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Duration of the Mission:

8th October to 31th October (23 days, both days inclusive)

Scientific report:

The aim of this scientific mission was to learn about “in vitro” production and preservation techniques for EPN (Entomopathogenic Nematodes) and also to learn about methods of EPN characterisation. All of these techniques are going to be applied in our Laboratory for the production, preservation and the characterisation of the EPN strains in the Mediterranean area. The techniques, object of the training during this mission, are the following:-

- 1) Regarding the production “in vitro”, the methodology for obtaining a monoxenic culture was carried out, following which the culture in quantity is initiated. This mass culture will be used in the trials against the different insect plagues. In this area, a diversity of work has been carried out:
 - a) Isolation of the EPN eggs starting from the cultures resulting from the infection of *Galleria mellonella*. Sterilization of the eggs and separation onto cellular culture dishes. Removal of the sterile eggs to petri dishes with Wouts agar. Observation and control of the progress of the new individuals up as far as their adult state.

- b) Isolation of the symbiotic bacteria (*Xenorhabdus* and *Photorhabdus*); techniques for the correct identification of the appropriate state for the initiation of the monoxenic culture. Methods of growth of the bacteria in a solid medium (NBTA Agar) and liquid medium (BSA medium).
 - c) Preparation of the diet for the production of the culture in large quantity. Control of the appropriate conditions for the creation of the monoxenic culture in BSA medium; optimum density of the bacteria and the nematodes; study of the appropriate EPN for inoculation; optimum conditions of temperature, agitation and humidity.
- 2) Improvement techniques for the in vivo preservation of EPN material, in liquid medium (Insect Ringer's solution) and in solid medium (Wouts Agar).
 - 3) Preservation of the EPN material in liquid nitrogen. These techniques include the manipulation and the preparation of the material for its optimum preservation; the preparation of suitable products; the verification of the biological activity and the infective capacity after being frozen.
 - 4) Preservation of the symbiotic bacteria (*Xenorhabdus* and *Photorhabdus*) at low temperatures (- 80°C) and selection of the most suitable phase. The isolation of the bacteria was achieved starting with *Galleria mellonella* infected by EPN and this was grown in NBTA Agar to isolate the colonies. A check on the identification of the bacteria was carried out to make a liquid culture to be frozen. Following this, the efficiency of its biological activity was studied after defreezing.
 - 5) Learning from protocols and nematode hybridation techniques for the identification of species in cellular culture boxes, in BSA medium.
 - 6) Techniques for the determination of the infectiousness of EPN using *Tenebrio molitor* in boxes with sterile sand.
 - 7) Learning from the characterisation protocols of the EPN strains against abiotic factors and adaptation to the requirements of the Mediterranean climate with regard to tolerance of EPN against different temperatures and the effect of humidity on the capacity of infection of the EPN. The range of temperatures and the values of humidity applicable in the Mediterranean region has been studied, adapting the methodology to the requirements.