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Entomopathogenic fungus *Metarhizium anisopliae* (strain NCAIM 362) effects on soil inhabiting *Melolontha melolontha* (Coleoptera) and *Duponchelia fovealis* (Lepidoptera) larvae in sweet potato (*Ipomoea batatas* L.)

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Abstract: The functional role of the insect pathogen fungus *Metarhizium anisopliae* strain NCAIM 362 against the white grub cockchafer (*Melolontha melolontha*) larvae and southern European marshland pyralid (*Duponchelia fovealis*) larvae in a sweet potato cultivation was tested under greenhouse conditions. As a positive control, the effect of the same strain of *M. anisopliae* were compared with the effect of the synthetic insecticide alpha-cypermethrin. According to the results, alpha-cypermethrin was more effective against both the Coleoptera and Lepidoptera larvae, a lower number of surviving individuals as well as less damaged tubers were detected after the chemical treatment, compared with *M. anisopliae.*

Keywords: biological management; pesticide control; insect damages; microbial effects

Fungal pesticides or mycoinsecticide products based on soil dwelling fungi have gained valuable research efforts in the past decades to control arthropod pests (Maina et al. 2018). The effects of this soil inhabiting fungi have long been known as a suitable agent for several arthropod pests including mites, ticks and species of the large insect orders as: Diptera, Coleoptera, Hemiptera, Lepidoptera, Isoptera, Orthoptera, Thysanoptera, Homoptera, Sternorrhyncha, Heteroptera (Jaber & Ownley 2018). Only a few studies have tested the effect on *Melolontha* larvae under the open field cultivation of sweet potatoes, but the effect was less significant compared with alpha-cypermethrin (Putnoky-Csicsó et al. 2020). Other species, such as the European pepper moth *Duponchelia*

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fovealis (Lepidoptera: Crambidae), may also infect the sweet potato, being an invasive insect, has not only had a large impact on several field crops in Europe, but also in other continents (Amatuzzi et al. 2018). Altogether, the efficacy of *Metarhizium anisopliae* against *Melolontha* and *Duponchelia* larvae in the sweet potato has not been sufficiently tested and compared under greenhouse conditions. Therefore, the present study has been designed to answer if the *M. anisopliae* strain NCAIM 362 can be a useful biological control fungi against these *Melolontha* and *Duponchelia* pests in sweet potato cultivations all over Europe.

MATERIAL AND METHODS

The experiments started in May 2019 under greenhouse conditions using potted sweet potato plants. The Beauregard variety was used in the experiment and purchased at the four-leaf stage from the Company 'Lajosmizse Sweet Potato' (Lajosmizse, Hungary). Altogether, 210 plants were used for one experimental plot, divided into three replicates: (1) 70 control plants with *Melolontha* larvae placed in the soil, without treatment; (2) 70 plants with *Melolontha* larvae and *Metarhizium* fungal treatment (wettable powder formulation). The *Metarhizium* treatment was purchased from the company Biovéd 2005 Kft. (Hungary), who isolated and formulated the treatment for commercial use, which was tested and whose effect was described for the first time by (Putnoky-Csicsó et al. 2020); (3) 70 plants with *Melolontha* larvae and alpha-cypermethrin (Merck, Germany) treatment. The whole design with these 3 × 70 plants was set-up again seven times. First, 30 L plastic dishes were filled using a 2 : 1 universal substrate : peat ratio with the same soil pH as is commercially recommended for sweet potato cultivation under open field conditions, each container containing one plant. When the plants started to have four leaves, the pots were randomly organised in rows (Figure 1A). Next, all the containers with plants were coupled to an Irritrol Junior Max controlled irrigation system. The temperatures inside the greenhouses were between 32–34 °C during the whole experimental period. Essential micro and macro elements were supplied to each plant, the first time after potting and the next time in mid-July. This was undertaken by using automatised Dosatron® systems. The soil moisture, pH and EC (electrical conductivity) were controlled randomly by ten plants every three days. The first instar larvae of *M. melolontha* were collected from the nearest forests, which were located approximately 100 km away from the experimental site. The larvae were first placed inside the previously potted and developed sweet potato containers

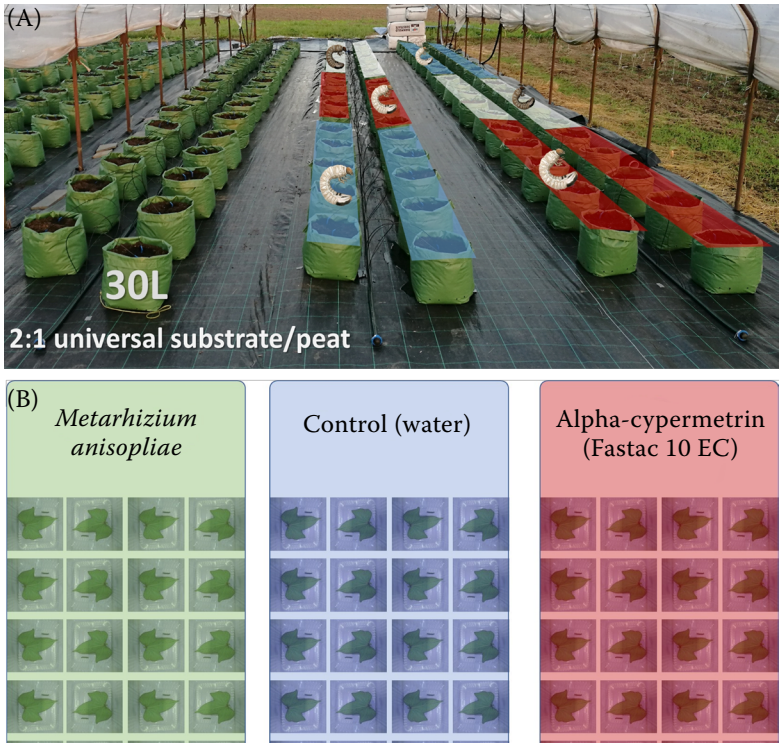

Figure 1. Experiment on *Melolontha* (A) and *Duponchelia* (B)
Blue represents the control, red represents the insecticide, the white (*Melolontha*) and green (*Duponchelia*) experiment represents the fungal treatment

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with tubers and allowed to develop until the third instar stage. Next, two larvae were added into each container with the plants used for experiment. As the treatment, 1 400 g of *M. anisopliae* was mixed into 12.6 L of water, which was then added to the fungal treatment plots at 20 mL/plant. The alpha-cypermethrin insecticide treatment was added in 0.1% concentration (10 mL insecticide/10 L water) at 2 L/plant. The larval infection can be detected by visual assessment, where all the infected larvae are covered by green hyphae. Additionally, laboratory analyses were made by placing larvae on an agar substrate, and the development of the fungus was followed. The damage to the tubers (70 tubers) was assessed using the following classification system: 0 = no damage; 1 = superficial damage, only on the epidermal surface of the tubers; 2 + deep damage, inside the tuber. The percentages of the dead and infected *M. melolontha* larvae were assessed at the end of the experiment during harvesting by manually searching for the larvae. The experiments with *Duponchelia* started in the summer of 2020 by adding larvae to the sweet potato soil. After three days, clear damage to the tubers and leaves was detected. Next, 200 larvae were collected from five different ornamental plant companies and each larva was kept separately in a petridish fed with sweet potato leaves. Fresh, untreated sweet potato leaves were offered three times/day for three days to ensure pesticide-free conditions for the larvae. Experiments were conducted on 120 larvae, from which 40 were treated with the *M. anisopliae* strain (NCAIM 362) suspension, another 40 were treated with the Fastac Active insecticide (active ingredient alpha-cypermethrin) and 40 were sprayed with distilled water only and served as the control served. The same 0.1% concentrations (10 mL insecticide/10 L water) at 2 L/plant were used as for *Melolontha* experiment. After 12 h and again after 24, 48 and 72 h, the number of dead larvae was assessed. The whole experiment was performed in three replicates using the same protocol (Figure 1B). As only the *Melolontha* mortality and survival data were normally distributed, an analysis of variance (ANOVA), followed by Tukey's HSD test, was used to compare the effect of the treatments (fungal, insecticidal treatment and control). For the analyses, the average data/treatment/blocks/replicates (n = 70) were computed. The data obtained from tuber damage data did not meet the assumption of normality, therefore, for this comparison, the non-parametric Kruskal-Wallis, followed by the Mann-Whitney U-test were used and the data were averaged on the plants/treatments/blocks (n = 70) computed for the analyses. Only the data of being damaged or not being damaged were considered. The *Duponchelia* data did not meet the assumption of normality also, so the same Kruskal-Wallis test followed by the Mann-Whitney U-test were used to compare the variables and the mean values of the treatments/replicates were used (n = 40). All the analyses were made in R (version 3.0.1; R Core Team 2013) and values equal or below $P \leq 0.01$ were considered as statistically significant differences.

RESULTS AND DISCUSSION

The rate of surviving larvae was the lowest when alpha-cypermethrin was used, significant differences were detected compared with the fungal agent and the control [alpha-cypermethrine (Cyperm)-Control U = 4.2, P < 0.01; Cyperm-*Metarhizium*(Metarh) U = 3.3, P < 0.01]. The effect of the *Metarhizium* treatment, however, was not different compared with the control (Metarh-Control U = 0.77, P < 0.43), where it was detected that only half of the larvae infected with the fungi died up to the end of the experiment. Contrary, the larval mortality rate was higher in the plots treated with alpha-cypermethrin (Cyperm-Control U = 4.4, P < 0.01; Cyperm-Metarh U = 4.1, P < 0.01) and again no differences were detected between the *Metarhizium* treatment and the control (Metarh-Control U = 0.90, P < 0.76) (Figure 2A). Fungi infections were hardly detected on the larvae, an average infection rate was one in ten in the *Metarhizium* treated pots at the end of the experiment (Figure 2A). The rate of damage to the sweet potato tubers also varied between the treatments, and this was correlated to the pesticide effects. The damage rate was almost unobservable in the pots with alpha-cypermethrin compared to the control and fungal treatments (Cyperm-Control U = 5.5, P < 0.01; Cyperm-Metarh U = 3.7, P < 0.01), and again no differences in the tuber damage between the *Metarhizium* treatment and the control were observed (Metarh-Control U = 0.54, P < 0.34) (Figure 2B). Accordingly, the number of dead larvae were significantly higher when alpha-cypermethrin was used to control the *Melolontha* larvae and,

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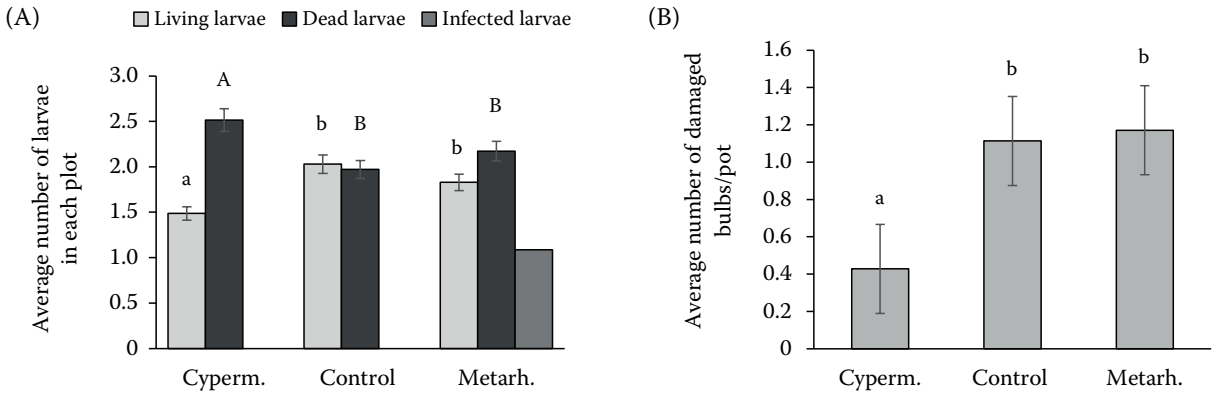

Figure 2. The mean number of surviving, dead and infected fungal *Melolontha* larvae (A); and the tuber damage (n = 70) detected under the treatments and the control (B)

Cyperm. – alpha-cypermethrin; Metarh. – *Metarhizium anisopliae*

[a,b/A,B]Different letters mean statistically significant difference

The ANOVA, followed by Tukey's HSD test, was computed to test the effect of fungal and insecticidal treatments. For the analyses, the average data/treatment/blocks/replicates (n = 70) were computed. The narrow lines mean the standard errors

similarly, no differences between the fungal treatment and the control were observed. These results can be explained in different ways. One possible reason of the apparent resistance of *Melolontha* larvae against *Metarhizium* may be some kind of evolutionary contact of the two species in the soil. A long interrelation in the soil may exist between the soil-inhabiting *Melolontha* larvae and *Metarhizium*, so that a genetic resistance may have occurred over time. Another problem can be the effects of the formulation through its commercial production. This can reduce the efficacy and caused alterations in the fungal characteristics including the conidial growth, conidial viability, and effectiveness to cause mortality (Yoder et al. 2017). This was, however, not the case in our research under greenhouse conditions as we managed to reduce ultra-violet light effects. By comparing the effect of the treatments on the *Duponchelia* larvae during the assessment periods, no differences in dead larvae were detected between the *Metarhizium* and insecticide treatments (Metarh-Cyperm U = 0.7, P < 0.21), however, the number of dead larvae were significantly lower in the control (Metarh-Control U = 2.7, P < 0.01, (Cyperm-Control U = 3.1, P < 0.01) (Figure 3). Overall, it can be stated that a low detrimental effect of *Metarhizium* on *Duponchelia* was detected, no differences in the dead larvae were detected between the *Metarhizium* and alpha-cypermethrin treatments, however, the number of dead larvae were significantly lower in the control. Altogether, it can be concluded that the effectiveness of alpha-cypermethrin against both species is significant and effective.

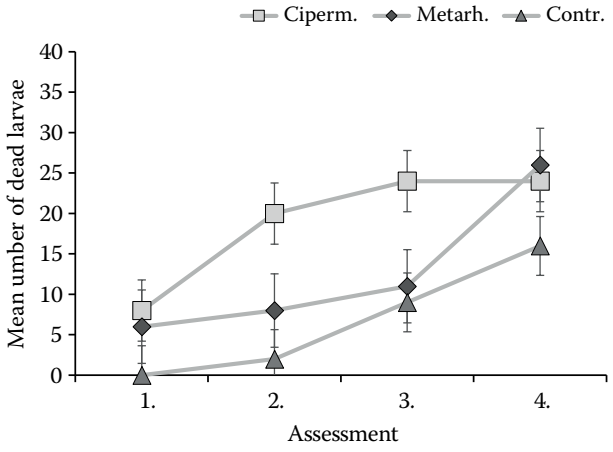

Figure 3. The average values of the dead *Duponchelia* larvae during the assessment

1. Assessment – 12 h after infection; 2. Assessment – 24 h after infection; 3. Assessment – 48 h after infection; 4. Assessment – 72 h after infection; Cyperm. – alpha-cypermethrin; Contr. – control; Metarh. – *Metarhizium anisopliae*

The data between the treatments were compared with Kruskal-Wallis, followed by the Mann-Whitney U-test (n = 40). The narrow lines mean the standard errors

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