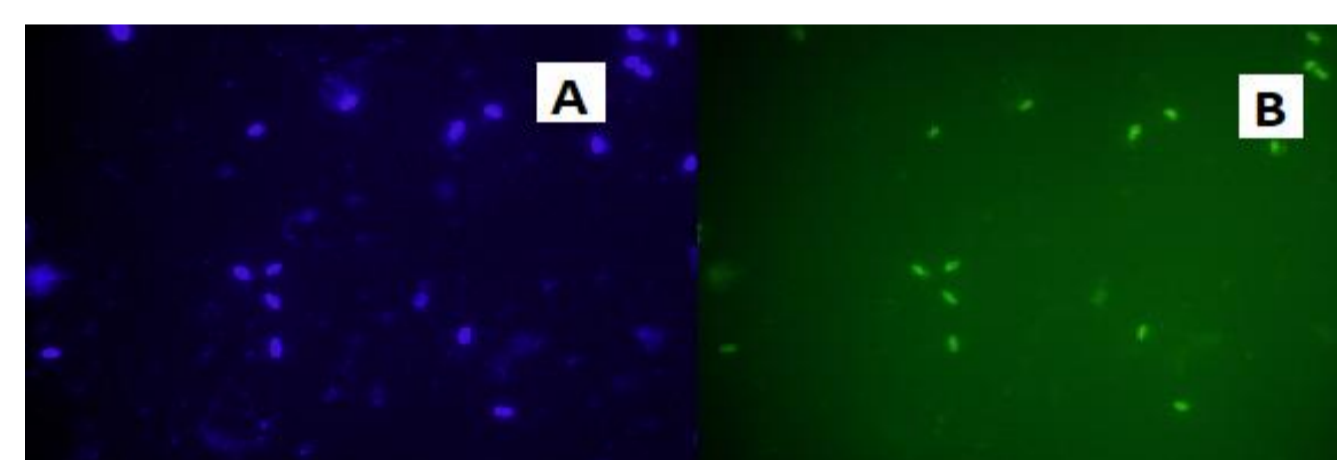
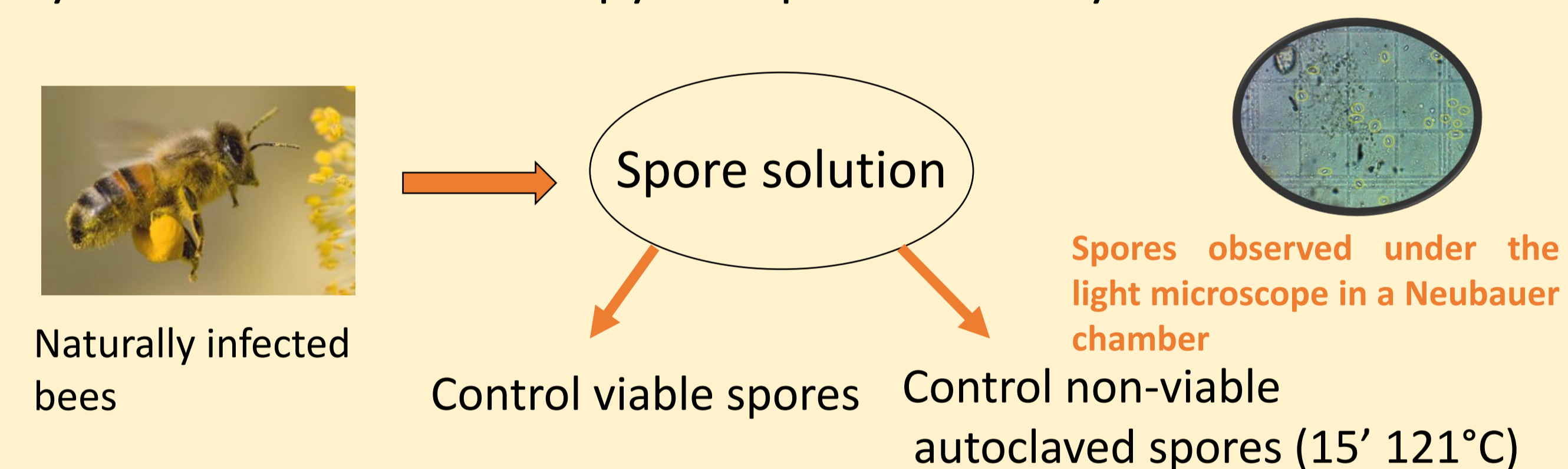
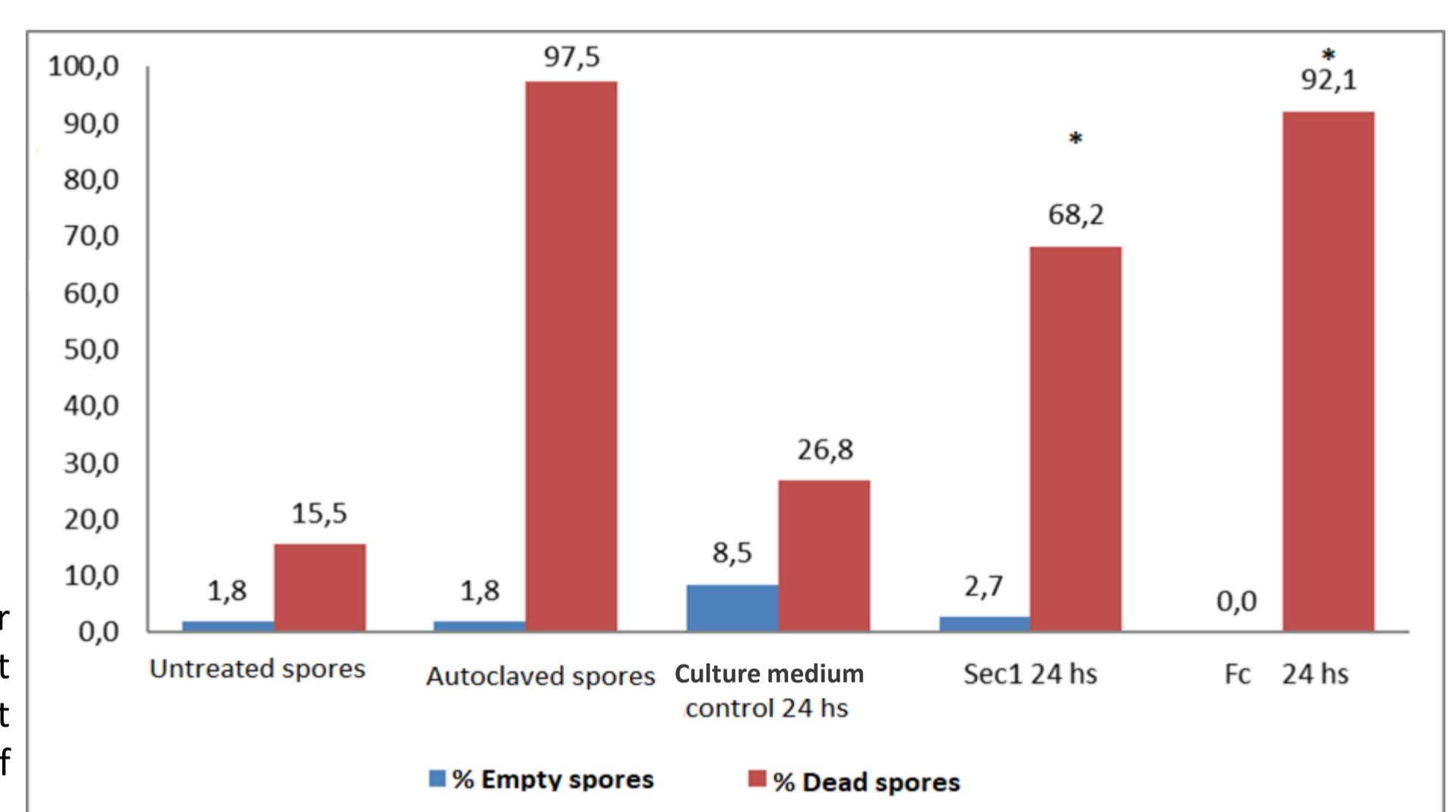


Figure 2. Autoclaved spores observed under a fluorescence microscope. Image of one field A) observed with the 395 to 415 nm filter to visualize the DAPI probe (100X) B) observed with the 470 to 490 nm filter to visualize the Sytox probe (100X).

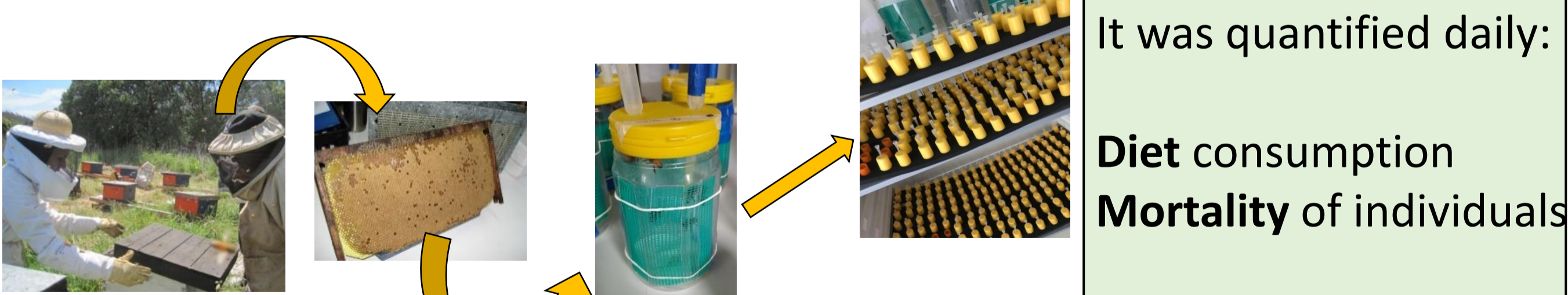
Membrane integrity of *N. ceranae* spores exposed to the complete secretome and the fraction of *Trichoderma harzianum*



Spore viability: Secretome ↓ 68.2%; fraction ↓ 92, 1% (H = 76.710; p < 0.001)

Antiparasitic activity

Worker bees were inoculated with *N. ceranae* spores or with a solution without spores, 3 replicates were made with 30 bees for each treatment



TREATMENTS

1. Control without infection "Ctrl"
2. Control infected "Ctrl inf"
3. Isolated fraction "Fc p'ev"
4. Lyophilized secretome "Liof1 prev"
5. Fumagillin, positive control "Fum" (6.8 mg / g)

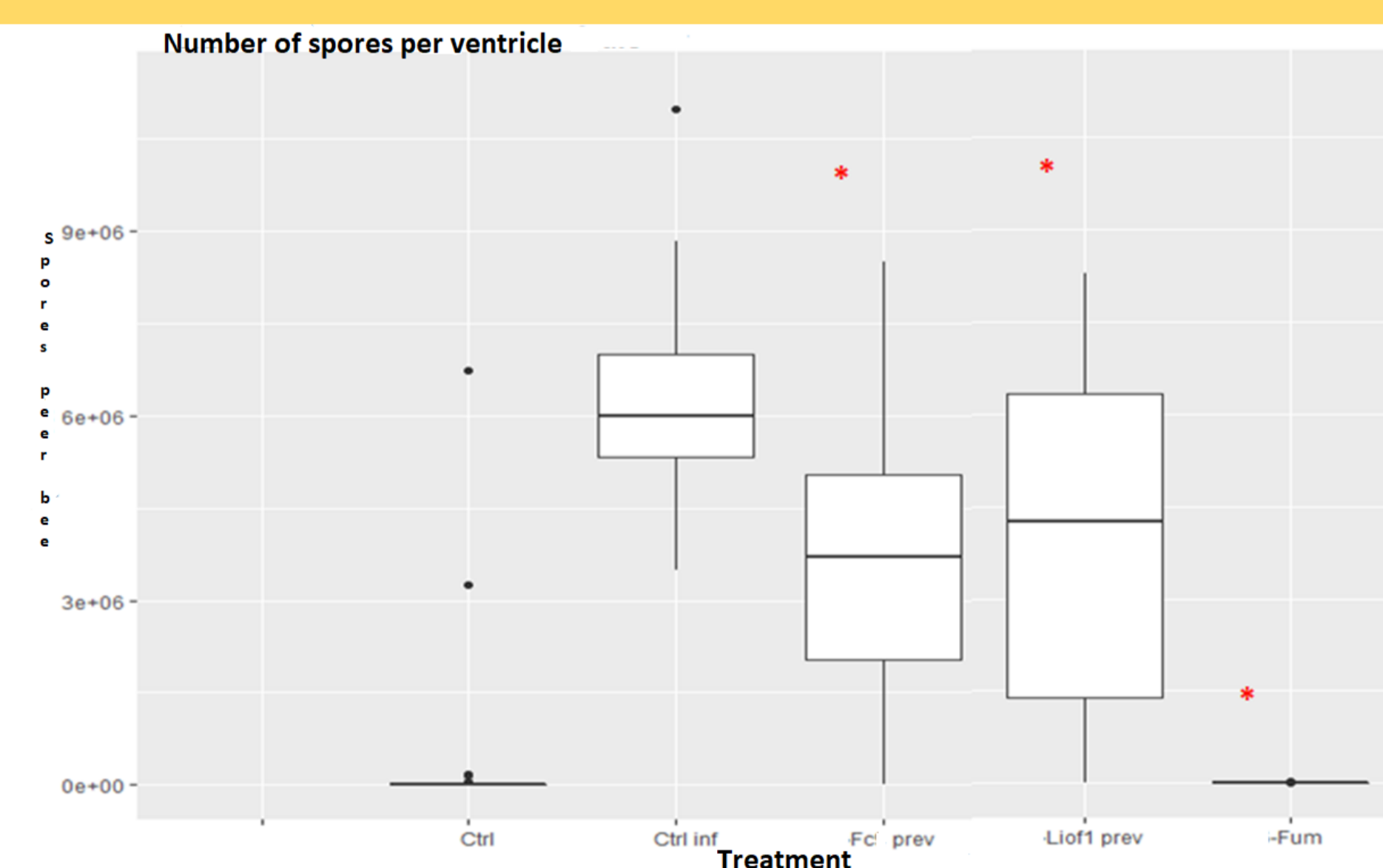


Figure 4. Estimation of the number of spores present in the different treatments at 17 days post-infection. Treatments: Preventive fraction, Preventive lyophilisate and Fumagillin.

Lyophilizate of *T. harzianum* caused a reduction of 36.3% and the fraction 46.6% in the production of spores per bee, estimated in individuals infected by *N. ceranae*.

Discussion

The experimental data suggests that the fraction tested may diminish the viability of *Nosema ceranae* spores present in infected bees without altering honeybee survival. Results of the research represent a further step towards the development of a possible therapeutic alternative for the control of this disease.