STUDIA ECOLOGIAE ET BIOETHICAE

2017, 15, 4: 31-39
ISSN 1733-1218
DOI: http://doi.org/10.21697/seb.2017.15.4.04

Susceptibility of lesser mealworm, *Alphitobius diaperinus* Panzer (Coleoptera: Tenebrionidae) to entomopathogenic fungi isolated from poultry houses litter and nearby soil

Elżbieta Popowska-Nowak[1]*, Dorota Tumialis[2], Elżbieta Pezowicz[2]

1 Cardinal Stefan Wyszynski University in Warsaw, Faculty of Biology and Environmental Sciences
Wojcickiego 1/3, 01-938 Warszawa, Poland

2 Warsaw University of Life Sciences SGGW, Department of Animal Environment Biology
Ciszewskiego 8, 02-786 Warsaw, Poland

* e.popowska@uksw.edu.pl

Summary

The lesser mealworm *Alphitobius diaperinus* is present in great numbers in poultry houses. These insects are especially dangerous as a potential carriers of pathogens such as bacteria, viruses and parasites. We explored the possibility of using local strains of entomopathogenic fungi isolated from litter and from soil to control lesser mealworm populations. Isolated fungi showed low pathogenicity to lesser mealworm beetles. Infection with a suspension at a concentration of 1×10^8 spores/ml resulted in only 4 *Metarhizium anisopliae sensu lato* isolates showing the highest insect mortality in the range of 30–36%. Still lower pathogenicity was found in isolates of *Beauveria bassiana,* with only 4 isolates of *B. bassiana* causing a mortality of 17–26%. Isolates of *Isaria fumosorosea* and *I. farinosa* did not cause mortality in beetles that differed significantly from that in the control variant. The larvae were more susceptible to infection. Except for *I. fumosorosea,* all species caused 100% mortality in larvae. For further studies, the *B. bassiana* 3K isolate (from the litter) could be selected because of its high mortality (100%) and high larval infectivity (50% overgrown with mycelium).

Key words

the lesser mealworm *Alphitobius diaperinus,* poultry houses, entomopathogenic fungi, biological control

1. Introduction

The lesser mealworm (*Alphitobius diaperinus*) Panzer occurs in natural conditions under tree bark (Burakowski *et al.* 1987). It can also be found in store-houses and larders in fodder or grains, most often in products that are mouldy (Lorenzo 1990). It is present in great numbers in poultry houses, where it finds optimum conditions for reproduction and growth (Rueda and Axtell 1997). Larvae and beetles of the lesser mealworm inhabit litter and gaps in walls (Geden and Axtell 1987). These insects damage polyurethane poultry insulation (Vaughan *et al.* 1984, Despins *et al.* 1989, Steelman 1996, Hinkle and Hickle 1999). The lesser mealworm feeds on fungi and also on small insects. Cannibalism is also possible – small larvae and eggs are eaten by larger larvae and beetles. It is also found in the bodies of

dead hens (Harding and Bissell 1958). The lesser mealworm is especially dangerous as a potential carrier of such pathogens as bacteria (mainly of the genera *Escherichia*, *Salmonella*, *Bacillus*, *Streptococcus,* and *Campylobacter*), viruses (causing Marek's, Gumboro, and Newcastle diseases, bird flu, and enteritis), and parasites (like *Eimeria* and larvae of the flatworms *Raillietina* spp. and *Choanotaenia* spp.) (Eidson *et al.* 1965, Lancaster and Simco 1967, De las Casas *et al.* 1972, 1976, Avancini and Ueta 1990, McAllister *et al.* 1994, 1996, Goodwin and Waltman 1996, Steelman 1996, Crippen and Sheffield 2006, Hazeleger *et al.* 2008, Crippen *et al.* 2009). Transmission of these pathogens occurs when a bird eats infected larvae. Hen broilers can eat *ca.* 450 larvae a day and turkeys *ca*. 200 larvae, even with permanent feeders with food present.

A known complication of insect control with insecticides is the emergence of resistance in *A. diaperinus* after some insecticide applications (Calibeo 2002). The various another risk factors associated with the use of chemical insecticides such as, accumulation of pesticide residue in a food chain, environmental pollution, contamination of stored food and residual effects. Therefore, it is not safe to use them in farms or stores. Moreover, the insecticides used thus far do not produce long-term effects (i.e., they do not persist in the environment and are effective for only one generation of insects). They also reach only a portion of the individuals in litter and in the isolation of farm houses or stores; hence, biological methods seem to be an attractive alternative (Voris *et al*.1994). One of the biological methods used in reducing the number of pest insects are preparations containing entomopathogenic fungi. Laboratory studies of the pathogenicity of these fungi with respect to *A. diaperinus* carried out in the United States, Denmark, Poland and Brazil found that the level of insect mortality depends on the strain and dose of the spores as well as the preparation's formulation, insect stage, and the type of litter (Steinkraus *et al*. 1991, Crawford *et al*. 1998, Steenberg *et al*. 2001, Popowska-Nowak 2003, Santoro *et al*. 2008). With highly pathogenic strains, the beetle mortality was 92% (Steenberg *et al*. 2001). However, so far, the only registered agent for the control of *A. diaperinus* is the U.S. preparation containing the spores and crystalline toxin of *Bacillus thuringiensis* (Hickle and Bradfish 1991, Hickle *et al*. 1991).

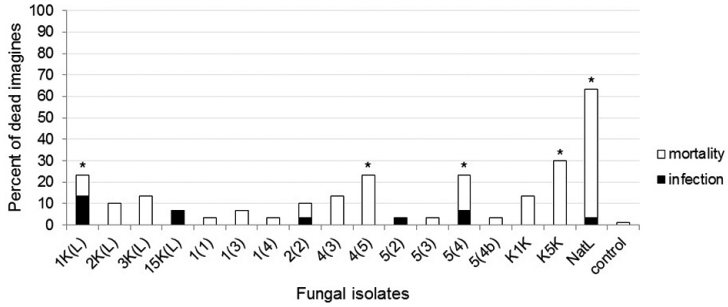
According to Bajan and Kmitowa (1997) and Bałazy (2000), entomopathogenic fungi representing the anamorphs of Hypocreales (Ascomycota) are potentially the most effective in the natural control of harmful insect populations in Poland. These are mainly *Beauveria bassiana, Isaria farinosa*, *I. fumosorosea*, *Metarhizium anisopliae* and *Verticillium lecanii*. They penetrate through insect exoskeletons and may cause infections even if the insects do not feed. By producing spores on the dead insect's body surface, they initiate new generations of the fungus, which penetrate insects contacting the pathogen. As facultative parasites, they may survive for long periods until the appearance of a host (insect) in a given habitat (Bałazy and Majchrowicz, 1978).

In view of the fact that *A. disperinus* also occurs in large numbers in poultry houses in Poland (Majchrowicz 1985, Wójcik *et al*. 2000), we explored the possibility of using local strains of entomopathogenic fungi to control lesser mealworms.

2. Material and methods

2.1. Isolation of entomopathogenic fungi

Fungi isolated from litter in a poultry house and nearby soil where litter was periodically stored were used in the study. Samples were taken from 5 localities: Słomczyn, Kąty, Góra Kalwaria, Uleniec and Białobrzegi in Poland. Fungi were isolated from litter with the dilution method onto Sabouraud and Czapek culture media with the addition of streptomycin (600 mg/l) and lithium chloride (4 g/l). Cultures were formed from a



* Significant difference relative to the control at p<0.05, (L) – isolates from litter.

Fig. 1. Mortality and infection of lesser mealworm adults after application of spores of *B. bassiana* strains isolated from poultry house litter or from nearby soil and the Naturalis-L preparation (NatL). Data represent cumulative beetle mortality. Beetle infection means overgrown with mycelium

dilution of 10^4. Fungi from soil were isolated according to the method for trapping insects described by Zimmerman (1986). The fungi were identified microscopically based on morphological characteristics using taxonomic keys (Samson *et al.* 1988, Humber 1997).

All cultures were deposited in the culture collection of the Department of Biology and Environmental Sciences (Cardinal Stefan Wyszynski University in Warsaw, Poland).

2.2. Pathogenicity test

To test the sensitivity of the various growth stages of the lesser mealworm to infection by entomopathogenic fungi, particular isolates of various species of fungi were cultured in Petri dishes on Sabouraud medium. Spores were collected from three-week fungal cultures, and a suspension was prepared with a density of 1×10^8 spores/ml in 0.01% Tween 20 solution. The concentration of conidia were determined in Thoma cell counting chamber. The control variant consisted of 0.01% Tween 20 solution. Ten beetles, two-week larvae or pupae were placed in Petri dishes lined with filter paper. Insects in dishes were infected with 1 ml of the above suspension or doused with 1 ml of 0.01% Tween 20 solution and kept at a temperature of 25°C. Insects were fed during the experiment.Over 21 days dead individuals and those covered with mycelium were counted and removed to separate wet chambers. Insects used for the experiment were taken from their own culture. Each experiment was repeated three times.

For comparative purposes, we used a liquid commercial preparation Naturalis-L based on *B. bassiana* recommended for controlling the silverleaf whitefly (*Bemisia tabaci*) and the greenhouse whitefly (*Trialeurodes vaporarium*). The preparation was used at a recommended dose of 10^7 spores/ml of water.

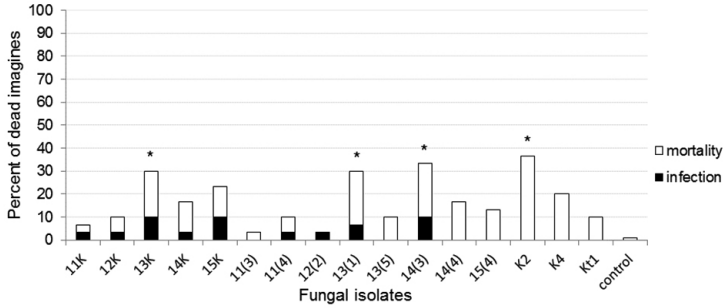
Spore viabilities of the tested strains was determined by counting germination propagules on SDA medium (Goettel and Inglis 1997).

2.3. Statistical analysis

Because the data were not normally distributed, the significance of the differences between the numbers of dead insects was calculated with the nonparametric Mann-Whitney (Wilcoxon) W test at $p<0.05$. The LT50 value was calculated with the log-probit analysis using the POLO PC programme (Russel *et al.* 1977).

3. Results

In total, we isolated 16 isolates of *B. bassiana* (4 from litter and 12 from soil), 16 of *M. anisopliae s.l.*(all from soil), 4 of *I. fumosorosea* (from soil) and 1 of *I. farinosa* (from soil). Spore viability of the individual isolates was high (95–98% of the spores germinated).



* Significant difference relative to the control at p<0.05.

Fig. 2. Mortality and infection of lesser mealworm adults after application of spores of *M. anisopliae s.l.* strains isolated from soil near the poultry house. Data represent cumulative beetle mortality. Beetle infection means overgrown with mycelium

3.1. Pathogenicity of isolated fungi to lesser mealworm beetles

Beetles of *A.diaperinus* were resistant to infection by *B. bassiana* isolates, and their mortalities ranged from 17 to 26%. Only 4 isolates caused mortality in beetles that were significantly different from the control variant (Fig. 1). Three of them were from soil. The highest number of dead beetles was observed after the infection by Naturalis-L (over 60% mortality), though the preparation was applied in a smaller dose. In only some cases of mortality, overgrowth of beetles with mycelium was observed (indicated in the figure as infection) (Fig. 1).

Beetles appeared slightly more sensitive to infections by the spores of *M. anisopliae*. Mortality in beetles differed significantly from that in the control variant only after the application of 4 isolates and ranged between 30 and 36%. The overgrowth of insects by mycelium was observed more often (Fig. 2).

The beetles were least sensitive to the isolates of *I. fumosorosea* and *I. farinosa*, with the number of dead beetles similar to that in the control. Overgrowth of the surface of dead insects by mycelium was not observed.

3.2. Pathogenicity of isolated fungi to lesser mealworm larvae and pupae

Lesser mealworm larvae were less resistant to the fungal isolates than the adult beetles. All isolates of *B. bassiana*, *M. anisopliae* and one isolate of *I. farinosa* caused 100% mortality in the larvae. Larvae infected by *I. fumosorosea* isolates showed at most 50% mortality. Table 1 presents the LT_{50} values for lesser mealworm larvae following infection by the spores of isolates whose mycelia overgrew more than 50% of the larvae. For *B. bassiana*, such results were obtained with the application of only one strain: 3K, isolated from litter and with commercial preparation Naturalis-L. The same effect was also obtained only with the *M. anisopliae* strain 2(2). The lowest LT_{50} (*ca.* 3 days) was observed following infection with commercial preparation and the highest (*ca.* 6 days) after infection with isolate 2(2) of *M. anisopliae* (Table 1).

We observed no mycelial overgrowth on dead larvae by *I. farinosa* and *I. fumosorosea*. Pupae of the lesser mealworm also appeared to be more susceptible to fungal infections than the beetles. The mortality in pupae was 100% after infection with the spores of isolates Bb3K, Ma2(2) and commercial preparation. However, the number of pupae overgrown by fungus exceeded 50% only after infections with Bb3K and commercial preparation. Differences also were seen in the LT_{50} values (Table 2). A lower LT_{50} (*ca.* 8 days) was observed after infection with the commercial preparation; higher values (*ca.* 11 days) occurred after infection with *M. anisopliae* isolate 2(2) (Table 2)

Table 1. LT_{50} values for lesser mealworm larvae after application of selected isolates of *Beauveria bassiana* (Bb), *Metarhizium anisopliae* (Ma) and commercial preparation Naturalis-L (NatL). The same letters after the brackets indicate no significant differences between treatments.

Isolate number	Number of larvae per treatment	Slope ± SE	LT50 ± confidence intervals at 95%	χ^2
Bb3K	30	7.244 ± 1.540	4.065 (3.310-5.558)a	2.61
BbNatL	30	2.157 ± 0.443	3.295 (1.294-4.589)a	10.10
Ma2(2)	30	9.974 ± 2.570	3.944 (3.183-4.352)a	9.06

Table 2. LT_{50} value for lesser mealworm pupae after application of spores of selected isolated of *Beauveria bassiana* and commercial preparation Naturalis-L (NatL).

Isolate number	Number of pupae per treatment	Slope ± SE	LT50 ± confidence intervals at 95%	χ^2
Bb3K	30	32.242 ± 5.475	11.575 (10.905-12.028)	13.69
BbNatL	30	6.743 ± 0.662	8.265 (6.834-9.583)	40.58

When comparing the LT_{50} values for larvae and pupae after application of the same isolates (Tables 1 and 2), one may note that *A. diaperinus* pupae are less susceptible to the fungal infection than the larvae.

4. Discussion and conclusions

All fungal isolate strains were characterised by relatively low pathogenicity to lesser mealworm adults. This study confirmed the results obtained by other investigators that lesser mealworm imagines are less prone to infections by the entomopathogenic fungus *B. bassiana* compared to the larvae and pupae (Geden *et al.* 1998). Out of 30 isolates of this species studied by Santoro *et al.* (2008), only 4 caused mortality in beetles exceeding 40% in 10 days if the density of the suspension was 10^8 spores/ml – the same as ours. Insect mortality, among other things, depends on the isolate applied and the dose of fungi. Fungi isolated under natural conditions from a given insect species are usually more pathogenic to that species than isolates obtained from other insect species (Poprawski *et al.* 1985). This pathogenicity of different strains was confirmed in relation to *A. diaperinus* (Steinkraus *et al.* 1991, Geden *et al.* 1998). However, some researchers found higher mortality in *A. diaperinus* beetles after infection with *B. bassiana* isolated from insects of the orders Lepidoptera (family Pyralidae) and Coleoptera (but from the family Chrysomelidae) (>60%) than with isolates from infected *A. diaperinus* (>49%) (Santoro *et al.* 2008).

In our study, the highest pathogenicity to beetles was found in 4 strains of *M. anisopliae* isolated from soil near the poultry house and in 4 strains of *B. bassiana* (one isolated from litter in the poultry house and the others isolated from soil).

Unfortunately, we did not succeed in finding a dead or infected lesser mealworm in or near the poultry house, although it is apparent from the relationship with chicken breeders that live beetles are found in these places. Though natural infections by *B. bassiana* and *M. anisopliae* are reported in poultry houses where the pest was present, e.g., in Denmark, the USA and Brazil (Steinkraus *et al.* 1991, Steenberg and Jespersen 1996, Castrillo and Brooks 1998, Alves *et al.* 2004), such infections are rare. This is an

effect of many unfavourable conditions like high temperatures and ammonia concentrations, which limit the occurrence of various organisms in a poultry house (Axtell and Arends 1990, Geden *et al*. 1998, Santoro *et al*. 2008). We found only 4 isolates of *B. bassiana* among 37 fungal strains isolated from poultry litter. Studies in Denmark showed that natural infections in poultry farms were very rare, being responsible for 5% of dead insects (adult infections by *B. bassiana*): 3.3% due to larval infections by *Paecilomyces farinosus* (now *I. farinosa*) and 1.9% from adult infections by *M. anisopliae* (Steenberg *et al.* 2001). However, with highly pathogenic isolates (obtained from infected lesser mealworm beetles), the mortality in beetles infected with *M. anisopliae* in the laboratory reached 92% (Steenberg *et al.* 2001). None of the isolates we used caused such high mortalities in beetles, but the adults were more susceptible to infection with *M. anisopliae* than with *B. bassiana*. On the other hand, we isolated only *B. bassiana* strains from the poultry litter, which indicates their greater resistance to extreme environmental conditions. In the US, this species caused a poultry house epizootic of *A. diaperinus*. Therefore, some investigators believe that this fungus can survive in intensive agricultural animal production systems (Steinkraus *et al.* 1991). Thus, it seems that this species has the greatest potential for the biological control of *A. diaperinus*.

One of the symptoms of fungal infections is the overgrowth of mycelium on the insect's surface. However, this does not always happen, especially in beetles. Some strains studied by us and used with commercial preparation Naturalis-L may serve as an example. Insect mortality after application of this preparation was relatively high, but mycelium developed on the surface of only a few dead insects. The reason for this could be that commercial preparations are prepared in a special way e.g., by the addition of chemicals that increase their viscosity and facilitate the adherence of spores to insects' bodies. Even if the strains used in the preparation were selected for application against other insect species, the spores could also infect the lesser mealworm. However, mycelium only poorly overgrew on the bodies of the infected insects.

The development of mycelium on insect surfaces is very important, such insects become the source of further infections, which facilitates the spread of the pathogen remaining in the habitat after its application in the form of a biopreparation. Therefore, we agree with the opinion of Hajek and St. Leger (1994) and Santoro *et al*. (2008) that during selection, one should choose such species or strains of entomopathogenic fungi that can develop on the surface of an insect's body.

Fungal strains isolated by us caused relatively low mortality in the beetles studied and poor development of mycelium on the surface of dead insects. Larvae of the lesser mealworm were more prone to infection by spores of the studied fungal species, with the exception of *I. fumosorosea*. It seems, however, that the pathogenicity of a given strain to resistant beetles of the lesser mealworm is the most important criterion regarding its selection. Considering the results of earlier studies (Popowska-Nowak 2003, Santoro *et al*. 2008), it seems that isolates with a high pathogenicity to *Alphitobius diaperinus* should not necessarily be searched for in the habitat of this pest. However, our study suggests that the local *B. bassiana* 3K (from litter) could be selected for further studies due to its high mortality and infectivity of larvae (100% dead, of which 50% overgrown with mycelium).

Acknowledgements

The study was financed from Ministry of Higher Education of Poland grant No 6 PO4G02116.

References

Alves L.F.A., Bressan D.F., Alves V.S., Neves P.M.O.J., Alves S.B., 2004, *Ocorrência de Metarhizium anisopliae (Metch.) Sorok. (Moniliales: Moniliaceae) em adultos de cascudinho (Alphitobius diaperinus)*

(Panzer) (Coleoptera:Tenebrionidae), em aviaries comerciais em Cascavel, PR [Natural occurrence of *Metarhizium anisopliae* (Metsch.) Sorok. on adults of the lesser mealworm (*Alphitobius diaperinus*) (Panzer) (Coleoptera: Tenebrionidae) in poultry houses in Cascavel, PR, Brazil], Neotrop. Entomol. 33, 793-795.

Avancini R.M., Ueta M.T., 1990, *Manure breeding insects (Diptera and Coleoptera) responsible for cestoidosis in caged layer hens,* J. Appl. Entomol. 110, 307–312.

Axtell R.C., Arends J.J., 1990, *Ecology and management of arthropod pests of poultry*, Annu. Rev. Entomol. 35, 101–126.

Bajan C, Kmitowa K., 1997, *Thirty years studies of entomopathogenic fungi in the Institute of Ecology PAS,* Pol. Ecol. Stud. 23, 133–154.

Bałazy S., 2000, *Zróżnicowanie grup funkcjonalnych grzybów owadobójczych* [*The diversity of functional groups of entomopathogenic fungi*] (in Polish), Biotechnologia, 3, 11–32.

Bałazy S., Majchrowicz I., 1978, *Grzyby – patogeny owadów i roztoczy* [*Fungi – pathogens of insects and mites*], in: Boczek J., Lipa J. (eds.) "Biologiczne metody walki ze szkodnikami roślin" [Biological control of plant pests] (in Polish), PWN Warszawa, 111–133.

Burakowski B., Mroczkowski M., Stefańska J., 1987, *Katalog. Fauny Polski* [*Polish fauna catalog*] (in Polish), p. XXIII, (14) p. 3, PWN, Warszawa, 309 pp.

Calibeo D.R., 2002. *Role and mitigation of two vectors of turkey coronavirus, Musca domestica L. and Alphitobius diaperinus Panzer,* Master thesis, Graduate Faculty of North Carolina State University.

Castrillo L.A., Brooks W.M., 1998, *Differentiation of Beauveria bassiana isolates from darkling beetles, Alphitobius diaperinus, using isosyme and RAPD analyses*, J. Invertebr. Pathol. 72, 190–196.

Crawford P.J., Brooks W.M., Arends J.J., 1998, *Efficacy of fields-isolated strains of Beauveria bassiana (Moniliales: Moniliaceae) as microbial control agents of the lesser mealworm (Coleoptera: Tenebrionidae),* J. Econ. Entomol. 91, 1295–1301.

Crippen T.L., Sheffield C.L., 2006, *External surface disinfection of the lesser mealworm beetle, Alphitobius diaperinus, (Coleoptera: Tenebrionidae),* J. Med. Entomol. 43, 916–923.

Crippen T.L., Sheffield C.L., Mowery S., Droleskey R.E., Esquivel J., 2009, *The acquisition and internal carriage of Salmonella by lesser mealworm beetles, Alphitobius diaperinus (Coleoptera: Tenebrionidae),* Vector- Borne and Zoonotic Diseases, 9, 65–71.

De las Casas E., Pomeroy B.S., Harein P.K., 1972, *Infestion and quantitative recovery of Salmonella typhimurium and Escherichia coli from within the lesser mealworm, Alphitobius diaperinus (Panzer),* Poult. Sci. 47, 1871–1875.

De las Casas E., Harein P.K., Deshmukh D.R., Pomeroy B.S., 1976, *Relationship between the lesser mealworm, fowl pax and Newcastle disease virus in poultry*, J. Econ. Entomol. 69, 775– 779.

Despins J.I., Turner E.C., Ruszler P.L., 1989, *Effects of poultry manure moisture and poultry house construction materials on the movements of the lesser mealworm, Alphitobius diaperinus (Panzer) (Coleoptera: Tenebrionidae), a structural insect pest in high rise caged layer houses,* Poult. Sci. 68, 1328–1331.

Eidson C.S., Schmittle S.C., Lal J.B., Goode R.B., 1965, *The role of the darkling beetle, Alphitobius diaperinus, in the transmission of acute leukosis in chickens*, Poult. Sci. 44: 1366– 1367.

Geden C.J., Arends J.J., Ruttz D.A., Steinkraus D.C., 1998, *Laboratory evaluation of Beauveria bassiana (Moniliales: Moniliaceae) against the lesser mealworm, Alphitobius diaperinus (Coleoptera: Tenebrionidae), in poultry litter, soil and pupal trap,* Biol. Control, 13, 71–77.

Geden C.J., Axtell R.C., 1987, *Factors affecting climbing and tunnelling behaviour of the lesser mealworm (Coleoptera: Tenebrionidae),* J. Econ. Entomol. 80, 1197–1204.

Goettel M.S., Inglis G.D., 1997, *Fungi: Hyphomycetes*, in: Lacey L.A. (ed.), Manual of techniques in insect pathology, Academic Press, San Diego, pp. 213–249.

Goodwin M.A., Waltman W.D., 1996, *Transmission of Eimeria, viruses and bacteria to chicks: darkling beetles (Alphitobius diaperinus) as vectors of pathogens,* J. Appl. Poultry Res. 5, 51–55.

Hajek A.E., St. Leger R.J., 1994, *Interaction between fungal pathogens and insect host.* Annu. Rev. Entomol. 39, 293–322.

Harding W.C., Bissell T.L., 1958, *Lesser mealworm in a brooder house*, J. Econ. Entomol. 51, 112

Hazeleger W.C., Bolder N.M., Beumer R.R., Jacobs -Reitsma W.F., 2008, *Darkling beetles (Alphitobius diaperinus) and their larvae as potential vectors for*

the transfer Campylobacter jejuni and Salmonella enteric a serovar Paratyphi B variant Java between successive broiler flocks, Appl. Environ. Microbiol. 74, 6887–6891.

Hickle L.A., Bradfisch G.A., Sick A.J., 1991, *Use of Bacillus thuringiensis microbe for controlling lesser mealworm, Alphitobius diaperinus*, Mycogen Cororation, San Diego, United States patent US 5,064,648.

Hinkle N.C., Hickle L.A., 1999, *California caged layer pest management evaluation*, J. Appl. Poultry Res. 8: 327–338.

Humber R.A., 1997, *Fungi: identification*, in: Lacey L.A. (ed.), Manual of techniques in insect pathology, Academic Press, San Diego, pp. 153–185.

Lancaster J.L., Simco J.S., 1967, *Biology of lesser mealworm, a suspected reservoir of avian leucosis,*. University of Arcansas Agricultural Experiment Station, Report No. 159, Fayetteville.

Lorenzo P., 1990, *Application of 60 Co gamma radiation to control insects in stored rice. Part IV. Lasioderma serricorne (Anobiidae: Coleoptera), Alphitobius diaperinus and Tenebrionido sp. (Tenebrionidae: Coleoptera)*, Ciencia y Tecnica en la Agricultura, serie Arroz, 13, 67–76

Majchrowicz I., 1985, *Alphitobius diaperinus (Panz.),* Med. Weter. 6, 336–338.

McAllister J.C., Steelman C.D., Skeels J.K., 1994, *Reservoir competence of the lesser mealworm (Coleoptera: Tenebrionidae) for Salmonella typhimurium (Eubacteriales: Enterobacteriaceae)*, J. Med. Entomol. 31, 369–372.

McAllister J.C., Steelman C.D., Skeeles J.K., Newberry L.A., 1996, *Reservoir competence Alphitobius diaperinus (Coleoptera: Tenebrionidae) for Escherichia coli (Eubacteriales: Enterobacteriaceae),* J. Med. Entomol. 33, 983–987.

Popowska-Nowak E., 2003, *Effects of various species and isolates of entomopathogenic fungi on Alphitobius diaperinus (Coleoptera: Tenebrionidae)*, IOBC wprs Bulletin, 26, 107–111.

Poprawski T.J., Marchal M., Robert P.H., 1985, *Comparative susceptibility of Otiorhynchus sulcatus and Sitoma lineatus (Coleoptera, Curculioniodae) early stage to five entomopathogenic Hyphomycetes*, Environ. Entomol. 14, 247–253.

Rueda L.M., Axtell R.C., 1997, *Arthropods in litter of poultry (broiler chicken and turkey houses)*, J. Agr. Entomol. 14, 81–91.

Russell R.M., Robertson J.L., Savin N.E., 1997, *POLO: a new computer program for probit analysis*, Bull. Entomol. Soc. Am. 23, 209–213.

Samson R.A., Evans H.C., Latge J.P., 1988, *Atlas of entomopathogenic fungi*, Springer-Verlag, Berlin, Germany, 187 pp.

Santoro P.H., Neves P.M.O.J., Alexandre T.M., Sartori D., Alves L.F.A., Fungaro M.H.P., 2008, *Selection of Beauveria bassiana isolates to control Alphitobius diaperinus,* J. Invertebr. Pathol. 97, 83–90.

Steelman D., 1996, *Darkling beetles are costly pests*, Poultry Digest, 55, 22–23

Steenberg T., Jespersen J.B., 1996, *Entomopathogenic fungi for control of litter beetles. Danish pest infestation laboratory*, Annual Report, 39: 65.

Steenberg T., Jensen K-M. V., Jespersen J.B., 2001, *Microbial control of hygiene pests.* DJF- rapport, 49, 101–104.

Steinkraus D.C., Geden C.J., Rutz D.A., 1991, *Susceptibility of lesser mealworm (Coleoptera: Tenebrionidae) to Beauveria bassiana (Moniliales: Moniliaceae): effect of host stage, substrate, formulation and host passage,* J. Med. Entomol. 28, 314–321.

Vaughan J.A., Turner E.C., Ruszler P.L., 1984, *Infestation and damage of poultry insulation by the lesser mealworm Alphitobius diaperinus (Panzer.),* Poultry Sci. 63, 1094–1100.

Voris J.C., Meyer J.A., Pfost R., Woodbury R., 1994, *Temperature affects lesser mealworm population in turkey brooder houses,* Calif. Agr. 48, 18–21.

Wójcik A.R., Grygoń-Francewicz B., Szczepanik M., Żbikowska E., Wasilewski L., 2000, *Inwazja pleśniakowca lśniącego, Alphitobius diaperinus Panzer, w niektórych fermach brojlerów regionu kujawsko-pomorskiego* [The lesser mealworm, *Alphitobius diaoerinus* Panzer, invasion in some poultry houses in the Pomorsko-Kujawskie region] (in Polish), Mag. Wet., Supl. Drób IV, 68–70.

Zimmerman G., 1986, *The "Galleria bait method" for detection of entomopathogenic fungi in soil,* J. Appl. Entomol. 102, 213–215.

Wrażliwość pleśniakowca lśniącego *Alphitobius diaperinus* Panzer (Coleoptera: Tenebrionidae) na infekcje grzybami owadobójczymi izolowanymi ze ściółki i gleby wokół brojlerni

Streszczenie

Pleśniakowiec lśniący (*Alphitobius diaperinus*) Panzer występuje w dużych liczebnościach w brojlerniach. Owady te są szczególnie niebezpieczne jako potencjalne nośniki patogenów, takich jak bakterie, wirusy i pasożyty. Badania dotyczyły możliwości wykorzystania lokalnych szczepów grzybów entomopatogennych izolowanych ze ściółki i gleby do ograniczania populacji tego owada. Izolowane grzyby wykazywały niską patogeniczność w stosunku do chrząszczy owada. Po infekcji zawiesiną w stężeniu 1×10^8 zarodników/ml tylko 4 izolaty *Metarhizium anisopliae sensu lato* wykazywały najwyższą śmiertelność owadów w zakresie 30–36%. Jeszcze mniejszą patogeniczność w stosunku do chrząszczy owada wykazywały izolaty *Beauveria bassiana*, tylko 4 z nich powodowały śmiertelność w zakresie 17–26%. Izolaty *Isaria fumosorosea* i *I. farinosa* powodowały śmiertelność chrząszczy na poziomie wariantu kontrolnego. Larwy były bardziej podatne na infekcję. Z wyjątkiem *I. fumosorosea* wszystkie badane gatunki grzybów powodowały 100% śmiertelność larw. Do dalszych badań można wyselekcjonować izolat *B. bassiana* 3K (ze ściółki) ze względu na wysoką śmiertelność (100%) i wysoką infekcyjność larw (50% porośniętych grzybnią).

Słowa kluczowe

pleśniakowiec lśniący *Alphitobius diaperinus*, kurniki, grzyby entomopatogeniczne, biologiczne zwalczanie