

## Research Project

Title: Novel biomarkers of health and nutritional status in honeybees

### State of the art:

Honeybees are one of the most important pollinating insects essential for the preservation and conservation of ecosystems and biodiversity. Over the last two decades, there have been losses of bee colonies, causing economic damage both to beekeepers and to agricultural production dependent on pollination. In fact, bees are exposed to numerous stressors, both biotic, such as new pathogens, parasites (*Varroa destructor*) and emerging predators (*Vespa velutina*), and abiotic, such as pollution by various chemicals (from pesticides to heavy metals and microplastics) and a reduction in plant biodiversity, which leads to a reduction in floral diversity and thus in the food resources available to honeybees, especially at certain times of the year. In addition, climate change has led to a climatic instability that is significantly reshaping ecosystems and landscapes, affecting flora and fauna and their interactions, including flowering phenology, honeybee behavior, and plant-pollinator interactions. Indeed, the lack of a balanced diet is a critical factor of considerable importance for bee health, negatively affecting survival, metabolism, immunity, resistance and tolerance to pathogens, and sensitivity to pesticides. Finally, plastic pollution is increasingly recognised as an emerging threat to human, animal, and environmental health; microplastics may modify agricultural landscapes and ecosystem services. In addition, microplastics may act synergistically with other pollutants as toxic elements or pesticides that negatively impact on honeybee health.

During the first and the second year of the activities carried out by the proposer (Isani Gloria) of this project in the framework of the task 2.1.3 of the PNRR CN - Agritech Spoke 2, a panel of biomarkers of health and nutritional status has been defined. This project aims to test these novel biomarkers under field conditions at the colony level in different agro-ecosystems of the Emilia-Romagna region. Particular attention will be paid to the relationships between microplastics and trace elements and the proposed biomarkers.

### Proposed activities:

Three apiaries located in different agroecosystems will be selected. This will make it possible to detect and highlight any possible correlation between the different agroecosystems and the defined panel of biomarkers, which include the most important proteins of the haemolymph. Where possible, innovative sensorised hives will be used, with sensors to measure internal temperature and number of flights. The samplings will cover the whole production season starting in spring 2025 and ending in autumn 2025.

Haemolymph will be collected to analyse both circulating proteins and antioxidant defences. Vitellogenin, apolipoprotein, transferrin and hexamerin 70a will be separated using polyacrylamide gel electrophoresis (SDS-PAGE) and then quantified, while antioxidant defences will be assessed using ELISA methods.

The most important essential trace elements (iron, zinc and copper) and the non-essential trace element cadmium will be analysed using atomic absorption spectrometry (AAS) after the digestion of honeybee samples. A method to detect microplastics in honeybees will be developed.

Finally, correlations between the selected biomarkers and both external (e.g. environmental temperature) and in hive factors (e.g. colony strength, trace elements and microplastic levels in honeybees) will be determined.