Scientific Bulletin. Series F. Biotechnologies, Vol. XXII, 2018
ISSN 2285-1364, CD-ROM ISSN 2285-5521, ISSN Online 2285-1372, ISSN-L 2285-1364

SUSCEPTIBILITY OF SOME MELOLONTHINE SCARAB SPECIES TO ENTOMOPATHOGENIC FUNGUS *Beauveria brongniartii* (Sacc.) Petch AND *Metarhizium anisopliae* (Metsch.)

Ana-Cristina FĂTU[1, 2], Mihaela-Monica DINU[1], Ana-Maria ANDREI[1]

[1]Research - Development Institute for Plant Protection, 8 Ion Ionescu de la Brad Blvd., District 1, 013813, Bucharest, Romania
[2]University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Mărăşti Blvd., District 1, 011464, Bucharest, Romania

Corresponding author email: anamaria_111@yahoo.com

Abstract

The melolonthine scarabs, Melolontha melolontha L., Amphimallon solstitiale L. and Anoxia villosa F. are well known as serious pest in orchards, vineyards, forests and fruit tree nurseries. The susceptibility of third instars larvae of M. melolontha, A. solstitiale and A. villosa to entomopathogenic fungus Beauveria brongniartii (three isolates) and Metarhizium anisopliae (three isolates) was evaluated in laboratory conditions by dipping the insects in aqueous suspensions of $1x10^7$ conidia/ml, respectively. The greatest mortality rates were observed to be caused by B. brongniartii isolates: 100% when M. melolontha larvae were treated and 60% mortality for A. villosa after 60 days of incubation. The most susceptible larvae species to M. anisopliae was A. villosa (35.5% mortality). The most resistant larvae species to all the fungal treatments was A. solstitiale at which mortality rate never exceeded 23.6% after 76 days of incubation.

Key words*: Melolontha melolontha, Amphimallon solstitiale, Anoxia villosa, Beauveria brongniartii, Metarhizium anisopliae, entomopathogenic fungus.*

INTRODUCTION

The melolonthine scarabs, *Melolontha melolontha* L, *Amphimallon solstitiale* L. and *Anoxia villosa* F. are well known as serious pest in Romanian forest nurseries (Simionescu et al., 2012; Arinton and Ciornei, 2015; Varga et al., 2015). Also called „white grubs" in larval stage, these polyphagous species feed on the roots of the plants and as adults they eat the leaves of young hardwoods and softwoods. Scarabs are a family of pest insects very difficult to control, on the one hand because of the resistance to chemical insecticides (Buss, 2006) and on the other hand because of the way of feeding, which makes difficult the penetration of insecticides in soil, to the root area. Considering these issues, the forest management added new regulations which prohibit or restrict the use of a large number of chemical insecticides.

Biological control using natural enemies of pest population has gained considerable attention over the past twenty years. Although entomopathogenic viruses, bacteria and nematodes have been investigated as potential insecticidal agents, entomopathogenic fungi were most often used in classical biological control programs (Hajek et al., 2007).

The entompathogenic fungi *B. brongniartii* and *M. anisopliae* are widespread soil borne pathogens which have been intensively studied for the biological control of a wide range of insect pests including scarabs (Rath, 1988; Raid and Cherry, 1992; Samuels et al., 1990; Yip et al., 1992; Ansari et al., 2006; Keller et al., 2003; Rodríguez-del-Bosque et al., 2005; Srikanth et al., 2010; Goble et al., 2015). Fungal infection begins with the attachment of conidia on insect's cuticle, which germinates by producing a germ tube and penetrate the integument by mechanical or enzymatical means, invading the host tissues. Once the fungus penetrates the host, it colonizes the haemocoele and multiplies, generating toxic metabolites and killing the host. Subsequently, the hyphae penetrate through the insect cuticle to the outside and, in proper conditions, form white (*B. brongniartii*) or green (*M. anisopliae*) mycelial masses on insect's body. In Europe,

biological products that are used in the biological control of whiteworms are Nema-green® (E-nema), B-Green® (Biobest), Terranem® (Koppert), to name a few. In Romania, the current strategy against whiteworm infestation includes forecasting measures (conducting soil surveys - during autumn), physico-mechanical measures (flooding uncultivated land, the use of trap plants) and agrotechnical measures (plowing and rotovating with rotary hoe) (Simionescu et al., 2000). Mechanical control is not possible in fields with young crops. In this study, laboratory bioassays were performed in order to select pathogenic isolates for subsequently field trials.

MATERIALS AND METHODS

Fungal isolates

The used fungal isolates belong to the entomopathogenic fungi collection of RDIPP and the place of isolation are described in Table 1.

Table 1. The origin of fungal isolates

Fungal name	Strain name	Host insect	Place of isolation
Metarhizium anisopliae	MaF	larva of *Anoxia villosa* Fabricius	Feteşti nursery (Ialomiţa county)
Metarhizium anisopliae	MaTVd	larva of *Anoxia* sp. Fabricius	Tudor Vladimirescu nursery (Galaţi county)
Metarhizium anisopliae	MaGp	adult of *Epicometis hirta* Poda	Timişoara county
Beauveria brongniartii	ICDPP# 2	larva of *Melolontha melolontha*	Roman nursery (Neamţ county)
Beauveria brongniartii	ICDPP# 3	larva of *Melolontha melolontha*	Dumbrava nursery (Neamţ county)
Beauveria brongniartii	ICDPP# 4	larva of *Melolontha melolontha*	Obicioara nursery (Bacău county)

To maintain the virulence, fungal isolates originated from pure cultures and maintained on the PDA medium were passed periodically through host insects.

For the preparation of conidian suspensions, 15 days age fungal slant cultures were used, resulting from stock cultures, subcultivated no more than 3 times, in order to avoid loss of virulence. Conidian suspensions were prepared by flooding the sporulated cultures with 5 ml aqueous solution of Tween 80 (0.01%) and vortexed for one minute at 1600 rpm with one gram of glass beads (2 mm in diameter), using a vortex-mixer (Velp Scientifica, Europa). The conidian suspensions were diluted for counting, using the Burker hemocytometer, under microscope (magnification 400 x).

Test insects

M. melolontha and *Amphimallon* sp. larvae were collected from nurseries in the counties Vâlcea and Vrancea, respectively, where the infestation was very high. *A. villosa* larvae were collected from a nursery in Feteşti (Ialomiţa county). Before being used in the experiment, larvae were quarantined for three weeks, with the exception of *A. villosa* larvae that were used immediately in the test. Only visible healthy individuals were used.

Biotesting

M. melolontha larvae were treated with *B.brongniartii*, respectively *Metarhizium* sp. by simultaneous dipping in 100 ml spores suspension of $1x10^7$ for 10 seconds, and then transferred into groups of 5, in disposable plastic food containers (8.5x10.5x5 cm). The boxes were filled with 300 g uncontaminated commercial soil-peat substrate and perforated for air exchange. Each replicate (x 4 boxes) consisted of five larvae from the same batch of insects. In each box were added small pieces of carrots for larval feeding. For the control variant, the larvae were treated with sterile distilled water and Tween 80 (0.05%). The boxes were incubated at 20 ± 2^0 C, in complete darkness. Mortality was recorded weekly for 60-76 days by overturning the entire contents of each box on sterile, single use substrates. The dead larvae were removed and placed in wet rooms to encourage the development of mycosis. The living larvae were returned in boxes and evaluated in the next week. Each larval species was treated in three different days. The entire experiment was performed on three different dates, using each time the conidian suspensions prepared in the test day, from the same batch of fungal cultures.

Statistical analysis

The mortality rates of fungal treated larvae were analyzed using the Kaplan Meier survival curves, and the log-rank test was applied to the significance threshold p <0.05 in the GrapfPadPrism version 5.00 for Windows (GraphPad Software, San Diego California USA). The individuals who survived until the end of the observation period were considered censored.

RESULTS AND DISCUSSIONS

Susceptibility of *M. melolontha* larva to *B. brongniartii* and *M. anisopliae* isolates

B. brongniartii (Sacc.) Petch fungus is well known to be the most important natural enemy of *M. melolontha* (Keller et al., 1997; Keller et al., 2003; Enkerly et al., 2004; Zimmermann, 2007) and a well-established active ingredient in bioinsecticide against insect pests (Faria and Wraight, 2007).

As expected, the highest *M. melolontha* larval mortality has been caused by the three strains of *B. brongniartii*.

Comparison of survival curves indicated an insignificant difference in susceptibility of *M. melolontha* larvae to treatment with different isolates of *B. brongniartii* (log-rank =3.54; p=0.17). A 100% mortality was recorded for larvae treated with ICDPP# 4 strain after 59 days of incubation. Those treated with the ICDPP# 3 strain recorded 89% mortality and the ICDPP # 2 strain determined a mortality of 68%. A single individual from the control variants was recorded with mycosis, 59 days post-treatment. The average survival periods for *M. melolontha* larvae treated with ICDPP *B. brongniartii* strains #2, #3 and #4 were 39, 37 and 38 days, respectively (Figure1).

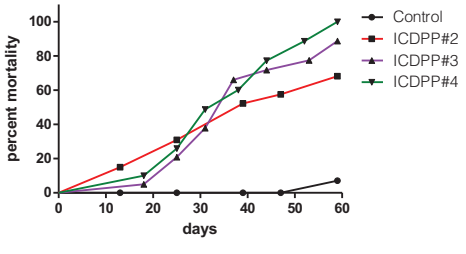

Figure 1. The mortality rate of *Melolontha melolontha* larvae (L3) treated with conidial suspension ($1x10^7$ conidia/ml) from different *Beauveria brongniartii* isolates

The mortality rate due to *M. anisopliae* infection was very low, ranging between 8% and 23% in the treated variants, after 53 days (Figure 2). For these values, average survival period could not be calculated because mortality rates were below 50%.

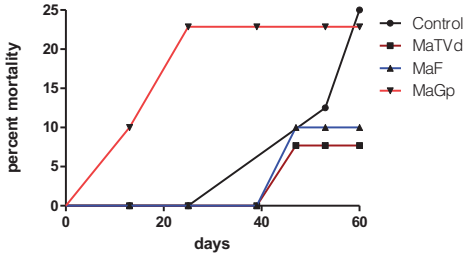

Figure 2. The mortality rate of *Melolontha melolontha* larvae (L3) treated with conidial suspension ($1x10^7$ conidia/ml) from different *Metarhizium anisopliae* isolates

Susceptibility of *A. villosa* larvae to *B. brongniartii* and *M. anisopliae* isolates

A. villosa larvae showed susceptibility to *B brongniartii* isolates and the highest mortality was of 60%, recorded among larvae treated with ICDPP # 4 isolate for which a median survival time of 59 days was obtained (Figure 3).

Comparison of survival curves indicated insignificant differences in the susceptibility of *A. villosa* larvae to treatment with different *B.brongniartia* isolates (log-rank = 0.10; p = 0.43).

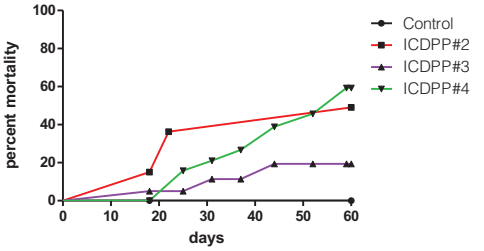

Figure 3. The mortality rate of *Anoxia villosa* larvae (L3) treated with conidial suspension ($1x10^7$ conidia/ml) from different *Beauveria brongniartii* isolates

M. anisopliae treatments led to infections of *A. villosa* larvae in very low percentages (<35.4%) and the mortality rate in control variant was 11.7% (Figure 4).

Comparison of survival curves showed insignificant differences in the susceptibility of

A. villosa larvae to treatments with various isolates of *M. anisopliae* (log-rank = 3.08, p = 0.21).

Although the MaF isolate originating from an *Anoxia* sp. larvae, its pathogenicity was very low under conditions of this study. This could happen because of an adaptation in time of this pathogen with his host. Also, no major larval mortality due to *M.anisopliae* was observed in field highly infested with *A. vilosa* from witch Maf isolate was obtained. Contrary to these results, when Raid and Cherry (1992) tested three isolate of *M. anisopliae* originated from different white grub species on *Ligyrus subtropicus* Blatchley larvae, only the isolate originating from *L. subtropicus* was pathogenic.

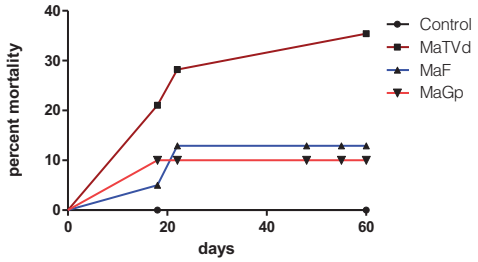

Figure 4. The mortality rate of *Anoxia villosa* larvae (L3) treated with conidial suspension ($1x10^7$ conidia/ml) from different *Metarhizium anisopliae* isolates

Susceptibility of *A. solstitiale* larvae to *B. brongniartii* and *M. anisopliae* isolates

Larvae of *A. solstitiale* showed a very low susceptibility to the *B. brongniartii* isolates, the highest mortality being 18%, after 60 days, and the mortality rate in the control variant was 5% (Figure 5).

Comparison of survival curves revealed insignificant differences in the susceptibility of *A. solstiliale* larvae to treatment with different isolates of *B. brongniartii* (log-rank = 1.07, p = 0.58). Pigmented points were observed on the cuticle of a live larva treated with *B.brongniartii.* These points could be melanin deposits occurring as a result of defense reactions at the penetration point of the fungus hyphae in the insect's cuticle (Gillespie et al., 1997). These defense reactions can explain the resistance of larvae to fungal attack.

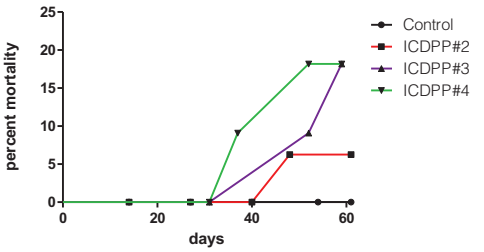

Figure 5. The mortality rate of *Amphimallon solstitiale* larvae (L3) treated with conidial suspension ($1x10^7$ conidia/ml) from different *Beauveria brongniartii* isolates

Treatments with *M. anisopliae* caused infection of *A. solstitiale* larvae in treated variants, but the mortality rate was very low. Mortality was also recorded in the control variants, which made the comparison of survival curves unjustifiable. After 60 days, the highest mortality (18%) was recorded in the variant treated with the MaTVd isolate, increasing to 23% after 76 days (Figure 6).

Results of laboratory studies on the screening and selection of virulent isolates of entomopathogenic fungi for the control of scarabs showed that the fungal isolates differed in pathogenicity and virulence against scarab-beetle species. In our tests both *B. brongniartii* and *M. anisoplie* endemic isolates proofed to be pathogenic for tested scarab species with different degrees of pathogenicity between fungal species of the same test insect species. No variation was found in pathogenicity of each isolate of the same fungal species.

Hadapad et al. (2005) reported differences in virulence of *B. brongniartii* isolates obtained from wide geographical and host origins, against the larvae of scarabs *M. melolontha* and *Holotrichia serrata* L. in screening and selection tests.

In our laboratory tests, *M. melolontha* and *A. villosa* larvae have shown greater susceptibility on *B. brongniartii* isolates compared with *M. anisopliae* isolates. Also, the results showed that *A. villosa* and *A. solstitiale* larvae (L3) are part of both *B. brongniartii* and *M. anisopliae* insect host spectrum. *B. brongniartii* is known to have a very narrow host specificity being a selective pathogen for *M. melolontha* (Hajek et al., 1994). In Romania, natural infected larvae of *M. melolontha* was reported by Ciornei et al. (2006). Larval stage diseases in field

experiments with endemic isolates of *B. brongniartii* and other scarabs such as *Anomala dubia* Scopoli and *Rhizotrogus aestivus* Olivier (Fătu et al., 2016) have also been reported.

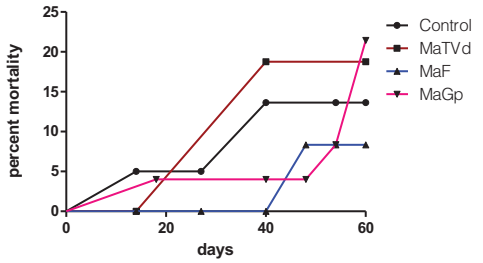

Figure 6. The mortality rate of *Amphimallon solstitiale* larvae (L3) treated with conidial suspension ($1x10^7$ conidia/ml) from different *Metarhizium anisopliae* isolates

In our experiments, *A. solstitiale* have proven to be resistant both to *Beauveria* and *Metarhizium* isolates. This could be explained by field insect populations that are continuously exposed to pathogens and may produce offspring with increased resistance to infection (Moret, 2006). So it is possible that *A. solstitiale* populations from Vrancea had been continuously confronted with *B. brongniartii* and *M. anisopliae* (data not shown). Another explanation might be the low susceptibility of the larval stage in which larvae of *A. solstitiale* were tested. Not all stages in an insect's life cycle are equally susceptible to infection by entomopathogenic Hyphomycetes (Inglis et al., 2001). Thus, in time mortality studies performed by Yubak Dhoj (2006) on fungus isolates indicated different pathogenicity of conidiospores and blastospores against three instars of white grubs *Maladera affinis* (Coleoptera, Scarabaeoidea), second instar larvae have shown greater susceptibility than first and third instars of infected grubs. The mortality percentage varied between 50%-80%. The most virulent isolates registered a LT_{50} of 2-4 weeks. Also Xiangcun Nong et al. (2011) founded no differences in susceptibility of all three instars of *Holotrichia oblita* Faldermann (Coleoptera: Scarabeidae) to some isolates of *M. anisopliae* and *B. brongniartii* but the younger instars of *A. corpulenta* (Coleoptera: Scarabeidae) were more sensitive than older instars to entomopathogenic fungi. In our experiment, the 3-rd instar larvae of *Melolontha* were very sensible to *B. brongniartii* isolates which is consistent with frequently literature reports, both in filed or laboratory (Hurpin and Vago, 1958; Ferron, 1967; Keller, 1997).

Although isolates of entomopathogenic fungi from *Amphimallon* larvae are reported (Keller, 2007; Kocacevik et al., 2015), reports on pathogenity of entomopathogenic fungus against this pest in field or laboratory are rare. Benker and Leuprecht (2005) obtained promising results in field trials with *B. brongniartii* (in the form of Melocont-Pilzgerste) against second larval stage of *M. melolontha* and *A. solstitiale*. The reduction of pest population was 80% of the starting number of the grubs in the variant treated with the fungus. Peters and Vlug (2005) have been successful in controlling L2 larvae of *A. solstitiale* using the nematode *Heterorhabditis bacteriophora*. Also Sezen et al. (2005) obtained high mortality for second and third instar larvae of *A. solstitiale* of 90% using *B. cereus* isolated from *A. solstitiale*, and of 100% using both mixture of this isolate of *B.cereus* with *B. sphaericus* and *B. thuringiensis,* within ten days.

Comparative studies on the susceptibility of soil-dwelling insect pests to *B. bassiana, B. brongniartii* and *M. anisopliae* led to different results. Beron and Diaz (2005) reported that different isolates of *B. bassiana* were generally more virulent to most soil-dwelling insect pests than *M. anisopliae*. Larvicidal bioassays on *Polyphylla fullo* (Coleoptera: Scarabaeidae) show also that *B. bassiana* is more effective than *M. anisopliae* both in young and older larvae (Erler and Ates, 2015). On the contrary, tests on *Anomala cincta* (Coleoptera: Melolonthidae) proved higher virulence of *M. anisopliae* compared with *B. bassiana* isolates (Guzman-Franco et al., 2012), according to the studies of Klein et al. (2000) which consider that *Metarhizium* species are better adapted to infect soil-dwelling insects than *Beauveria* species.

Besides pathogenic microorganisms, parasitoids like *Dexia rustica* F., *Dexiosoma caninum* F. (Walker 1944; Vanhara, 2009) and *Pexopsis aprica* Meig (Lutovinovas et al., 2014) were found to attack larvae and adults of *M. melolontha*, respectively. *Dexia rustica* also

infect *Amphimallon* species (Huiting et al., 2006). Also the protozoa *Nosema melolonthae* Krieg and *Adelina melolonthae* Tuzet (Hurpin, 1968) and neogregarian protozoa (Yaman et al., 2016) have been recorded from these hosts. Larvae of wasps like *Scolia hirta* Schrank develop within soil larvae of scarab beetle including *M. melolontha* (Banazsak and Twerd, 2009).

CONCLUSIONS

The third instar larvae of melolonthine scarabs, *M. melolontha*, *A. villosa* and *A. solstitiale* that were tested under laboratory conditions by immersion in conidial suspensions showed a different susceptibility to different entomopathogenic fungi: the larvae of *M. melolontha* and A. *villosa* showed a higher degree of susceptibility to *B.brongniartii* compared to *M. anisopliae*; *A. solstitiale* showed a low susceptibility to both fungal species. No significant differences in susceptibility of scarab larvae to different isolates of the same entomopathogenic species were registered.

The high mortalities of *Melolontha* and *Anoxia* third instar larvae indicate that isolates of *B. brongniartii* can be considered as future candidates for biological control of these white grub species. Further tests on young instar larvae of *Amphimallon* must be performed.

REFERENCES

Ansari M. A., Shah F. A., Tirry L., Moens M., 2006. Field trials against *Hoplia philanthus* (Coleoptera: Scarabaeidae) with a combination of an entomopathogenic nematode and the fungus *Metarhizium anisopliae* CLO 53. Biological Control, 39(3), 453-459.

Arinton M., Ciornei C., 2015. Muzeul Olteniei Craiova. Oltenia.Studii şi comunicări. Ştiinţele Naturii. Tom. 31, (1), 105-113. biozoojournals.ro/oscsn/cont/31_1/14_Arinton.pdf.

Banaszak J. Ó. Z. E. F., Twerd L., 2009. Historical and current records of Scolia hirta Schrank, 1781 (Hymenoptera: Scoliidae) in Poland. Polish Journal of Entomology, 78, 101-110.

Benker U., Leuprecht B., 2005. Field experience in the control of Common Cockchafer in the Bavarian region Spessart. Insect Pathogens and Insect Parasitic Nematodes: *Melolontha*. IOBC/wprs Bulletin 28(2): 21-24.

Berón C. M., Díaz B.M., 2005. Pathogenicity of hyphomycetous fungi against *Cyclocephala signaticollis*. BioControl 50: 143-150.

Buss E. A., 2006. White grub biology and management. EDIS. http://edis.ifas.ufl.edu/LH037.

Ciornei C., Andrei A.M., Lupăştean D., Pavel O., Tudorache M., 2006. Occurrence of *Beauveria brongniartii* (Sacc.) Petch in Romanian forest nurseries infested with *Melolontha melolontha* (L.) IUFRO Working Party 7.03.10 Proceedings of the Workshop 2006, Gmunden, Austria, 253-258.

De Faria M.R., Wraight S.P., 2007. Mycoinsecticides and mycoacaricides: a comprehensive list with worldwide coverage and international classification of formulation types. BiolControl, 43:237–256.

Enkerly J., Widmer F., Keller S., 2004. Long-term field persistence of *Beauveria brongniartii* strains applied as biocontrol agents against European Cockchafer larvae in Switzerland. Biol Control, 29:115–123.

Erler F., Ates A.F.O., 2015. Potential of two entomopathogenic fungi, *Beauveria bassiana* and *Metarhizium anisopliae* (Coleoptera: Scarabaeidae), as biological control agents against the June beetle. J Insect Sci., 15(1): 44.

Fatu A.C., Dinu M.M., Ciornei C., Andrei A.M., 2016. Testing Biomelcon bioinsecticide in some forest nurseries from Bacău County. Romanian Journal for Plant Protection., 9: 1-4.

Ferron P., 1967. Etude au laboratoire des conditions écologique favorisant le développement de la mycose a *Beauveria tenella* du ver blanc. Entomophaga, 12: 257-293.

Gillespie JP, Kanost M.R., Trenczek T., 1997. Biological mediators of insect immunity. Annu Rev Entomol, 42:611-643.

Goble T.A., Conlong D.E., Hill M.P., 2015. Virulence of *Beauveria brongniartii* and *B. bassiana* against *Schizonycha affinis* white grubs and adults (Coleoptera: Scarabaeidae). Journal of Applied Entomology, 139(1-2), 134-145.

Guzmán-Franco A.W., Hernández-López J., Enríquez-Vara J.N., Alatorre-Rosas R., Tamayo-Mejía F., Ortega-Arenas L.D., 2012. Susceptibility of *Phyllophaga polyphylla* and *Anomala cincta* larvae to *Beauveria bassiana* and *Metarhizium anisopliae* isolates, and the interaction with soil properties. BioControl, 57: 553–563.

Hajek, A. E., St. Leger R. J.,1994. Interactions Between Fungal Pathogens and Insect Hosts. Annu. Rev. Entomol. 39, 293–322.

Hajek A.E., McManus M.L., Delalibera I. Jr., 2007. A review of introductions of pathogens and nematodes for classical biological control of insects and mites. Biol. Control, 41,1-13. http://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.564.467&rep=rep1&type=pdf.

Huiting H.F., Moraal L.G., Griepink F.C., Ester A., 2006. Biology, control and luring of the Cockchafer, *Melolontha melolontha*: literature report on biology, life cycle and pest incidence, current control possibilities and pheromones. http://library.wur.nl/WebQuery/wurpubs/fulltext/121073

Hurpin B., 1968. The influence of temperature and larval stage on certain diseases of *Melolontha melolontha*. Journal of Invertebrate Pathology, 10(2), 252-262.

Hurpin B., Vago C., 1958. Les maladies du hanneton commun (*Melolontha melolontha* L.). Entomophaga, 3(4), 285-330.

Hadapad A.B., Reineke A., Zebitz, C.P.W., 2005. Screening and selection of virulent isolates of the entomopathogenic fungus *Beauveria brongniartii* (Sacc.) Petch for the control of scarabs. Insect pathogens and insect parasitic nematodes: *Melolontha*. IOBC/wprs Bull, 28(2), 63-69.

Inglis G.D., Goettel M.S., Butt T.M., Strasser H., 2001. Use of hyphomycetous fungi for managing insect pests. In, T.M. Butt, C. Jackson and N. Magan (eds.) Fungi as biocontrol agents, CABI Publishing, Oxon, UK, 23-69.

Keller S., Schweizer C., Keller E., Brenner H., 1997. Control of white grubs (*Melolontha melolontha* L.) by treating adults with the fungus *Beauveria brongniartii.* Biocontrol Science and Technology, 7(1), 105-116.

Keller S., Kessler P., Schweizer C., 2003. Distribution of insect pathogenic soil fungi in Switzerland with special reference to *Beauveria brongniartii* and *Metarhizium anisopliae*. BioControl 48: 307-319. https://doi.org/10.1023/A:1023646207455.

Keller S., 2007. List of arthropod-pathogenic mitosporic fungi from Switzerland. Mitteilungen-Schweizerische Entomologische Gesellschaft, 80:1-5. https://www.e-periodica.ch/cntmng?pid=seg-001:2007:80::346.

Klein M.G., Grewal P.S., Jackson T.A., Koppenhöfer A.M., 2000. Lawn, turf and grassland pests. In Lacey L.A., Kaya H.K. (eds.), Field manual of techniques in invertebrate pathology. Springer, Dordrecht, 655–675.

Kocaçevik S., Sönmez E., Demirbağ Z., Demir I., 2015. Isolation, Characterization and Virulence of Highly Promising Entomopathogenic fungi from *Amphimallon solstitiale* (Coleoptera: Scarabaeidae). The Fifth International Entomopathogens and Microbial Control Congress, ·Ankara/Turkey. https://www.researchgate.net/publication/281814536_Isolation_Characterization_and_Virulence_of_Highly_Promising_Entomopathogenic_fungi_from_Amphimallon_solstitiale_Coleoptera_Scarabaeidae.

Lutovinovas E., Tschorsnig H.P., Barták M., Kubík Š., 2014. Tachinidae (Diptera) of Vráž nr. Písek (Czech Republic). In 6th workshop on biodiversity, Jevany. Česká zemědělská univerzita, Praha, 106-137. https://www.researchgate.net/publication/270760137_Tachinidae_Diptera_of_Vraz_nr_Pisek_Czech_Republic.

Moret Y., 2006. „Transgenerational immune priming": specific enhancement of the antimicrobial immune response in the mealworm beetle, *Tenebrio molitor*. Proc R Soc B273: 1399-1405.

Peters A., Vlug H., 2005. Timing of nematode application to control white grubs (Scarabaeidae) IOBC Insect Pathogens and Insect Parasitic Nematodes: Melolontha. IOBC/wprs Bulletin, 28(2), 51-55.

Raid R.N., Cherry R.H., 1992. Pathogenicity of *Metarhizium anisopliae var. major* (Metschnikoff) Sorrokin to a sugarcane grub *Ligyrus subtropicus* (Coleoptera: Scarabaeidae). J. Agric. Entomol., 9, 11–16. http://scentsoc.org/Volumes/JAE/v9/1/00091011.pdf.

Rath A.C., 1988. Developments in the use of *Metarhizium anisopliae* for control of subterranean red-headed Cockchafer (Coleoptera: Scarabaeidae: *Adoryphorus couloni*). In Proceedings of the 5th Australasian Conference on Grassland Invertebrate Ecology, 15-19.

Rodríguez-del-Bosque L.A., Silvestre F., Hernández V.M., Quiroz H., and Throne J.E., 2005. Pathogenicity of *Metarhizium anisopliae* and *Beauveria bassiana* against *Phyllophaga crinita* and *Anomala flavipennis* (Coleoptera: Scarabaeidae). Journal of Entomological Science, Vol. 40(1), 67-73.

Samuels K.D.Z., Pinnock D.E., Bull R.M., 1990. Scarabeid larvae control in sugarcane using *Metarhizium anisopliae*. Journal of Invertebrate Pathology, 55(1), 135-137.

Sezen K., Demir I., Kati H., Demirbag Z., 2005. Investigations on bacteria as a potential biological control agent of summer chafer, *Amphimallon solstitiale* L.(Coleoptera: Scarabaeidae). Journal of microbiology (Seoul, Korea), 43(5), 463-468.

Simionescu A., Mihalciuc V., Chira D., Lupu D., Vlăduleasa A., Vişoiu D., Rang C., Mihalache G., Ciornei C., Olenici N., Netoiu C., Iliescu M., Chira F., Tăut I., 2000. Protecţia pădurilor. Editura Muşatinii, Suceava, 45-46.

Srikanth J., Easwaramoorthy S., Santhalakshmi G., 2010. Field efficacy and persistence of *Beauveria brongniartii* (Sacc.) Petch applied against *Holotrichia serrata* F. (Coleoptera: Scarabaeidae) infesting sugarcane in southern India. Sugar Cane International, 28(4), 151-156.

Vanhara J., Tschorsnig H.P., Herting B., Mückstein P., Michalková V., 2009. Annotated host catalogue for the Tachinidae (Diptera) of the Czech Republic. Entomologica fennica, 20(1), 22-48.

Varga M.I., Oltean I., Muntean V., Florian T., Mândru M., 2014. Monitoring the species *Melolontha melolontha* L. in the nursery of Gurghiu Forestry High School. Bulletin UASVM Horticulture, 71(1), 128-129.

Walker M. G. 1944. Notes on the Biology of *Dexia rustica* F., a Dipterous Parasite of *Melolontha melolontha* L.. Proceedings of the Zoological Society of London, A113: 126–176.

Xiangcun Nong, Chunquin Liu, King Lu, Quinglei Wang, Guangjun Wang, Zehua Zhang, 2011. Laboratory evaluation of entomopathogenic fungi against the white grubs, *Holotrichia oblita* and *Anomala corpulenta* (Coleoptera: Scarabaeidae) from the field of peanut, *Arachis hypogaea*. Biocontrol Science and Technology 21(5):593-603.

Yaman M., Eroğlu M., Radek R., 2016. Occurrence of a microsporidium in the predatory beetle *Calosoma sycophanta* L. (Coleoptera: Carabidae). Turkish Journal of Agriculture and Forestry, 40(3), 420-424.

Yip H.Y., Rath A.C., Koen T.B., 1992. Characterization of *Metarhizium anisopliae* isolates from Tasmanian pasture soils and their pathogenicity to redheaded Cockchafer (Coleoptera: Scarabaeidae: *Adoryphorus couloni*). Mycological Research, 96(2), 92-96.

Yubak Dhoj G.C., 2006. White grubs (Coleoptera: Scarabaeidae) associated with Nepalese agriculture and their control with the indigenous entomopathogenic fungus *Metarhizium anisopliae* (Metsch.) Sorokin (Doctoral dissertation, University_of_Basel).

Zimmermann G., 2007. Review on safety of the entomopathogenic fungi *Beauveria bassiana* and *Beauveria brongniartii*. Biocontrol Sci Technol 17:553–596.