SUMMARY

of the doctoral thesis entitled:

EXPLORING THE BIOTECHNOLOGICAL POTENTIAL OF SOME ENTOMOPATHOGENIC FUNGI FOR THEIR USE IN BIOLOGICAL CONTROL

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Biological control is an ecological alternative to chemical crop protection, and some species of entomopathogenic fungi are successfully used for the biological control of harmful arthropods.

The aim of this doctoral thesis was to obtain a source of biological material for bioproducts based on entomopathogenic fungal strains, for use in the biological control of pests.

With the aim of achieving the proposed goal, the research conducted for this doctoral thesis focused on the following **general objectives**:

Objective 1: Isolation of entomopathogenic fungi;
Objective 2: Evaluation of specific mass cultivation requirements and insecticidal activity of obtained fungal isolates;
Objective 3: Selection of isolates for the production of mycoinsecticides;
Objective 4: Genetic characterization of selected isolates.

The thesis is structured into seven chapters. Chapters I and II present information on the field of biological control, entomopathogenic fungi, and the production and formulation of bioinsecticides. Chapters III-VI present the original research, and Chapter VII presents the general conclusions, novel elements, and recommendations for future research directions.

In order to identify entomopathogenic fungi, 198 biological samples, including adult and larval stages of insects, soil samples from diverse locations, and plant fragments, were analyzed. Classical microbiological methods were used to isolate, purify, and cultivate entomopathogenic fungi and a total of 26 fungal isolates were obtained: 2 through endophytic isolation, 5 through soil washing, 5 through insect

baits, and 14 through direct isolation from dead insects. These isolates were identified based on morphological characteristics as belonging to the Order Hypocreales, specifically the genera *Beauveria*, *Metarhizium*, *Lecanicillium*, and *Purpureocillium*, and the Order Entomophthorales, specifically the genera *Pandora* and *Entomophthora*.

Following the evaluation, 12 entomopathogenic fungal isolates were selected for the next step: nine monosporal isolates belonging to the Order Hypocreales, exhibiting robust growth on solid media and whose conidia did not germinate at 37^0C, and three isolates from the Order Entomophthorales. Classical microbiological methods were employed to evaluate vegetative growth, sporulation, and insecticidal activity against laboratory-reared insects, with techniques adapted to the specific taxonomic requirements of each group. For isolates identified as belonging to the Order Hypocreales, insecticidal activity was evaluated against larvae of *Plodia interpunctella* (Hubner) (Lepidoptera: Pyralidae), while for isolates belonging to the Order Entomophthorales, insecticidal activity was evaluated against adults and larvae of *Myzus persicae* (Sulzer) (Hemiptera: Aphididae). The highest mortality rates were recorded for insects infected with fungi belonging to the Order Entomophthorales, but vegetative growth on the selected culture medium was reduced, confirming previous research in the field. Considering the results of this screening, three isolates belonging to the genus *Beauveria* were selected for further studies: ICDPP22-1 (isolated directly from an unidentified lepidopteran), ICDPP22-2 (isolated from potato plants *Solanum tuberosum* L. – endophytic growth) and ICDPP23-2 (directly isolated from an adult of *Leptinotarsa decemlineata* Say (Coleoptera: Chrysomelidae).

The subsequent phase of the study assessed the impact of abiotic factors on propagule number, spore germination, and insecticidal activity against *Tenebrio molitor* L. (Coleoptera: Tenebrionidae). In order to evaluate the influence of the culture medium on the production of propagules, eight carbon sources (molasses, glucose, fructose, maltose, starch, arabinose, cellulose) and five nitrogen sources (peptone, wheat bran, corn extract, yeast extract, and flaxseed meal) were tested. The molasses-based medium demonstrated the highest efficacy as a carbon source for the isolates under investigation, while varying the nitrogen source exhibited no significant impact on the propagule titer of the tested isolates. As the fungal colonies grew, the impact of the culture medium on their development became more significant.

In order to assess the effect of the initial medium pH on propagule production, the pH was adjusted between 4.0 and 6.5, while maintaining a constant temperature of 24^0C. The study revealed that the optimal pH range for propagule production in the three isolates tested was 5.0 to 5.5. The optimal pH range for propagule production was similar for all three isolates, ranging from 5.0 to 6.5, with no significant differences among them. Spore germination was assessed at temperatures between 5^0C and 35^0C, and at relative humidity levels of 29.8%, 52.6%, 75.3%, 84.7%, 92.7%, and 100%. For all isolates studied, the optimal temperature range for germination was found to be between 20^0C and 22^0C, with maximum germination rates exceeding 99%. ICDPP21-1 isolate demonstrated thermotolerance at 28^0C and 30^0C, while ICDPP22-2 and

ICDPP23-2 isolates exhibited enhanced germination at 15^{0}C. Conidia of these fungal isolates were unable to germinate at 5^{0}C, 10^{0}C, and 35^{0}C. Conidia germination initiated at a relative humidity of 52.6% or higher. Analysis of the insecticidal activity of fungal isolates cultivated in different liquid media revealed a significant impact of the carbon source on insect mortality. However, statistical analysis did not show significant variations among the three fungal strains.

Molecular analyses confirmed the assignment of the three entomopathogenic isolates, initially identified based on morphological characteristics, to the genus *Beauveria*. Sequencing of the ITS regions of isolate ICDPP22-1 led to its identification as belonging to the species *Beauveria pseudobassiana* S.A. Rehner & Humber, while sequencing of both the ITS regions and the D1/D2 domain of the large subunit (26S) ribosomal RNA (rRNA) of isolates ICDPP22-2 and ICDPP23-2, led to the conclusion that the isolates are highly genetically similar and could be clones of the same strain of *Beauveria bassiana* (Bals.) Vuill. The endophyte isolate was recovered from young potato plants at a time when the crop was free of *L. decemlineata*.

The elements of novelty of the doctoral thesis entitled "Exploring the biotechnological potential of some entomopathogenic fungi for their use in biological control" are: (1) Isolation, identification, and characterization of three new indigenous entomopathogenic fungal strains with biotechnological potential for the development of bioinsecticides; (2) Biotechnological potential assessment of an entomopathogenic fungal strain colonizing potato plants, recorded for the first time in Romania; (3) This is the first study in Romania to present a step-by-step isolation, identification, and selection of entomopathogenic fungal strains with biotechnological potential for use in biological control of pests.