

BIOASSAYS WITH ISOLATES OF BEAUVERIA BASSIANA (BALS.) VUILL. AND PAECILOMYCES FARINOSUS (HOLM.) BROWN & SMITH AGAINST IPS SEXDENTATUS BOERNER AND IPS ACUMINATUS GYLL. (COLEOPTERA: SCOLYTIDAE)

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Abstract: Bioassays with conidial suspensions (1×10^8 conidia/ml) of isolates of the entomopathogenic fungi *Beauveria bassiana* (Bals.) Vuill. and *Paecilomyces farinosus* (Holm.) Brown & Smith have been proved against adults of *Ips sexdentatus* Boer. and *Ips acuminatus* Gyll. (Coleoptera: Scolytidae). Lethal effect of each fungal isolate was estimated by values of the cumulative mortality (%) in treated populations during ten days.

It has been established that the isolate 426 of *B. bassiana* caused the highest lethal effect to adults of *Ips sexdentatus* – 96.67%, following by isolates 412 and 422 of *B. bassiana* – 90.67% and 89.33%, respectively. The isolate 426 of *B. bassiana* was obtained into pure culture from imago of *Ips sexdentatus*. For the first time in Bulgaria the fungal pathogen *B. bassiana* has been found to occur in natural populations of the pest. Isolates 290 and 290re of *P. farinosus* showed less lethal effect to the same host – 45.00% and 66.67%. It has been established that adults of *Ips acuminatus* were not susceptible to the studied fungal isolate 290 of *P. farinosus*. The isolates 412, 422 and 426 of *B. bassiana* were highly virulent to adults of *Ips sexdentatus*. Their values of the median lethal time (LT₅₀) varied within overlapped narrow confidence intervals from 4.374 to 4.867, from 3.659 to 6.112 and from 4.110 to 4.550 days (at p -level < 0.05). Significant differences between virulence of *B. bassiana* isolates have not been proved. Isolates of *B. bassiana* could be distinguished from *P. farinosus* isolates according to their virulence to *Ips sexdentatus* evaluated as median lethal time.

Key words: entomopathogenic fungi, *Beauveria bassiana*, *Paecilomyces farinosus*, *Ips sexdentatus*, *Ips acuminatus*, lethal effect, virulence

С. ДРАГАНОВА, Д. ТАКОВ¹, Д. ДОЙЧЕВ², Институт за защита на растенията, 2230 Костинброд; ¹Институт по зоология, 1000 София; ²Лесотехнически университет, 1756 София. БИОЛОГИЧНО ИЗПИТВАНЕ НА ИЗОЛАТИ НА BEAUVERIA BASSIANA (BALS.) VUILL. И PAECILOMYCES FARINOSUS (HOLM.) BROWN & SMITH СПРЯМО IPS SEXDENTATUS BOERNER И IPS ACUMINATUS GYLL. (COLEOPTERA: SCOLYTIDAE)

Резюме: Проведени са проучвания с конигиални суспензии (1×10^8 конигии/ml) на изолати на ентомопатогенните гъби *Beauveria bassiana* (Bals.) Vuill. и *Paecilomyces farinosus* (Holm.) Brown & Smith спрямо възрастните насекоми на короядите *Ips sexdentatus* Boer. и *Ips acuminatus* Gyll. (Coleoptera: Scolytidae). Леталният ефект на всеки гъбен изолат е оценяван чрез смъртността (%) в третираната популация за 10 дни.

Установено е, че изолат 426 на *B. bassiana* предизвиква най-висок летален ефект спрямо имагото на *Ips sexdentatus* – 96,67%, следван от 412 и 422 на *B. bassiana* – 90,67% и съответно 89,33%. Изолат 426 на *B. bassiana* е получен в чиста култура от възрастно насекомо на *Ips sexdentatus*. Намирането на гъбния патоген *B. bassiana* в природна популация на неприятеля е първо съобщение за България. Изолати 290 и 290re на *P. farinosus* показват по-нисък летален ефект спрямо същия гостоприемник (45,00% и 66,67%). Установено е, че възрастните индивиди на *I. acuminatus* не са чувствителни спрямо изолат 290 на *P. farinosus*.

Изолати 412, 422 и 426 на *B. bassiana* са високовирulentни спрямо имагото на *I. sexdentatus* със стойности на средното летално време (LT₅₀), вариращи в препокриващи се доверителни

интервали съответно: от 4,374 до 4,867, от 3,659 до 6,112 и от 4,110 до 4,550 дни (при $p < 0,05$). Статистически не се доказват разлики между вирулентността на изпитаните изолати на *B. bassiana*. Изолатите на *B. bassiana* могат бъдат разграничени от изолатите на *P. farinosus* според тяхната вирулентност спрямо *I. sexdentatus*.

Ключови думи: ентومопатогенни гъби, *Beauveria bassiana*, *Paecilomyces farinosus*, *Ips sexdentatus*, *Ips acuminatus*, летален ефект, вирулентност

The bark beetles (Coleoptera: Scolytidae) are widely recognized as one of the most damaging groups of forest insects. They require weakened or recently felled tree boles for reproduction but they could infest health trees as well, especially when climate conditions promote outbreak in pest population density. Some years ago about 300 ha stand of spruce in the forest reserve Bistrishko branishte have been destroyed due to damages caused by *Ips typographus* (L.) (Georgiev, 2006).

Biology of the bark beetles complicates their control. In order to avoid application of toxic pesticides now efforts are directed to searching for natural enemies of the pests – predators and parasitoids, nematodes and entomopathogens and combination of some of them with pheromone traps (Vaupel and Zimmermann, 1996; Kreutz et al., 2001). During several years of investigations, different infections with pathogens were found in bark beetles – viroses (Entomopoxvirus), mycoses (Deuteromycota and Ascomycota) and protozoan infections (Rhizopoda and Sporozoa) (Fuhrer and Purrini, 1981; Wegensteiner and Weiser, 1995, 1998, 2005; Ryall, 1997; Handel et al. 2003; Sosnowska et al., 2004; Takov et al. 2006).

Beauveria bassiana (Bals.) Vuillemin was the fungal pathogen frequently occurring in populations of bark beetles while species *Paecilomyces fumosoroseus* (Wize) Brown et Smith and *Metarhizium anisopliae* (Metsch.) Sorokin appeared only in single samples (Wegensteiner, 1992; Sosnowska et al., 2004; Jurc and Milijasevic, 2006). Bioassays with conidia of *B. bassiana* against *Scolytus scolytus* F. and *Trypodendron lineatum* Ol. (Doberski, 1981 a, b) or against *I. typographus* (Wegensteiner, 1992, 1996; Kreutz et al., 2004 a) have been proved under various conditions – at different temperatures, relative humidities and spore concentrations. Wegensteiner (1992) compared *Beauveria* spp. according to their efficacy against *I. typographus* and established that *B. bassiana* showed the best results. Wegensteiner (1996) in laboratory evaluation of *B. bassiana* against adults of *I. typographus* concluded that a concentration of 3×10^6 conidia/cm² bark seemed to be a sufficient infection dose to kill more than 90% of beetles.

Similar studies on the lethal effect of *B. bassiana* or another entomopathogenic fungi against representatives from family Scolytidae except *I. typographus*, *S. scolytus* and *T. lineatum* have not been found.

In Bulgaria there are not any records deal with bark beetles as hosts of the entomopathogenic fungi in bioassays with them.

The aim of the study was to establish the lethal effect of one isolate of *Paecilomyces farinosus* (Holm.) Brown & Smith to adults of *Ips acuminatus* Gyll. (Coleoptera: Scolytidae) and to estimate the virulence of three isolates of *B. bassiana* and by one isolate and re-isolate of *P. farinosus* to adults of *Ips sexdentatus* Boer.

MATERIALS AND METHODS

Bioassays were conducted under laboratory conditions in 2005–2006.

Two isolates of *B. bassiana* (412 and 422) and 290 of *P. farinosus* used in the study were from the Culture Collection of Entomopathogenic Fungi maintained in the Department of Biological and Integrated Pest Control, Plant Protection Institute, Bulgaria. The isolates 412 and 422 of *B. bassiana* were initially isolated from caterpillars of *Cydia pomonella* L. (Lepidoptera: Tortricidae), 290 of *P. farinosus* – from imago of *Leptinotarsa decemlineata* Say (Coleoptera: Chrysomellidae). The isolate 290re of *P. farinosus* have been obtained from dead adult of *I. sexdentatus* after laboratory bioassays with 290 of *P. farinosus* against the same insect host. The isolate under number 426 used in bioassays was isolated into pure culture on SDAY (Sabouraud dextrose agar with yeast extract) from dead adult of *I. sexdentatus* found in pine bark in the Malashevskia Mountain in 2006. Fungal pathogen was identified according to Samson et al. (1988) and Humber (1997) according to morphological characteristics as on the host as on a culture.

Conidia of the isolates used as infectious units in bioassays were obtained after fifteen-days cultivation on SDAY in tubes at 25° C following by washing down with sterilized water. The concentrations of conidia were determined by counting in haemocytometer. Aqueous suspensions applied in bioassays were prepared by dilution at concentration of 1×10^8 conidia/ml.

Adults of *I. sexdentatus* and *I. acuminatus* used in bioassays were from natural populations of the pests collected from pine bark in Maleshevskia Mountain. They were treated by a surface contact with 1 ml of conidial suspensions for 24 h placed on filter paper discs (100 mm in diameter) in Petri dishes (Draganova and Staneva, 1988). Experiments were carried out in three replicates with 20–30 adults per replicate at temperature $25 \pm 2^\circ$ C and 60% RH. Insects were fed on pine bark 24 h after the treatment. Adults in control variants were treated with water instead of conidial suspension.

Insect mortality was noted daily for 10 days. Dead insects were placed into a humid chamber for fungal pathogen exhibition expressed as produced hyphal growth. Results of the bioassays were evaluated as percentages of cumulative daily mortality due to mycosis.

Virulence of each fungal isolate was estimated by values of the median lethal time (LT₅₀), calculated by probit analysis (Finney, 1971).

RESULTS AND DISCUSSION

It has been established that according to its morphological characteristics the isolate marked under number 426 belongs to species *B. bassiana*. On the host mycelium grew emerging through insect exoskeleton and formed dense white covering on surface. Conidiogenous cells were densely clustered in whorls, colorless, short, with globose base and extended denticulate apex, growing apex repeatedly formed conidium and re-grew just below the new conidium, giving a distinctly zig-zag appearance. Conidia were one-celled, hyaline, thin-walled, globose, with length 1.8–2.0 μm. Obtained isolate 426 of *B. bassiana* was used in conducted bioassays against adults of *Ips sexdentatus*. For the first time in Bulgaria the fungal pathogen *B. bassiana* has been found to occur in natural populations of the pest.

Finding and isolation of the fungal pathogen from *Ips sexdentatus* in Bulgaria and its identification as belonging to species *B. bassiana* confirmed reports about biodiversity of the species (Sosnowska et al., 2004; Jurc and Milijasevic, 2006).

Results obtained in bioassays with isolates of *B. bassiana* and *P. farinosus* against adults of *Ips sexdentatus* and *Ips acuminatus* were exhibited in figure 1, table 1 and table 2.

As it has been shown in Figure 1 the isolates of *B. bassiana* were with higher lethal effects to adults of *Ips sexdentatus* than isolates of *P. farinosus*.

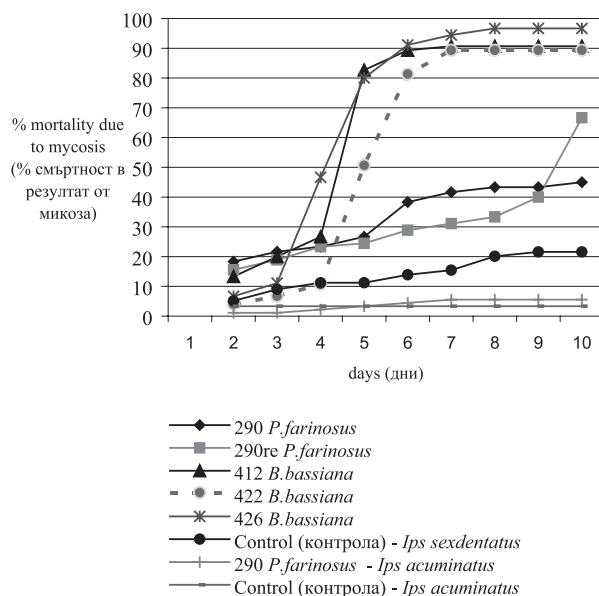


Fig. 1. Lethal effect of isolates of entomopathogenic fungi to adults of *Ips sexdentatus* Boerner and *Ips acuminatus* Gyll. (Coleoptera: Scolytidae)

Фиг. 1. Летален ефект на изолати на ентомопатогенни гъби спрямо възрастните насекоми на *Ips sexdentatus* Boerner и *Ips acuminatus* Gyll. (Coleoptera: Scolytidae)

Differences between cumulative mortality due to mycoses in the treated populations began to be visible after the 3rd day. The curves of mortality in the variants treated with conidia of the isolates 412, 422 and 426 of *B. bassiana* jumped up between the 4th and 5th day from 26.67 to 82.67% ± 0.72, from 10.67 to 50.67% ± 0.72, and from 46.67 to 80.00% ± 0.83, respectively. It could be explained with the lethal effect of produced toxic metabolites by the isolates.

According to Roberts (1981) some strains of *Beauveria* species produce toxic compounds, which rapidly debilitate the host after invasion of the haemolymph. Some of them are beauvericin, beau-

Table 1/Таблица 1

Lethal effect and efficacy of isolates of entomopathogenic fungi in bioassays with adults of *Ips sexdentatus* Boerner and *Ips acuminatus* Gyll. (Coleoptera: Scolytidae)

Летален ефект и ефективност на изолати на ентомопатогенни гъби при експерименти с възрастни насекоми на *Ips sexdentatus* Boerner и *Ips acuminatus* Gyll. (Coleoptera: Scolytidae)

Species of insect host Вид на насекомия гостоприемник	Treated insects (number) Третирани насекоми (брой)	Isolates and species of entomopathogenic fungi Изолати и видове ентомопатогенни гъби	Mean percentage mortality due to mycoses ± SE ten days after the treatment with isolates Средна смъртност от микоза 10 дни след третирание с изолатите (% ± SE)
<i>Ips sexdentatus</i>	60	290 <i>Paecilomyces farinosus</i>	45.00 ± 3.80
<i>Ips sexdentatus</i>	90	290re <i>Paecilomyces farinosus</i>	66.67 ± 0.96
<i>Ips sexdentatus</i>	75	412 <i>Beauveria bassiana</i>	90.67 ± 0.72
<i>Ips sexdentatus</i>	75	422 <i>Beauveria bassiana</i>	89.33 ± 0.72
<i>Ips sexdentatus</i>	90	426 <i>Beauveria bassiana</i>	96.67 ± 0.83
<i>Ips sexdentatus</i>	25	Control	21.59 ± 0.68
<i>Ips acuminatus</i>	90	290 <i>Paecilomyces farinosus</i>	5.56 ± 0.64
<i>Ips acuminatus</i>	30	Control	3.33 ± 0.64

Table 2/Таблица 2Virulence of isolates of entomopathogenic fungi to adults of *Ips sexdentatus* Boerner (Coleoptera: Scolytidae)Вирулентност на изолати на ентомопатогенни гъби спрямо възрастните насекоми на *Ips sexdentatus* Boerner (Coleoptera: Scolytidae)

Isolate and species of entomopathogenic fungus Изолати и видове ентомопатогенни гъби	Values of the median lethal time (LT ₅₀), days Стойности на средното летално време (LT ₅₀), дни			Regression coefficient (b ± S _{eb}) Коефициент на регресия
	Average values of LT ₅₀ средни стойности на LT ₅₀	Confidence intervals* доверителен интервал		
		from/от	to/до	
290 <i>Paecilomyces farinosus</i>	17.5	11.26	27.17	1.1210 ± 0.1960
290re <i>Paecilomyces farinosus</i>	11.7	9.156	14.96	2.4968 ± 0.2559
412 <i>Beauveria bassiana</i>	4.614	4.374	4.867	6.0096 ± 0.3255
422 <i>Beauveria bassiana</i>	4.73	3.659	6.112	4.4545 ± 0.7584
426 <i>Beauveria bassiana</i>	4.324	4.11	4.55	5.4564 ± 0.2207

*Confidence intervals at statistical significance level p < 0.05

verolides, bassianolide, pigments and oxalic acid. Production of toxic metabolites is a step of development of the mycosis followed by death of the host. *Paecilomyces* spp. produce pyridine-2,6-dicarboxylic acid but it is with less lethal effect.

The isolates 290 and 290re of *P. farinosus* caused low mortality to adults of *Ips sexdentatus*. The calculated cumulative mortality due to mycoses on the 9th day was 43.33% ± 3.80 and 40.00% ± 0.96, respectively (Figure 1).

Bioassays with adults of *Ips acuminatus* showed that the host was not susceptible to the examined isolate 290 of *P. farinosus* (figure 1, table 1). The calculated mean percentage mortality due to mycosis was 5.56 ± 0.64 on the 10th day. Mortality in control variant was 3.33% ± 0.64 (table 1).

It has been established that the isolate 426 of *B. bassiana* caused the highest lethal effect to adults of *Ips sexdentatus* for 10 days – 96.67% ± 0.83, following by isolates 412 and 422 of *B. bassiana* – 90.67% ± 0.72 and 89.33% ± 0.72, respectively (table 1). Mortality in control variant was 21.59% ± 0.68. Isolates 290 and 290re of *P. farinosus* showed less lethal effect to the same host – 45.00% ± 3.80 and 66.67% ± 0.96 in comparison with the other tested isolates.

As it could be seen the passage of the fungal pathogen through the host following by isolation into pure culture led to slight increasing in the lethal effect of the isolate of *P. farinosus*.

Comparison between examined fungal pathogens could be better done according to their virulence estimated as median lethal time (LT₅₀). The isolates 412, 422 and 426 of *B. bassiana* were highly virulent to adults of *Ips sexdentatus* with values of the median lethal time varying within overlapped narrow confidence intervals from 4.374 to 4.867, from 3.659 to 6.112 and from 4.110 to 4.550 days (at p-level < 0.05). Significant differences between virulence of the examined isolates of *B. bassiana* have not been proved (table 2).

Evaluation of the virulence of the isolate 290 and the re-isolate (290re) of *P. farinosus* to adults of *Ips sexdentatus* showed that both isolates were with low to median virulence (table 2). Calculated values of the median lethal time (LT₅₀) varied within overlapped confidence intervals from 11.26 to 27.17 and from 9.16 to 14.96 days, respectively, but they could not be distinguished according to their virulence.

Comparison between confidence intervals of LT₅₀ of *B. bassiana* isolates and isolates of *P. farinosus* showed that they were not overlapped. Isolates of *B. bassiana* could be distinguished from isolates of *P. farinosus* according to their virulence to *Ips sexdentatus* evaluated as median lethal time. Significant differences between them have been proved at p-level < 0.05.

Regression coefficient showed sharpness of the lethal effect of the mycosis caused by the certain isolate. It's values calculated for the isolates of *B. bassiana* were higher than values for isolates of *P. farinosus* (Table 2).

Kreutz et al. (2004b) in laboratory and field experiments demonstrated that transmission of *B. bassiana* between beetles of *I. typographus* and dissemination of the fungus within a bark beetle population were possible.

Lavalee et al. (2005) conducting a field experiment achieved encouraging results about application of *B. bassiana* as a potential control method against the pine shoot beetle (*Tomicus piniperda*, Scolytidae).

The results obtained in the current bioassays with the examined isolates 412, 422 and 426 of *B. bassiana* against *Ips sexdentatus* were hopeful of the future investigations about application of fungal pathogens in control of the pest.

CONCLUSIONS

The isolate 426 of *B. bassiana* was obtained into pure culture from imago of *Ips sexdentatus*. For the first time in Bulgaria the fungal pathogen *B. bassiana* has been found to occur in natural populations of the pest.

It has been established that the isolate 426 of *B. bassiana* caused the highest lethal effect to adults of *Ips sexdentatus* – 96.67% ± 0.83, following by isolates 412 and 422 of *B. bassiana* – 90.67% ± 0.72 and 89.33% ± 0.72, respectively.

Isolates 290 and 290re of *P. farinosus* showed less lethal effect to *Ips sexdentatus* – 45.00% ± 3.80 and 66.67% ± 0.96 in comparison with the other tested isolates.

It has been established that adults of *Ips acuminatus* were not susceptible to the studied fungal isolate 290 of *P. farinosus*. The calculated mean percentage mortality due to mycosis was 5.56 ± 0.64.

The isolates 412, 422 and 426 of *B. bassiana* were highly virulent to adults of *Ips sexdentatus* with values of the median lethal time (LT₅₀) varying within overlapped narrow confidence intervals from 4.374 to 4.867, from 3.659 to 6.112 and from 4.110 to 4.550 days (at p-level < 0.05). Significant differences between virulence of examined isolates of *B. bassiana* have not been proved.

Isolates of *B. bassiana* could be distinguished from isolates of *P. farinosus* according to their virulence to *Ips sexdentatus* evaluated as median lethal time.

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