Mice and rat models are commonly use din biological research; however, their use is associated with high costs and ethical dilemmas and other solutions are being sought. An increasingly attractive alternative is that of insects, including wax moth (*Galleria mellonella*). While the moth is a pest in beehives, with the larvae being able to digest wax and destroy brood patches, it can be used in the laboratory to study the functioning of the immune system. The insect is easy to culture, large enough to provide various tissues and organs, and its immune system is similar to the human system in many respects.

Wax moth (*G. mellonella*) larvae and imago

In addition, as the larvae can be incubated at a wide range of temperatures, they can be used to study a number of pathogenic microorganisms. One such organism is the fungus *Conidiobolus coronatus*, which is pathogenic to insects and, in some cases, humans. Preliminary studies conducted by our scientific team have shown that it produces two alkaloids: harman and norharman. These substances have a great impact on the nervous system causing, among others, an increase in a neurotransmitter called serotonin, which affects the functioning of the immune system in both mammals and insects by regulating the secretion of cytokines. Hence, studies are needed to understand the full impact of infection caused by *C. coronatus* and metabolites of this fungus on selected elements of the *G. mellonella* immune system.

The aim of the proposed study funded by the grant will be to examine whether hemocytes of *G. mellonella* larvae contain proteins similar to mammalian cytokines after infection with *C. coronatus* and the administration of its metabolites (harman and norharman). It will also identify the subpopulations in which they are present and how they influence cell activity.

The study will use three groups of three-day-old last instar *G. mellonella* larvae. The first will be directly infected with the fungus, the second will receive harman and norharman in their food, and the third will receive the two compounds topically as spots on the back. After an appropriate incubation period (24 or 48 hours), hemolymph will be taken from the insects. Three analytical techniques will be used to identify the following cytokines: IL-1α, IL-1β, IL-2, IL-3, IL-6, IL-7, IL-8, IL-12, IL-13, IL-15, IL-17 , IL-19, IFN-γ, TNF-α, TNF-β, GM-SCF, M-SCF, G-CSF
- ELISA immunoassay
- immunocytochemical analysis using a fluorescence microscope
- flow cytometry

The groups in which cytokines are detected will be subjected to further tests. Briefly, individual groups (subpopulations) of cells containing cytokines will be extracted using a flow cytometer with a sorter. The cells will be incubated individually and in various configurations to learn about their interactions. The detected cytokines will be added to cell cultures, and their effects on cell migration, viability, morphological changes and phagocytosis will be determined after incubation. The cell culture medium will then be sent to another laboratory to identify the proteins produced by the cells in various systems. The next step will be the identification of cytokine-like insect proteins: the samples will be separated by 2-D electrophoresis and the resulting spots will be sent to a commercial laboratory for identification based on chromatographic analysis and bioinformatics programs.

The planned research is innovative and will deepen knowledge in various scientific fields, including entomology, immunology and cytology. Although the functions, interactions and effects of cytokines on individual immune pathways are well recognized in mammals, these mechanisms are not well known in insects. The research planned as part of the Sonata 15 project will allow the first comprehensive investigation of this issue.