Hungarian University of Agriculture and Life Sciences

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Entomopathogenic fungus (*Metarhizium anisopliae*) effect on soil dwelling arthropod pests in sweet potato cultivation

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1. Scientific background and objectives

The effect of fungal entomopathogen *M. anisopliae* strain NCAIM 362 against *M. melolontha* larvae in sweet potato was tested under open field conditions when crop management included compost supply and soil cover (agro-foil or agro-textile). Also the role of the *M. anisopliae* strain NCAIM 362 against *Duponchelia fovealis* in sweet potato cultivation was tested under greenhouse conditions. The effect of *M. anisopliae* same strain against *M. melolontha* was compared with the effect of alpha-Cypermethrin. Soil microbial community using Illumina sequencing and soil biological activity were tested as possible parameter influencing *M. anisopliae* effect.

Microbial pesticides, and especially mycoinsecticides, products based on living fungi to control arthropod pests, were given valuable research efforts in the past decades (Bateman, 2004; Hussain *et al.*, 2014; Kergunteuil *et al.*, 2016; Maina *et al.*, 2018; Skinner *et al.*, 2014). Metarhizium strains are soil-dwelling organisms detected extensively all over the world, regardless of climatic and soil limitations (Bidochka and Small, 2005; Bischoff *et al.*, 2009). Members of the genus are facultative saprophytes and may either live freely within the topsoil or in the presence of a suitable arthropod host act as parasites (Kepler *et al.*, 2014; Skinner *et al.*, 2014). The number and scope of research on *Metarhizium* species suggest that strains and isolates of *M. anisopliae* have been given the highest scientific attention within the genus, and also, they are the most widely used organisms in microbial pest control (Mascarin *et al.*, 2018; Zimmermann, 2007). The first scientific recognition of *Metarhizium anisopliae* dates to Russia in 1879, when E. Metchnikoff discovered a fungus that not only covered the cadaver of a chafer, but was evidently the cause of death of the arthropod (Bidochka and Small, 2005). It was then named *Entomophthora anisopliae,* referring to the chafer, *Anisoplia austriaca*. Later, N.V. Sorokin repositioned this species to the genus *Metarhizium* (Zimmermann, 1993, 2007). When the species finds an arthropod to parasite, its conidial growth is predominantly green, giving the reason for the original name of "green muscardine" to the condition induced by the fungus (Bischoff *et al.*, 2009; Driver *et al.*, 2000).

Strains and isolates of *M. anisopliae* have long been recognized as entomopathogens, with a wide range of targeted (host) arthropods including mites, ticks and members of the following insect orders: Diptera, Coleoptera, Hemiptera, Lepidoptera, Isoptera, Orthoptera, Thysanoptera, Homoptera, Sternorrhyncha, Heteroptera. Ongoing research of the past two decades, however, has shown that the position and effect of *M. anisopliae* is more complex. The fungus was found to colonize plants within rhizosphere, have a symbiotic relationship with plants, promote plant growth and may act as an antagonist to plant diseases (Akello and Sikora, 2012; Barelli *et al.*, 2016; García *et al.*, 2011; Jaber and Ownley, 2018; Liao *et al.*, 2014; Vega *et al.*, 2009). Commercialized products based on strains and isolates of *M. anisopliae* dominate the selection of mycoinsecticides worldwide. Formulation, application methods, targeted environment (arable or protected production), targeted crops, targeted pests, and strategies of use (inundative and non-inundative, or conservative way) are varying (Kergunteuil *et al.*, 2016; Maina *et al.*, 2018; Mascarin *et al.*, 2018; Shah and Pell, 2003). The potential of *M. anisopliae* isolates against pests of sweet potato (*Ipomoea batatas*) has been tested for more than three decades. One of the earliest virulence tests was performed in 1984, where the efficacy of three *M. anisopliae* strains were investigated in laboratory conditions against adult individuals of the sweet potato weevil (*Cylas formicarius*) (Ondiaka *et al.*, 2008). A subsequent study compared 12 isolates of three fungal entomopathogens including *M. anisopliae*, also on adults of the same pest (Burdeos and Villacarlos, 1989). This resulted in one of the *M. anisopliae* isolates giving the lowest LD_{50} values. In another laboratory experiment, *M. anisopliae* isolates were found not only to infect and destroy coleopterans, but to have effect on the feeding and reproduction characteristics of *Cylas puncticollis* as well (Ondiaka *et al.*, 2008). It was only in 2014, when the pathogenicity of *M. anisopliae* against *C. formicarius* was evaluated not only as a standalone treatment, but in a combination with *Beauveria bassiana* (Reddy *et al.*, 2014). *The possible ways of transmitting the fungal disease in sweet potato beetle was investigated, when fecundity, expressed in the number of eggs and the rate of viable eggs was significantly hindered even when the eggs themselves had no contact with the fungus. It appeared that the presence of M. anisopliae altered the behaviour of the pest, resulting in less eggs being positioned appropriately* (Dotaona *et al.*, 2017).

One of the earliest accounts of testing the efficacy of the fungus in field conditions dates to 1998, when damage by the Banded Cucumber Beetle (*Diabrotica balteata*) and White grub (larvae of *Phyllophaga* spp.) were evaluated using *M. anisopliae* (Story *et al.*, 1999). Although a single application before planting was found to have promising results against *D. balteata*, the effects on the other pest (i.e. Melolontha larvae) were uncertain, which may suggest that more research should be focusing on finding the conditions to enhance the efficacy of *M. anisopliae* on *M. melolontha* larvae (Story *et al.*, 1999).

Laboratory essays and open field experiments together suggests that, there are many abiotic and biotic factors contributing to the success and failure of using *M. anisopliae* in pest control. Among them several factors needs further attention, such us soil chemical composition, soil microbiota and biological activity (Jackson *et al.*, 2010; Skinner *et al.*, 2014). Soil properties are governed by a complexity of factors, so in order to obtain helpful suggestions that can be used in the practice of IPM or organic production, complex studies are required, with a set-up of complex models, and their viability must be trialed in realistic situations as well (Jaronski, 2010). Altogether more information is needed on what mechanisms endophytes establish and interact within a plant, the *M. anisopliae* on circumstances that favour the establishment of endophytism, so as to utilize its benefits (Jaber and Ownley, 2018; Kepler *et al.*, 2017; Vidal and R Jaber, 2015). Since the effect of *M. anisopliae* against *M. melolontha* larvae in sweet potato has not been a widely researched topic, we set up the present study to find answers to the following questions. (1) Can the fungal entomopathogen *M. anisopliae* strain NCAIM 362 (commercialized against coleopteran larvae) serve as an effective biological control agent against *M. melolontha* larvae in sweet potato?; (2) In sweet potato production, which soil parameters can significantly influence the efficacy of *M. anisopliae* ? (3) Is *M. anisopliae* more effective in sweet potato than the chemical insecticide?

The aims of my PhD work were the following:

What impact does the use of mulches in open-field cultivation have on the yield average and tuber damage of sweet potatoes, particularly when soil-dwelling pests (scarab larvae) are treated with the entomopathogenic fungus *M. anisopliae*?

How effective is the entomopathogenic fungus *Metarhizium anisopliae* in controlling soil-dwelling scarab larvae compared to the traditional use of cypermethrin?

What is the significance of the infection and mortality rates caused by the entomopathogenic fungus on scarab larvae, compared to control plants that are untreated?

To what extent does the presence of the entomopathogenic fungus *Metarhizium anisopliae* influence the microbial community and biological activity in the soil?

What effect does the entomopathogenic fungus under study have on the larvae of other pests, such as the *Duponchelia fovealis* (Zeller), a potential pest larva of sweet potatoes?

2. Materials and methods

2.1. Experimental set-up under open field and greenhouse conditions

Open field experiments were conducted between 2018 and 2019. Sweet potato plants Beauregard variety were obtained in 4-leaf stage from the Lajosmizse Sweet Potato Company, Hungary, and planted in eight rows/block, each row containing 22 plants. The soil was chernozem (6.5 pH). The field was chosen for our experiment because the soil inhabiting pests was dominated by *M. melolontha* larvae. This was determined before experiment, with an average of one 3rd instar larvae/m^2 detected. *M. melolontha* larvae infection was also influenced by the nearby (200 m distance) oak forests and orchards (100 m distance, mostly apple, pear, and plum trees at a 1.4 ha area). Since open field sweet potato production in the temperate zone usually involves the application of compost and soil cover systems (using foil or textile), we followed and tested this routine. The eight rows and 22 plants within each row were also treated or not with compost and covered by foil or textile (Figure 1 and S1 and S2 supplementary online materials). From each row, half of the plants were treated with *M. anisopliae* strain NCAIM 362 and the other half served as control (no *M. anisopliae*). There were 4 replications to each treatment, resulting in a total of eight replicates for each type. The presence of compost was marked K+ or K-; the presence of foil and textile; and the presence or absence of *M. anisopliae* (M+ or M-) (Figure 1A and S1 and S2 supplementary online materials). The whole system was set up at the end of May, 2018, connected to automatic irrigation system (Irritrol junior max, placed below the soil cover systems, so each plant got the same amount of water), while the *M. anisopliae* treatment in Wettable Powder

(WP) formulation (as it was commercially recommended) was added in June 27, after all plants were carefully checked. No plant pathogen symptoms or pest damages were detected on plants, and all plants were in the phenophase when the fungal entomopathogen treatment was added. This was done by preparing a 10% fungal solution (1400 g *M. anisopliae* to 12.6 liter of water) transferred to all 700 plants. Treatment was added to each plant separately using a 20 ml syringe. The whole system was daily controlled until harvest. Crop harvest started at October 1, with leaves and stems harvested first Next, all soil covers were removed, and tubers were mechanically harvested. Each tuber from each treatment and cover system were separately collected, and tuber weights for each plant were measured and assigned to cover systems and treatments (M+ or M). Because synthetic pesticides (i.e. alpha-cypermethrin) against soil inhabiting insects' larvae are not allowed in open field sweet potato control in Europe, this treatment was only used under well controlled conditions in a greenhouse experiment. Next, the damage made by soil inhabiting insects' larvae were evaluated using the following classification system: 0 – no damage, 1 – superficial damage, found only on the epidermal surface of tubers, 2 – deep damage, found in deeper tissues (Figure 2). As no severe damages were detected, there was no reason to set up more levels in our classification system. The weight of missing tuber parts at level 2 damages were assessed by the following method: using gelatinized plastic with the same density as that of sweet potato tubers. Each hole in was filled with this plastic. After drying (24 h), the plastic was removed and its weight (g) was measured (Figure 2). Yield was also measured at the end of the experiment by measuring every tuber under each plant. Weight results were averaged per compost use, soil cover systems, treatments and blocks. The whole experiment was replicated again in next year, using the same cover systems, treatments, and methods.

Experimental set-up under greenhouse conditions were conducted in 2019, starting from May, parallel with the second-year field experiments. Soil properties, its chemical and microbial compositions and biological activities were monitored under standardized and controlled conditions. The same sweet potato variety was obtained and used from the same company. For one experimental plot, there were 210 plants in three treatments (control 70 plants, fungal treatment 70 plants and alpha-cypermethrin 70 plants); each divided in two sections (35 plants for each treatment) with (P+) and without (P-) *M. melolontha* larvae, all treatments replicated seven times again. Plants first were potted in 30l plastic containers using 2:1 universal substrate / peat ensuring the same soil pH as under open field conditions. Pots were then organized in rows (Figure 1B, and S3, S4 Supplementary online materials). The whole system was connected to an automatic irrigation system, controlled by Irritrol junior max. Temperature inside the greenhouses were controlled and kept around 35 ^{o}C during the vegetation period. Micro and macro elements were added twice, first after potting and later, in mid-July, to each plant using automatized Dosatron® systems. During the course of the whole experiment soil moisture, pH and EC were tested every third days. *M. melolontha* larvae were collected from natural environment (forest soil) from about 100 km from the experimental site and placed into the sweet potato containers when tubers were already developed, on 2 September. Two third-instar larvae were placed into each *M. melolontha*-treated container. The soil insecticide alpha-cypermethrin and *M. anisopliae* in a same WP formulation were added on 13 September. The insecticide was added in a concentration of 10ml/10liter to each treated plant. The fungus was applied the same way and in the same concentration as described for the open field experiment. Tuber damage and yield weight were evaluated as described above, too. The ratio of survived, dead and infected *M. melolontha* larvae were counted at the end of the experiment by manually searching for larvae from containers after the plants were removed during harvest.

For soil chemical analyses, microbial assay and biological activity measurements, soil samples were collected twice: one month after planting (first week in June) and again, a month later. The same soil samples were divided and used for chemical assay, microbial analyses, and biological activity. From the soil of each treated and control plants (6 plants soil sample / treatments) 100 g soil was put into sterile pots and deposited at -70 °C until analyses. Damages on sweet potato tubers were assessed in a same way as under open field conditions.

2.2. 16S rRNA gene amplicon sequencing of soil bacterial community and biological activity assay

The soil bacterial community analysis was performed based on amplicon sequencing of the 16S rRNA gene as in our previous work (Benedek *et al.*, 2019). Briefly, total genomic DNA was extracted using the DNeasy PowerSoil Kit (Qiagen), a part of the 16S rRNA gene was amplified with primers containing the Bacteria-specific sequences Bakt_341F (5’-CCT ACG GGN GGC WGC AG-3′; (Herlemann *et al.*, 2011)) and Bakt_805NR (5’-GAC TAC NVG GGT ATC TAA TCC-3′; (Apprill *et al.*, 2015)), and DNA sequencing was performed on an Illumina MiSeq platform using MiSeq standard v2 chemistry as a service provided by the Genomics Core Facility RTSF, Michigan State University, USA. There, illumina compatible, dual indexed adapters were added by PCR with primers targeting the CS1 and CS2 sites. PCR products were then batch normalized using SequalPrep DNA Normalization plates (Invitrogen) and all product recovered from the normalization plate was pooled. Subsequently a clean-up of this pool was performed with Agencourt AMPure XP magnetic beads (Beckman Coulter). Quality control and quantification was carried out using a combination of Qubit dsDNA HS (Thermo Fisher Scientific), Fragment Analyzer High Sensitivity DNA (Advanced Analytical) and Kapa Illumina Library Quantification qPCR (Kapa Biosystems) assays. The pool was then loaded onto a standard MiSeq v2 flow cell (Illumina). Sequencing was performed in a 2 × 250 bp paired end format using a v2, 500 cycle MiSeq reagent cartridge. Custom sequencing and index primers complementary to the CS1/CS2 oligomers were added to appropriate wells of the reagent cartridge. Base calling was done by Illumina Real Time Analysis (RTA) v1.18.54 and output of RTA was demultiplexed and converted to FastQ format with Illumina Bcl2fastq v2.19.1. Raw sequence data were submitted to NCBI under BioProject ID PRJNA632727.

For biological activity assays homogenized soil samples, sieved through a 1.6 mm sieve to remove stones and plant debris, were used. For the FDA hydrolysis 1 g of soil was measured, placed in a 500 ml conical flask, 50 ml of 100 mM potassium phosphate buffer (pH 7.6) and 0.15 ml 12.01µM FDA was added to start the reaction. Blank was prepared without the FDA substrate along with a control probe without soil sample. Time was monitored, and the hydrolysis took place at 37°C for 1 h, hand-stirring every 5 minutes. After the hydrolysis 2 ml of acetone were added to each probe to stop the reaction. Then the probes were centrifuged on 4000 rpm for 10 minutes and sieved through Whatman nr. 1 filter papers. Fluorescein concentrations were determined with spectrophotometer (PG Instruments T60 UV/VIS Spectrophotometer) on 490 nm. The obtained absorbance values were placed in the equation of calibration graph obtained by 0.03 – 10 μg/ml fluorescein standards, from where we obtained the FDA enzyme activities of the soil probes in μg/g soil/h. Determination was replicated three times for each sample and treatment.

2.3. Data analyses

Sweet potato damage data from the field experiment were first tested for the normality of errors and homogeneity of variances. Because data were normally distributed, analysis of variance (ANOVA) was used, followed by Tukey’s HSD (Honestly Significant Difference) test to compare the effect of *M. anisopliae* on tuber damages (deep damages only) using average damage / tuber / plant / compost application / soil cover / block (n=22). Data were first compared between years, then block and side effects were tested using multivariate ANOVA; MANOVA). Because no significant differences were detected between years, and no blocks and side effects detected, pooled and averaged data between years were used for further analyses. Next, crop yield (average tuber weight / plant / compost application / soil cover / fungal treatment / block (n=22) were compared between control and fungal treatment using the same method (data were normally distributed).

Data from greenhouse experiment were again tested for the normality of errors and homogeneity of variances. Here only the crop weight data was normally distributed, therefore analysis of variance (ANOVA) was used, followed by Tukey's HSD test to compare the effect of treatments (fungal, insecticidal treatment and control) using average tuber weight (g) / plant / treatment / block (n=35). Tuber damage data and *M. melolontha* larval survival and infection data did not meet the assumption of normality, therefore the nonparametric Kruskal-Wallis test was used, followed by a Mann-Whitney U test to compare damages (averaged on plants / treatments / blocks (n=35)) and average survived and dead larvae (average number / plant / treatment / block (n=35)). All analyses were made using R version 3.0.1 (R Core Team, 2013) and values below $p \leq 0.01$ were considered as statistically significant different.

Chemical composition values of the soil were compared between collection dates and between treatments using analysis of variance (ANOVA), followed by Tukey's HSD test (data of five measurements / treatments and control).

Statistical analyses of soil bacterial communities were described in Benedek et al. (Benedek *et al.*, 2019), the differences were that the resulting sequence reads were processed using the mothur v1.41 software ((Schloss, 2020); based on the MiSeq standard operating procedure, downloaded on 03/04/2020) and the removal of chimeric sequences was performed using VSEARCH (Rognes *et al.*, 2016). OTUs (operational taxonomic units) were defined at a 97% nucleotide sequence similarity level. For the statistical analysis of amplicon sequencing data, the subsampling of reads was performed to the read number of the smallest dataset (n=19,791). Microbial alpha diversity (estimated using the Shannon-Wiener and Inverse Simpsons's (1/D) diversity indices) and species richness values (using the Chao1 and the ACE richness metrics) were calculated using mother v1.38.1. Linear regression was used to assess the variation in total bacterial diversity indices (Shannon and Simpson) under different treatments and control, R^2 values computed using PAST. Variation in bacterial community composition was also compared between genera for each treatment and control with ANOVA followed by Welch F test using mean percentages of DNA from total samples.

Data of soil biological activity was again normally distributed, thus analysis of variance (ANOVA) was used, followed by Welch F test to compare the biological activity under different treatments and control using average data / plant / block (n=6). Analyses were made using R version 3.0.1 (R Core Team, 2013).

3. Results

The presence and development of *M. anisopliae* was detected both under compost and soil cover management systems. While no differences in crop weight (an average of 1600 gr/plant) were detected between treatments, there were differences in tuber damage, and significantly higher damage (only at level 2 – deep damage) was detected when the crop was covered with foil, and not treated with compost and fungus. These damage figures however, were not different from those obtained by foil and compost cover, with or without fungal treatment, and textile cover without compost and *M. anisopliae*. Altogether, a tendency of lower damage of Melolontha larvae was detected when sweet potato was treated with *M. anisopliae* strain NCAIM 362 and covered by agro-textile.

Again, the presence and development of *M. anisopliae* was detected in all containers M+. While no differences in crop weight (an average of 1700 gr/plants) were observed, variations in *M. melolontha* larvae survival and damage were detected between treatments. Significantly lower number of survived larvae was detected in plots treated with alpha-cypermethrin (CipermP+ControlP+ U=3.2, p<0.01; CipermP+MetarhP+ U=3.0, p<0.01) and no differences were detected between *Metarhizium* treatment and control (MetarhP+ControlP+ U=0.67, p<0.23), where generally half of the larvae died before the end of the experiment. The numbers of dead larvae were higher in plots treated with alpha-cypermethrin (CipermP+ControlP+ U=4.1, p<0.01; CipermP+MetarhP+ U=3.9, p<0.01) and again no differences were detected between *Metarhizium* treatment and control (MetarhP+ControlP+ U=0.88, p<0.56). Signs of fungal infection among larvae were hardly observed at all (an average of one infected larva was found in 10 *Metarhizium* treated pots) at the end of the experiment in M+ treatments, and this did not made statistical analysis possible. The damage rate of tubers also varied between treatments. Significantly lower damage rates were detected in pots with alpha-cypermethrin (CipermP+ControlP+ U=5.2, p<0.01; CipermP+MetarhP+ U=3.9, p<0.01), while no differences between *Metarhizium* treatment and control were observed (MetarhP+ControlP+ U=0.66, p<0.45).

Representative elemental composition of sweet potato soil results were averaged out from five measurement each. Since a small amount of sample was used, results below 0.5 are considered as qualitative information, given that these elements only appear in trace amount. No differences in the chemical composition of sweet potato soil were detected between treatments.

A total of 697,221 high-quality bacterial 16S rRNA gene sequences were obtained from the samples (38,734±9 336 reads per sample). Good's coverage values were higher than 0.94 in all cases, which indicated that sequencing depth was sufficient to recover all major bacterial taxa. The average length of sequences was ~450 nt, which allowed genus-level taxon identification. No significant differences between bacterial community were detected when the soil was treated with insecticide or the fungus, and control with and without *M. melolontha* larvae (Welch F test F=0.0006, df=22.37, p<0.9). Also, no significant differences in soil biological activity were detected when treatments and control were compared Welch F test F=0.03, df=6, p<0.76.

4. Conclusions and recommendations

According to the results, the effect of fungal entomopathogen *M. anisopliae* strain NCAIM 362 in WP formulation against *M. melolontha* larvae in sweet potato is not an effective biological control method. Even if the soil parameters are identical, the effect of alpha-cypermethrin against *Melolontha* larvae is more significant, and less survived larvae and damaged tubers can be detected after the insecticidal treatment. Under open field conditions, some soil management methods such as compost supply and textile cover may enhance the effect of *M. anisopliae*, but further research is needed to test other species of the *Metarhizium* genus to find as effective agent in sweet potato sustainable pest control.

According to the results, compost supply and textile cover may enhance the effectiveness of *M. anisopliae* under open field conditions, while no effect of fungal treatment was detected under greenhouse conditions. Even if soil parameters (chemical composition, bacterial and biological activity) were identical, the effect of alpha-Cypermethrin against *M. melolontha* larvae was significant: lower ratio of larval survival and less damaged tubers were detected after the chemical treatment. Our results suggests that *M. anisopliae* strain NCAIM 362 is not effective to control *M. melolontha* larvae, further researches are needed to test other species of the *Metarhizium* genus to find an effective agent for sustainable pest control in sweet potato. Also we detected that the alpha-Cypermethrin was more effective against Lepidoptera larvae, a lower number of survived individuals as well as less damaged tubers were detected after the chemical treatment, compared with *M. anisopliae*.

5. New scientific results

1. Under open field conditions, soil covering technologies had no effect on sweet potato production, however the rate of damage was lower when soil-textile was used as soil covering method.

2. The treatments with *Metarhizium anisopliae* did not influenced significantly the development of the *Melolontha* larvae, comparing with the synthetic pesticide alpha-cypermethrin treatment.

3. The whole soil microbial flora did not differed between treatments, therefore these had no effect on *Metarhizium anisopliae*.

4. No differences in biological activity were detected after using the *Metarhizium anisopliae* treatment.

5. It was demonstrated that the European pepper moths (*Duponchelia fovealis*) larvae are significant pest on sweet potato production.

6. Treatments with *Metarhizium anisopliae* in sweet potato production had significant mortality on the European pepper moths (*Duponchelia fovealis*) larvae, comparing with *Melolontha* larvae.

6. Scientific publications related to the topic of the dissertation

ISI Journals

1. Putnoky-Csicsó, B., Tóth, F., Bálint, J., Kentelky, E., Benedek, K., Fora, GC., Nyárádi, II., Balog, A. (2022). 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.). Plant Protection Science 58(3), 264-268. https://doi.org/10.17221/2/2022-PPS. **(IF. 1,30).** Scimago ranking **Q3.**

2. Putnoky-Csicsó, B., Tonk, Sz., Szabó, A., Márton, Zs., Tóthné Bogdányi, F., Tóth, F., Abod, É., Bálint, J., Balog A. (2020). Effectiveness of the entomopathogenic fungal species *Metarhizium anisopliae* strain NCAIM 362 treatments against soil inhabiting *Melolontha melolontha* larvae in sweet potato (*Ipomoea batatas* L.). Journal of Fungi 6(3), 116, https://doi.org/10.3390/jof6030116. **(IF. 5,816).** Scimago rank **D1**.

3. Tóthné Bogdányi F, Petrikovszki R, Balog A, Putnoki Csicsó B, Gódor A, Bálint J, Tóth F (2019). Current knowledge of the entomopathogenic fungal species *Metarhizium flavoviride* sensu lato and its potential in sustainable pest control. Insects 10(11), 385. https://doi.org/10.3390/insects10110385 **(IF. 2,22)**. Scimago rank **Q1**.

Cumulated IF: **9,336**

Conference papers

1. **Putnoky Csicsó Barna, Bálint János, Balog Adalbert, Tóth Ferenc,** *Metarhizium* anisopliae entomopatogén gomba alkalmazása édesburgonya (Ipomoea Batatas) talajlakó kártevőivel szemben Marosvásárhelyen – előzetes vizsgálatok. In: Haltrich Attila, Varga Ákos (szerk.): 65. Növényvédelmi Tudományos Napok, Budapest, Tóbiás István Magyar Növényvédelmi Társaság elnöke, 2019, pp. 86–86., ISSN 0231-2956

2. **Kentelky Endre, Lukács Zalán, Tekla Amália Lunka, Klára Benedek, Domokos Erzsébet, Barna Putnoky-Csicsó, Zsolt Szekely-Varga,** Mycorrhization of corylus avellana l. And quercus robur l. Seedlings with tuber aestivum vittad, *Scientific Papers. Series B,* Vol. Vol. LXVI, No. 1, 2022, 2022, ISSN 2285-5653, pp. 701–705. (**IF. 0,4**).

3. **Csorba Artúr-Botond, Putnoky Csicsó Barna, Demeter Antal**, **Nyárádi Imre-Istvan, Bálint János,** Insecticide efficacy on ticks (Dermacentor spp.) - Case study from an infested territory from Transylvania, *Acta Universitatis Sapientiae, Agriculture and Environment*, Vol. 13, 2021, pp. 23–35., SCIENDO

4. **Csorba Artúr-Botond, Putnoky Csicsó Barna, Koncz Róbert, Bandi Attila, Nyárádi Imre István, Bálint János,** Biological control of thrips pests (Thysanoptera: Thripidae) under greenhouse conditions in Transylvania, *DRC Sustainable Future*, Vol. 1, No 2, 2020, pp. 147–154., Publons, Scilit, Crossref

5. **Csorba Artúr-Botond, Putnoky Csicsó Barna, Nyárádi Imre-Istvan, Bálint János,** Testing insecticides efficacy on pollen beetles (Meligethes aeneus F.), *Journal of Horticulture Forestry and Biotechnology*, Vol. 24, No 3, 2020, pp. 53–57.,

7. References

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