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The use of entomopathogenic fungi in biological control of pests

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The efficacy of entomopathogenic fungi from genus *Lecanicillium*, *Isaria*, *Beauveria,* and *Purpureocillium* were tested against larvae and adults of bean weevil (*Acanthoscelides obtectus* Say.). Some strains of *Lecanicillium*, *Isaria* and *Beauveria* showed obvious efficacy against adults of bean weevil but no treatment of bean seeds prevented the reproduction of adults, oviposition nor subsequent infestation of bean seeds.

Keywords: entomopathogenic fungi, bean weevil, biological control

1 Introduction

In recent decades intensive development of biological control of pests plays an important role in integrated pest management. The reason is to reduce using of chemicals and to protect the environment. Currently there are more than 750 species of fungi pathogenic for plant pests (Koubová, 2009). Entomopathogenic fungi were detected on members of all insect orders, mostly on *Hemiptera*, *Orthoptera*, *Thysanoptera*, *Homoptera*, *Lepidoptera*, *Coleoptera* and *Diptera* (Koubová, 2009). Some of them are polyphagous, that means they attack members of many insect orders at different development stages, others are specialized to one group of pests or only to certain species.

Entomopathogenic fungi belonging to the genera *Beauveria*, *Lecanicillium*, *Metarhizium*, *Isaria* and *Hirsutella* are the best known and most frequently used in biological control of pests (Grent, 2011; Koubová, 2009). The advantage of entomopathogenic fungi is little likelihood of insect resistance development to them. Their disadvantage is that efficacy of fungi against pests is dependent on environmental conditions, particularly on temperature and humidity. *Beauveria bassiana* is soil fungus spread worldwide. *B. bassiana* can parasite on range of pest hosts, and have also the ability of endophytic colonization of plants (Koubová, 2009; Domsch et al., 2007). Fungi of *Lecanicillium* genus are important pest pathogens. These genus was formerly classified in the genus *Verticillium* including species *V. lecanii*. These species has been dividend into a number of new taxonomic entities, including *L. lecanii*, *L. longisporum*, *L. attenuatum*, *L. nodulosum* and *L. muscarium*. Most isolates, formerly identified as *V. lecanii*, belong to *L. muscarium*, according to Zare and Gams (2001). Some isolates are effective against phytopathogenic nematodes or fungi. For instance, the cucumber powdery mildew is susceptible to infection by *L. muscarium* (Askary and Yarmand, 2007). Genus *Isaria* (formerly *Paecilomyces*) represents polyphagous species of fungi. The most important members of the genus are *I. fumosorosea* and *I. farinosa*. *I. fumosorosea* was detected as a naturally occurring pathogen of whiteflies populations, but it is also highly virulent against aphids, thrips, larvae of some butterfly species, larvae and pupae of leafminers, and even against red spider mite (Koubová, 2009). Under certain circumstances, this strain acts as a mycoparasite, it can infect the rust or powdery mildew of some crops. *I. farinosa* has related host spectrum as *I. fumosorosea*. *Purpureocillium lilacinum* (formerly *Paecilomyces lilacinus*) is soil fungus known for its efficacy against soil nematodes. The efficacy of entomopathogenic fungi was investigated against various species of insect, but against been weevil very few records are available. (Dal Bello et al.,

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2006; Padin et al. 2002). Bean weevils affect all been varieties, sometimes, chickpea and soybean. Bean weevil belongs to major storage pest. According to their size, 1–28 larvae could develop into bean grain. (Săpunaru. et al.)

The aim of this study was to investigate the efficacy of entomopathogenic fungi *Lecanicillium*, *Isaria*, *Beauveria,* and *Purpureocillium* against mealworm larvae (*Tenebrio monitor*) as a model organism and then on larvae and adults of bean weevil (*Acanthoscelides obtectus*).

2 Material and Methods

Thirteen strains of entomopathogenic fungi were tested against mealworm larvae, from which 6 strains were *Lecanicillium* (*LM* OP1, *LM* CCF 3297, *LM* CCF 1888, *LM* CCF 1674, *L*sp. AGTA2, *L*sp. AGTA1), 1 strain *Verticillium lecanii* (*VL* CCM F805), 2 strains *Beauveria bassiana* (*BB* AGTA5, *BB* AGTA6), and 4 strains *Isaria fumosorosea* (*IF* AGTA7, *IF* CCF 2790, *IF* CCF 3272, *IF* CCF 1650). Six strains of entomopathogenic fungi were tested against adults and larvae of bean weevil, from which 2 strains were *L. muscarium* (*LM* OP1 and *LM* CCF 3297), 1 strain *B. bassiana* (*BB* AGTA5), 2 strains *I. fumosorosea* (*IF* AGTA7, *IF* CCF 2790), and 1 strain *P. lilacinum* (*PL* CCF 3531). Above mentioned strains marked as AGTA or OP were obtained by own isolation from death insects, other strains were obtained from the Czech collections of microorganisms (CCF, CCM).

White coloured common bean (*Phaseolus vulgaris* L.), variety Perlička were used in all experiments.

Mealworms were put into the plastic tubes (50 ml) filled with slightly damp horticultural substrate enriched with wheat grains and breadcrumbs in an amount of 5 individuals per tube. Horticultural substrate was inoculated with 2 ml of conidial solution of each entomopathogenic fungus with spore titer/ml at least 1×10^7. Each treatment was 8 times repeated. The larval mortality was evaluated every 3–4 days.

Fifty seeds of common bean and four visibly infested seeds with larvae or pupae of bean weevil were put into each plastic tube (50 ml). Four spore-mycelial plugs of 20 days old pure culture of each fungus (5mm in diameter) were applied to seeds and shaken to evenly cover the seed. Moisture was ensured with moistened filter paper that was placed on the lid. Four repetitions per each treatment were used. Adults were counted during hatching time on an ongoing basis. After oviposition the adults were removed from tubes and after 68 days the number of visible infested seeds was counted. Into the each plastic tube (10 ml) 5 white coloured common bean seeds were placed. At first the seeds were inoculated with 2 spore-mycelial plugs of 20 days old pure culture of each fungus (5mm in diameter) and shaken. Then the seeds were infested with 5 adults of bean weevil not older than 5 days after hatching. The lid of each tube was perforated with red-hot needle because of the air circulation. The moisture was ensured with moistened filter paper that was placed on the lid. The mortality was evaluated every 3–4 day. The experiment was repeated 3 times.

The experimental data were processed statistically by STATISTICA 12 using the program ANOVA. Treatment means were compared with Tukey LSD test at P = 0.05.

3 Results

Thirty four days after the experiment establishment the highest mortality of mealworms was observed in 6 treatments – *IF* AGTA 7, *IF* CF 2790, *BB* AGTA5, *VL* CCM F-805, *IF* CCF 3272, *LM* OP1 and *LM* CCF 3297. The mortality reached 75–85 % in comparison with untreated control where the mortality reached 22.5 % (Figure 1). The first statistically significant differences in mortality between treatments and untreated control were observed 10^{th} day at treatments *LM* CCF 3297, *VL* CCM F-805 and *BB* AGTA5 (mortality 35, 30 and 27.5 %, respectively versus 1.3 % in control). Thirteen days after the experiment establishment the statistically significant differences were observed additionally in two

treatments – *IF* CCF 3272 a *LM* CMF 2703 (mortality 47.5 and 42.5 % versus 8.8 % in control).

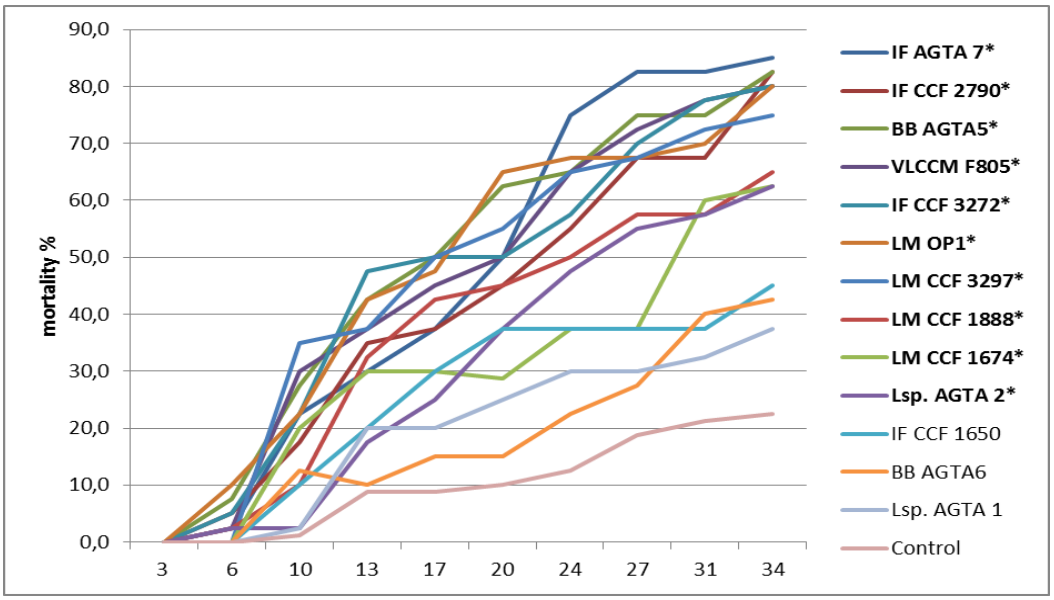

Figure 1 The effect of entomopathogenic fungi *Lecanicillium muscarium* or *Lecanicillium* sp. (LM, Lsp.), *Verticillium lecanii* (VL), *Isaria fumosorosea* (IF), and *Beauveria bassiana* (BB) on mortality of mealworms (*Tenebrio monitor*) during 34 days in laboratory test. Statistical significant differences (p = 0.05) between values of each treatment and untreated control on the 34th day after establishment of the experiment are marked with * in the legend.

From four infected bean seeds hatched on average 21 adults of bean weevil during 31 days. In these adults was determined the ability to infest new spore inoculated bean seeds by ova and subsequently by larvae. The least adults hatched in treatments *IF* CCF 2790 and *BB* AGTA5 (on average 14.3 and 16.3 pcs) and the most in treatments *LM* OP1 and *PL* CCF 3531(on average 27.7 and 24.7 pcs). Approximately 52 days after oviposition the amount of infested seeds was evaluated. The least infested bean seeds were observed at treatments *IF* CCF 2790 and *BB* AGTA5 (both 26 %), which is by 40 % less than in control (60.7 % infestation), see Figure 2. The statistical significant differences between treatments and untreated control were not obtained due to the broad ranges. The efficacy of entomopathogenic fungi on mortality of adults was difficult to monitor in a time interval longer than 16 days because of their quick death observed also in control variants. The first significant differences obtained from mortality evaluation were recorded after six days from the start of experiment between treatments *LM* OP1, *LM* CCF 3297 and untreated control. On the ninth day obtained differences were between treatments *LM* OP1, *BB* AGTA5 and control, on the 13th day between *LM* OP1, *LM* CCF 3297, *BB* AGTA5 and control, and on 16th day moreover between *IF* CCF 2790 and control. Sixteenth day the mortality of adults reached 80.6–97.2 % (Figure 2).

4 Conclusions

Selected stains of *Lecanicillium* genus effectively reduced adults of bean weevil six days from the establishment of the experiment, while species *B. bassiana* and *I. fumosorosea* did later. However, any treatment did not prevent from the reproduction of adults, oviposition nor subsequent infestation of bean seeds. The efficacy on larvae was not sufficient. The efficacy of fungi *Lecanicillium* or *Isaria* on bean weevil is not mentioned in reports, but in biological control of this pest are rather tested plant products or predators (Renuka et al., 2014; Schmale et al. 2006). The efficacy of fungus *B. bassiana* was tested rarely, however, no significant differences were found in the fresh weight of seed infested with bean weevil in

treated or untreated grain nor in the percentage weight loss of grains infested with these insect, with and without *B. bassiana* (Padin et al. 2002).

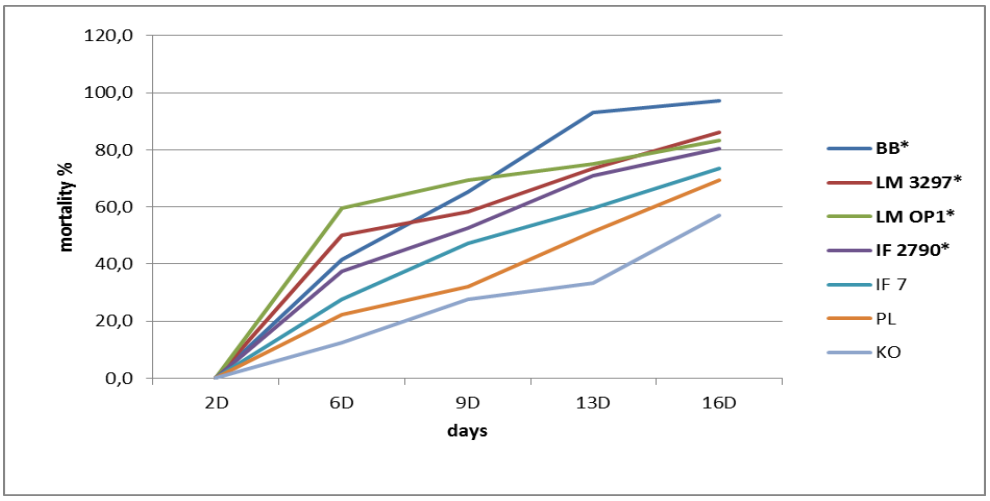

Figure 2 The effect of entomopathogenic fungi *Lecanicillium muscarium* (LM), *Isaria fumosorosea* (IF), *Purpureocillium lilacinum* (PL), and *Beauveria bassiana* (BB) on mortality of bean weevil adults during 16 days in the laboratory test. Statistical significant differences (p = 0.0.5) between values of each treatment and untreated control on the 16th day after establishment of the experiment are marked with * in the legend

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