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Pathogenicity of four fungal species to Indian meal moth *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae)

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The effect of four fungal species (*Beauveria bassiana* (*B. b.*), *Lecanicillium* (*Verticillium*) *lecanii* (*L. l.*), *Metarhizium anisopliae* var *anisopliae* (*M. a.*) and *Paecilomycers farinosus* (*P. f.*)) isolates from forest soil in Lithuania was tested on adults and one species tested on mature larvae of Indian meal moth, *Plodia interpunctella* Hübner (Lepidoptera: Pyralidae). Under laboratory conditions, the insects were sprayed with conidial suspension (concentration 2.6×10^6 ml^{-1} conidia/ml). All the fungal isolates tested were pathogenic, however, with a different dynamics of their effect. During the first-three-day period after spraying, the highest mortality (35–40% versus control) caused by *P. f.* and *M. a.*, and there was no significant difference in the survival as compared to control when *B. b.* and *L. l.* were sprayed. The median lethal time period when mortality reached 50% (LT_{50}) or 100% (LT_{100}) varied depending on fungus species from 1 to 5 days and from 9 to 12.3 days, respectively. *P. f.*, was effective to *P. interpunctella* adults, but not to larvae of the species, as during 14 days of testing no 50% of mortality was reached.

Key words: insects, entomopathogenic fungi

INTRODUCTION

Interactions among organisms in nature are the main research tasks in ecology. This concerns interactions between entomopathogenic fungi and insects as well. At least 90 genera and more than 700 species of fungi, dispersed in various taxonomic groups, have been identified as insect pathogens (Roberts, Humer, 1981; Inglis et al., 2001). Most genera of the entomopathogenous fungi belong either to the class Entomophthorales in the phylum Zygomycota or to the class Hyphomycetes in the phylum Deuteromycota (Samson et al., 1988; Bałazy, 1993; Pell, 2003). Insect-pathogenic fungi have been extensively studied as key regulatory factors in insect populations and as agents for biocontrol (Roberts, Humer, 1981; Samson et al., 1988; Inglis et al., 2001; Quesada-Moraga et al., 2006). During the last four decades, ca. 80 companies worldwide developed and / or manufactured 144 mycoinsecticides (Faria, Wraight, 2007). However, approximately only 13 species or subspecies of fungi have been used as active ingredients of those mycoinsecticides, the most common being *Metarhizium anisopliae* (Metschn.) Sorokin (53 products), *Beauveria bassiana* (Bals.-Criv.) Vuill. (46), *Lecanicillium* spp. (11), *Paecilomyces fumosoroseus* (Wize) A. H. S. Br. & G. Sm. (8), and *Beauveria brongniartii* (Sacc.) Petch. (7 products). Several commercial products are based on the *Verticillium lecanii* (Zimm.) Viégas strains isolated from cosmopolitan insect hosts (see Mini Review of Shah and Pell, 2003).

Fungal species, and sometimes even their strains or isolates, behave in a very different way depending both on biotic (insect host range, infection level, germination rate) and abiotic (humidity and especially temperature) factors (Sierotzki et al., 2000; Todorova, 2000, 2002; Pell et al., 2001; Shaw et al., 2002; Lanza et al., 2004; Aslantas et al., 2008). Entomopathogenic fungi exhibit a high genetic variability, thus being able to inhibit pest resistance to the pathogens. The stability of their pathogenic effect depends on the species as well as on the strain and / or their geographical origin (Bidochka et al., 2005; Rehner, Buckley, 2005; Thakur et al., 2005; Meyling, Eilenberg, 2007; Gauthier et al., 2007). Like any other organisms, both fungi and insects adapt themselves to their habitat conditions. Thus, searching for the most efficient fungal strains suitable to control some local insect populations (mycoinsecticides), it seems reasonable to carry out a search under natural conditions. In Lithuania, still no mycoinsecticide has been registered, as the data available from the State Plant Protection Service indicate (State Plant Protection Service: http://www.vaat.lt/index.php). Soil is a natural reservoir of insect-infecting fungi; therefore, many fungal species as potential pathogens of insects have been isolated from soil. Investigations of soil microorganisms revealed 72 strains belonging to 9 genera of entomopathogenic fungi have been isolated by the end of 2006 in Lithuania (Pečiulytė, Dirginčiutė-Volodkienė, 2008). The biological properties of these fungi were investigated extensively, but not their interactions with insects. None of alive fungal collections living in Lithuania have been systematically tested for fungal strains' potential as mycoinsecticides.

To establish the pathogenic efficiency of isolated local fungi strains, a comparison with standard strains available from a fungal bank is needed. However, before starting such comparisons, preliminary data on the entomopathogenicity of the isolated strains is needed. To establish this for a few local fungus isolates is the main aim of the present paper. The Indian meal moth *Plodia interpunctella* Hübner (Lepidoptera: Pyralidae), either at the adult or larval stages, was chosen as the test object. The insect species is widespread and known as pest of stored food products of plant origin in many countries, including Lithuania.

MATERIALS AND METHODS

Fungi. The fungal species and strains used in this study were the following: *Beauveria bassiana* (strain DPK-02-d), *Lecanicillium* (= *Verticillium) lecanii* (strain DPK-08-d), *Metarhizium anisopliae* var. *anisopliae* (strain DPK-06-d) and *Paecilomyces farinosus* (DPK-12-d). They were isolated from forest soils in Lithuania (*B. bassiana* and *L.* (*V.*) *lecanii* in Kėdainiai district, *M. anisopliae* and *P. farinosus* in Vilnius district). Fungal strains were maintained in the collection of live cultures at the Institute of Botany (Vilnius, Lithuania). To obtain conidia for bioassays, all isolates were cultured in Petri dishes on PDA (potato-dextrose-agar) medium for 14 days at 25 ± 1 °C and used immediately or stored at 4 °C for 3–4 days. Conidia were harvested by scraping the surface of agar plates with a sterile loop and by suspending conidia in sterile distilled water. The content was passed through two layers of cheesecloth to remove any large particles and hyphae, and then conidia were suspended using a vortex. Before each assay, a hemacytometer was used to determine the concentration of conidia in the mixture. The concentration of viable conidia was calculated as germination percentage × the concentration of conidia established by means of a hemocytometer. For estimating the conidial germination, 0.5 ml aliquot from each mixture was spread on water-agar medium in four Petri dishes with a Drigalski spatula. The dishes were incubated at 20 ± 1 °C. After 24 h the percentage of germinated conidia was counted. Conidial mixtures were diluted to obtain a necessary concentration of viable conidia in each fungal suspension used for the bioassay. For insect infection, we used suspensions of 2.6×10^9 conidia/ml were used. The dosage applied was 1 ml per cage with 10 insects.

Insects. Indian meal moths were reared under laboratory conditions on natural diet (mix of oat flakes, corn, raisins) in glass vials 0.75 l in volume. A single vial contained ca 100 g of diet, and 5 pairs of moths were introduced for egg laying. The culture was maintained in thermostatic chambers in the dark at 25 ± 0.6 °C and 55 ± 10% relative humidity. Stage 4 larvae or freshly emerged adults were used for testing.

Bioassay. Either adults or larvae of *P. interpunctella* were exposed to the fungal conidia in glass cages 750 cm^3 in volume. A single cage contained either 10 adult insects or 10 larvae. Three replications of each treatment and control were used. Fungal suspensions (test) and distilled water (control) were sprayed using a glass spray apparatus. Both adults and larvae were treated with the same concentration and dosage of conidia (2.6×10^6 conidia/ml vu). A cage was sprayed with one ml of a test fungus suspension (2.6×10^9 conidia/ml) or 1 ml of distilled water (control). Adults were sprayed in the cages and larvae were moved into the cages already sprayed either with fungus conidia or distilled water (control). Adult insects were treated with all four fungal isolates tested and the larvae only with the fungus *Paecilomyces farinosus* (strain DPK-12-d), i. e.with species most virulent for adults of Indian meal moth adults. The cages of both tests were kept under 20 ± 1 °C, 65 ± 5% relative humidity (RH) and a 16 : 8 h light / dark photoperiod. Each cage was supplied with a cotton lump moistened with distilled water to achieve a higher humidity inside the cage. The survival of adults and larvae was recorded daily during two weeks. Cadavers of adults and larvae were removed from the cages, placed on moistened filter paper in a Petri dish and kept at 20 ± 1 °C for observation of fungal extrusion. To establish whether mortality in Indian meal moth adults and larvae had been caused by the test fungi, all cadavers were checked under a microscope, and presence or absence of hyphae and conidia on cadaver surface was recorded. The LT_{50} and LT_{100} were calculated.

Statistical analysis. To obtain statistically significant data, groups of 10 insects per test were used for spraying. Every test and control was performed in 3 replicates. Standard error was estimated for every experimental point and marked in Figures as an error bar. Statistical differences in viability were evaluated according to Student's *t* criterion. The median lethal time period (LT_{50}) and 100% lethal time period (LT_{100}) were determined from regression lines (Sokal, Rohlf, 1995).

RESULTS

The viability of all fungi conidia incubated on water agar for 24 h at 20 °C ranged from 95.6 to 98.7% and remained stable when maintained for 2–3 weeks at 4 °C; therefore, it could be used for the preparation of conidial suspensions repeatedly as inoculums to continue the tests.

Effect on *P. interpunctella* adults. All four fungal isolates caused a higher mortality of *P. interpunctella* adults as compared to that in control. However, there were differences in the dynamics of survival depending on the fungal isolate applied.

During the initial two-day period after treatment, a high mortality was observed in the *P. farinosus* and *M. anisopliae* fungi test. The survival of moths varied from 50% to 40%, i. e. was by 40% to 35% lower as compared to that in control ($P < 0.05$). During this period, there was no effect of spraying with *B. bassiana* and *L. lecanii* as compared to control, and *P. farinosus* was the most virulent fungus.

A significant effect of all fungi isolates tested was revealed starting with the third day of the test (Fig. 1). The time period

Table 1. **LT_{50} and LT_{100} mortality of *P. interpunctella* adults sprayed with 2.6×10^6 fungus conidia / ml vu of the culture**

Fungi tested	Effect	
	LT_{50} (days)	LT_{100} (days)
Beauveria basiana	5.0	12.3
Lecanicillium (*V.*) *lecanii*	4.5	–
Metarhizium anisopliae var. *anisopliae*	3.2	9.0
Paecilomyces farinosus	1.0	11.1

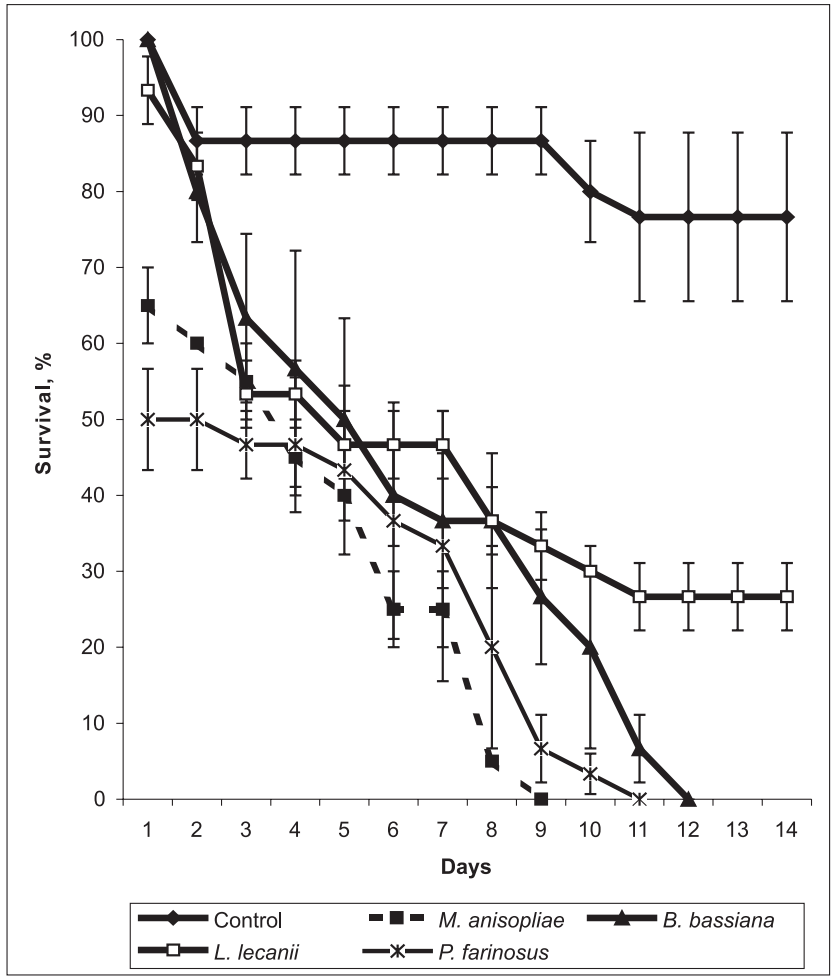

Fig. 1. Survival dynamics of adult *P. interpunctella* moths in the control and treatment following application of different fungal cultures

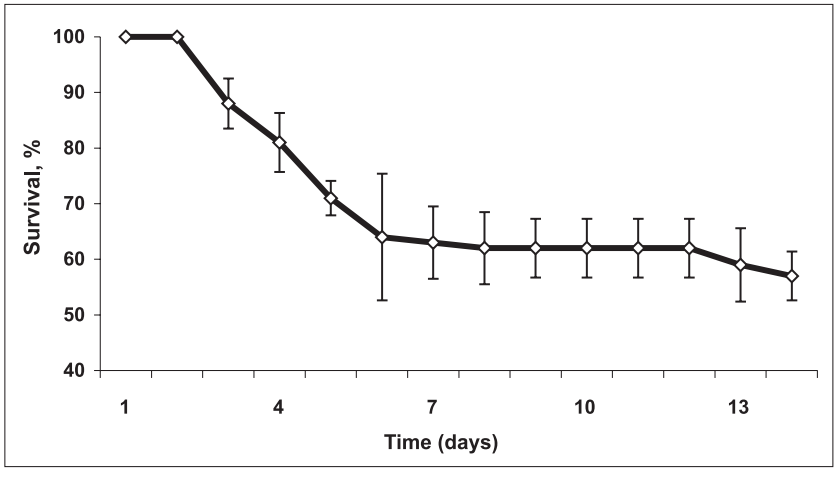

Fig. 2. Survival dynamics of *P. interpunctella* larvae following *Paecilomycers farinosus* application. The survival was counted as a difference between control and treatment. Vertical bars represent standard error

required to cause 50% insect mortality (median lethal time, LT_{50}) ranged from 1.0 to 5.0 days depending on the isolate applied (Table 1). The shortest LT_{50} was recorded for *P. farinosus* isolate and the longest ones for those of *B. basiana* and *L. lecanii.* Among the four fungi strains tested, only *L. lecanii* did not increase mortality in adults during this 3-day period (from 5th to 7th day after spraying), remaining at the LT_{50} level (Fig. 1).

The lethal time period when 100% mortality was recorded differed depending on the fungi. LT_{100} ranged from 9.0 to 12.3 days for three fungal isolates (Table 1) and was not established for *L.* (*V.*) *lecanii* as it exceeded the test period of two weeks. Spraying of the latter species reduced the survival of *P. interpunctella* adults rapidly (within 3 days after spraying) from 100% to ca 55% (Fig. 1), however, later the dynamics of the process changed, resulting in a significantly lower total mortality of insects as compared to any fungal isolate tested ($P < 0.001$ starting with day 10 of exposure).

Effect on *P. interpunctella* larvae. As the experiment on adult moths of *P. interpunctella* revealed the highest mortality caused by *P. farinosus* and *M. anisopliae* isolates, the isolate of fungus *P. farinosus* was chosen to test its effectiveness towards the larval stage of the insect species.

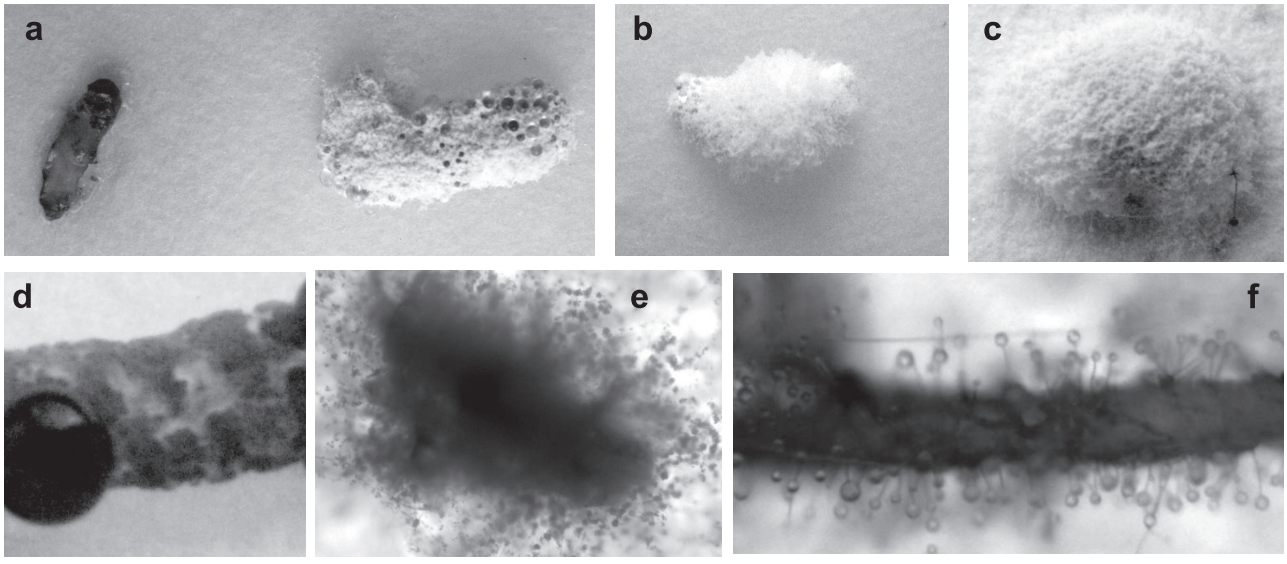

Fig. 3. Symptoms of fungus-infected Indian meal moth *P. interpunctella* (adults or larvae): *A* – cadavers of larvae (left – contro, right – infected with *B. bassiana*); *B* – cadaver of larva; *C* and *E* – cadavers of adults infected by *L. lecanii, P. farinosus* and *B. bassiana*, respectively; *D* – fragment of larval cadaver; *F* – segment of adult insect cadaver infected by *M. anisopliae*

The dynamics of larval survival following spraying with *P. farinosus*, based on daily counts and calculation as a difference in viability between control and test groups, is presented in Fig. 2. The larvae were susceptible to fungus *P. farinosus*, however, the same conidial suspension established as effective towards the adult stage of the insect was not effective enough towards the larval stage of the same insect species. During the two-week period, even a 50% mortality (LT_{50}) of larvae was not reached. The survival during six days after spraying decreased approximately to 62% and remained nearly the same until the next one-week period of observations, with a very slow decreasing trend. Only approximation allowed to evaluate the lethal time period required to achieve a 50% mortality (LT_{50}), and it was found to be ca 15 days.

Analysis of cadavers. Disease symptoms and analysis of cadavers suggested that both larvae and adults of *P. interpunctella* (95% and 100% of those analysed, respectively) were attacked by fungi, and it was fungal infection that reduced their survival. Some larvae tended to succumb to bacterial infection as well. All the four fungi tested infected *P. interpunctella* adults and utilized nearly all their internal organs by the time of insect death. The life cycle of any entomopathogenic fungus starts with a conidia attachment to the host cuticle, formation of an aspersorium, followed by a penetration peg and entering a cuticle (Scholte et al., 2004). After infection, a fungus produces hyphae inside the insect's tissues and invades its internal organs, causing death. Diseased insects, depending on the fungus, changed their coloration from white to green and became progressively darker, respectively fluffy white or smooth yellow in case of infection with *M. anisopliae* var. *anisopliae*, *B. bassiana* and *P. farinosus* (Fig. 3).

DISCUSSION

The great majority of insect pathogens were isolated from cadavers of soil-inhabiting insects or those found in litter. Successful isolation of entomopathogenic fungi from soil samples was carried out by adding Zn ions to the isolation media (Pečiulytė, 2001; Pečiulytė, Dirginčiutė-Volodkienė, 2008). More than 75–80% of Zn-resistant fungi isolated from various soil samples were attributed to potential entomopathogens. In populations of Zn-contaminated soil fungal species from the genera *Beuveria, Metarhizium*, *Paecilomyces* and *Verticilium* prevailed (Pečiulytė, 2001; Pečiulytė, Dirginčiutė-Volodkienė, 2008). The ento-

Table 2. **Insect species from the Pyralidae family (Lepidoptera) recorded as hosts of four fungal species (after Humber and Hansen, 2005)**

Host insect	Fungus species isolated			
	B. bassiana	***L. (V.) lecanii***	***M. anisopliae***	***P. farinosus***
Lepidoptera: Pyralidae	*Acigona sp.* *(2)			
	Cnaphalostrismedinalis (1)			
	Coniesta sp. (1)	None		
	Diatrea saccharalis (8)			
	Dioryctria sylvestrella (1)			
	Galleria mellonella (34)		*Galleria mellonella* (8)	*Galleria mellonella* (1)
	Gymnancyla canella (1)			
	Ostrinia nubilalis (3)		*Ostrinia nubilalis* (1)	*Ostrinia nubilalis* (1)

* The number in brackets indicates how many fungus records on the cadavers of the corresponding insect species have been registered.

mopathogenicity of local isolates of four fungal species identified from soil samples collected in Lithuania was tested, and the first data on their effect on pyralid moth, *P. interpunctella*, were presented. According to the catalogue of entomopathogenic fungi (Humber, Hansen, 2005), the fungal species we had tested were known as capable to affect Lepidopteran insects from 25 families. Comparing this number with the total number of families equal to approximately 130 within the order Lepidoptera (Scoble, 1995), we can conclude that the spectrum of the potential target Lepidopteran groups still remains quite poorly investigated. Based on the data of the catalogue, the fungal species we have tested are known to be able to infest Lepidopterans from 25 families: *B. bassiana* those from 19 families, *L. lecanii* from 3 families, *M. anisopliae* from 10 and *P. farinosus* from 11 families. As for insects from the family Pyralidae, data on the entomopathogenicity of the fungal species we have tested remain to be very scarce. These data are presented in Table 2.

Based on the records presented in the Table, one can conclude that data on the pathogenic effect on insects from the family Pyralidae caused by the fungi are scarce. As far as we know, the entomopathogenic effect of the fungal species used in our test was not established for *P. interpunctella*, although this insect species is an economically important pest of stored plant products with a worldwide distribution. Insecticide application with the aim to control this pest in storage and other facilities is undesirable, so search for biological control means is needful.

Among isolates of the fungal species used in our tests, two belong to species widely used as biological control agents. Those are *B. bassiana* and *M. anisopliae* (Faria, Wraight, 2006, 2007; Thomas, Read, 2007). It is interesting to note that our results indicate *B. bassiana* being not among the most effective fungus towards *P. interpunctella* moths. *L. lecanii* and *M. anisoplia* caused more lethal effects as compared with those caused by *B. bassiana.* It indicates *B. bassiana* to be not among the fungal species potentially suitable for a successful application in *P. interpunctella* control programs.

Fungi of the four study species tested (*B. bassiana*, *L.* (*V.*) *lecanii*, *M. anissopliae* and *P. farinosus*) were isolated from cadavers of *P. interpunctella* for the first time. Results obtained in our tests revealed a high level of pathogenicity of local isolates of the three fungal species tested (*B. bassiana*, *L. lecanii*, and *M. anisopliae*) to adults of Indian meal moth, *P. interpunctella*. However, their effect differs in time. In the period from 1 to 3 days after spraying, the most pronounced lethal effect was recorded for *P. farinosus* and *M. anisopliae*. A hundred percent lethal effect was recorded on 9, 11 and 12 days for *M. aniso-pliae, P farinosus* and *B. bassiana* correspondingly. Among the four fungal isolates tested, *L. lecanii* was least effective towards adults *P. interpunctella* adults.

It should be noted an essential difference in effects caused by the fungus depending on the stage of a target insect. For example, *P. farinosus*, though recorded as one of the most pathogenic fungi for Indian meal moth adults, was little effective for larvae of the same species. In our test, 4th instar larvae were used. They were mature enough, not feeding and ready to start pupation. Thus, infection with fungi by feeding was limited. We assume that the difference in adult / larva infestation could be attributed to differences in cuticle composition, which influences spore attachment.

Analysis of publications as well as data obtained in the present research indicate the lack of entopmopathogenic fungi effective enough for pyralid *P. interpunctella*. Furthermore, there are very few fungal species toxic for many other insect pests of economic importance from the family Pyralidae. A search for such pathogens is necessary. The possible differences in the interaction of fungi with adult and larval stages of the same species, as demonstrated in the case of *P. farinosus* and Indian meal moth *P. interpunctella,* should be taken into consideration.

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KETURIŲ MIKROMICETŲ RŪŠIŲ PATOGENIŠKUMAS PIETINIAM UGNIUKUI, *PLODIA INTERPUNCTELLA* (LEPIDOPTERA, PYRALIDAE)

S a n t r a u k a

Tirtas keturių grybų rūšių (*Beauveria bassiana* (*B. b.*), *Lecanicillium* (*Verticillium*) *lecanii* (*L. l.*), *Metarhizium anisopliae* var *anisopliae* (*M. a.*) ir *Paecilomycers farinosus* (*P. f.*)), išskirtų iš miško dirvožemio Lietuvoje, poveikis *Plodia interpunctella* (Lepidoptera: Pyralidae) drugių suaugėliams ir vienos iš šių grybų rūšies poveikis vikšrams. Laboratorijos sąlygomis veikiant 2.6×10^6 ml^{-1} konidijų/mL suspensija parodyta, kad visos tirtos padermės yra patogeniškos, tačiau jų veikimo dinamika skirtinga. Per 1–3 dienas po kontakto su grybais apdorotu paviršiumi didžiausius letalius efektus suaugėliams (35–40%, lyginant su kontrole) sukelia *P. f.* ir *M. a.*, tuo tarpu *B. b.* ir *L. l.* poveikis nuo kontrolės nesiskiria. Laikas, per kurį letalus poveikis pasiekia 50% (LT_{50}) arba 100% (LT_{100}), įvairavo priklausomai nuo grybo rūšies atitinkamai nuo 1 iki 5 dienų ir nuo 9 iki 12,3 dienos. *L. l.* nesukėlė 100% mirtingumo drugiams per 14 testavimo dienų. Parodyta, kad *P. f.*, efektyviai veikiantis *P. interpunctella* suaugėlius, yra mažai efektyvus šios rūšies vikšrams: per 14 dienų nesukėlė 50% mirtingumo.

Raktažodžiai: mikromicetai, vabzdžiai, entomopatogeniniai grybai