POTENTIAL OF THE STRAIN OF ENTOMOPATHOGENIC FUNGUS *Isaria fumosorosea* CCM 8367 AS A BIOLOGICAL CONTROL AGENT AGAINST *Cameraria ohridella* AND OTHER PESTS

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Background

Entomopathogenic fungus *Isaria fumosorosea* (syn. *Paecilomyces fumosoroseus*) (WIZE) Brown & Smith (Deuteromycota) is potentially usefull for the biocontrol of economically important agricultural and forest insect pests. Selection of effective, highly virulent strain is prerequisite for development of successfull biopesticide.

Our strain of *I. fumosorosea* was isolated in the Czech Republic from the horse chestnut leaf miner, *Cameraria ohridella* (Fig. 1), an invasive species that has spread rapidly through central and Western Europe [1]. The strain is deposited under number CCM 8367 (CCEFO.011.PFR) as a patent culture in the Czech Collection of Microorganisms in Brno.

FIGURE 1: Adult of the horse chestnut leaf-miner, *Cameraria ohridella*.

Objectives

The aim of our study was to evaluate the effect of *Isaria fumosorosea* (syn. *Paecilomyces fumosoroseus*) CCM 8367 on:

1. *C. ohridella* (Lepidoptera)
   - naked pupae,
   - pupae in horse chestnut leaves.
2. Larvae of *Leptinotarsa decemlineata* (Coleoptera).
3. Larvae of *Spodoptera littoralis* (Lepidoptera).

Comparison with *I. fumosorosea* isolated from commercial biopesticide PreFeRal® WG (Biobest, Belgium; active ingrediet: *I. fumosorosea* strain Apopka 97) was made.

Materials and Methods

Entomopathogenic fungus

Blastospores of *I. fumosorosea* CCM 8367 [2] and the strain isolated from PreFeRal® WG (Biobest) were obtained after 120 hours submerged cultivation in grow media using orbital shaker. The concentration of blastospores was adjusted to 5×10^7 spores/ml of suspension. Soaking agent Tween 80 was added to the suspension at concentration 0.02%.

Design of experiments

The effect of the fungus on naked pupae of *C. ohridella* and larvae of *L. decemlineata* and *S. littoralis* was evaluated using standard dip test.

In case of *C. ohridella* pupae hibernating in horse chestnut leaves, the leaves (200 g per replication) were immersed for five minutes into the suspension of blastospores, while control was immersed into distilled water with Tween only. After two minutes of drying, the leaves were put into large metal cylinders.

All experiments were conducted at 23±1°C and 100% R.H.

Results

The effect on *Cameraria ohridella* naked pupae

Results of bioassays revealed faster virulence of CCM 8367 strain compared to PreFeRal® WG strain (Fig. 2).

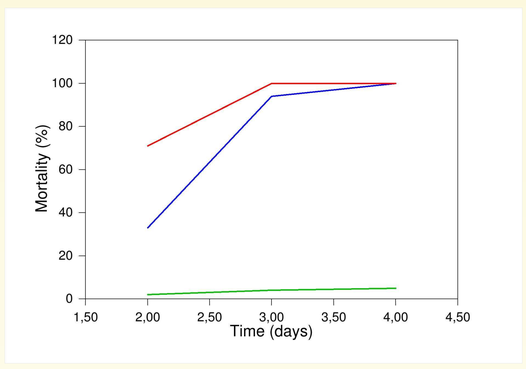

FIGURE 2: Cumulative mortality of *C. ohridella* pupae treated with *I. fumosorosea* blastospores. Red line: CCM 8367; blue line: PreFeRal® WG; green line: control; n=200.

The effect on *Cameraria ohridella* pupae in horse chestnut leaves

Mortality of *C. ohridella* pupae in leaf samples was significantly higher when the leaves were treated with *I. fumosorosea* blastospores (Table 1). Mycosis of *I. fumosorosea* was visible on pupae inside the cocoon since the 12th day after the application of the fungus (Fig. 3).

TABLE 1: Percentages of dead *C. ohridella* pupae in control and *I. fumosorosea*-treated horse chestnut leaves.

Day	Control	Treatment	n	P (Fisher's test)
10	10.0	76.7	30	<0.0001
12	13.3	86.7	30	<0.0001
14	16.7	86.7	30	<0.0001

FIGURE 3: Sporulation of *I. fumosorosea* CCM 8367 inside the *C. ohridella* pupal chamber.

The effect on *Leptinotarsa decemlineata* larvae

Results of bioassays revealed high virulence of CCM 8367 strain to the larvae of Colorado potato beatle (Figs. 4 and 5).

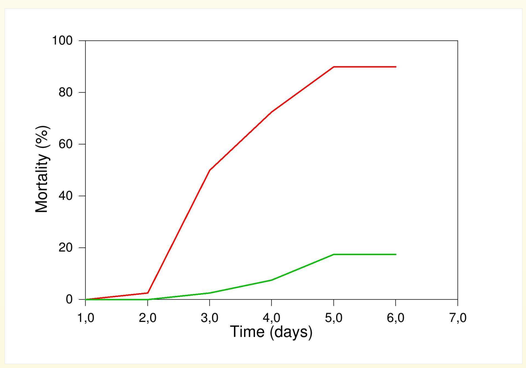

FIGURE 4: Cumulative mortality of *L. decemlineata* larvae treated with *I. fumosorosea* blastospores. Red line: CCM 8367; green line: control; n=40.

FIGURE 5: Mycosis of *I. fumosorosea* CCM 8367 on *L. decemlineata* larva.

The effect on *Spodoptera littoralis* larvae

Results of bioassays revealed higher virulence of CCM 8367 strain compared to PreFeRal® WG strain (Figs. 6 and 7).

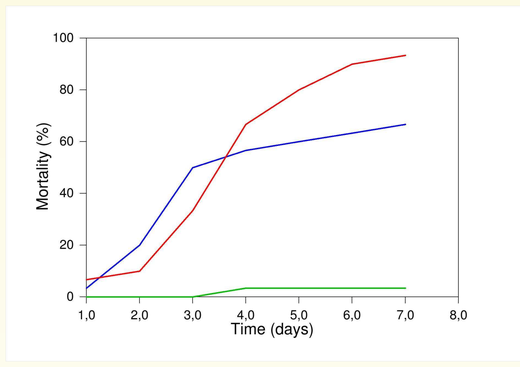

FIGURE 6: Cumulative mortality of *S. littoralis* larvae treated with *I. fumosorosea* blastospores. Red line: CCM 8367; blue line: PreFeRal® WG; green line: control; n=30.

FIGURE 7: Mycosis of *I. fumosorosea* CCM 8367 on *S. littoralis* larva.

Conclusions and perspectives

- **Strain *Isaria fumosorosea* CCM 8367 has strong insecticidal effect on *C. ohridella*, *L. decemlineata* and *S. littoralis*.**
- **Strain *Isaria fumosorosea* CCM 8367 acts faster compared to strain isolated from PreFeRal® WG.**
- **Implementation of the new strain as a novel biocontrol agent is recommended.**

In this stage of research we have already elaborated technology of preparation of the new bioagent based on spores of the strain CCM 8367 (i.e. its cultivation and finalization). **We invite and appreciate any kind of cooperation with both R&D and commercial organizations.**

Acknowledgements

This work was supported by the Czech Ministry of Education, Youth and Sports (grant No. 2B06005). The authors thank Prof. Z. Landa for providing sample of PreFeRal® WG and Mrs. J. Jabůrková for her technical assistance.

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