Pathogenicity of *Beauveria bassiana* Strains Isolated from *Ostrinia nubilalis* Hbn. (Lepidoptera: Pyralidae) to Original Host Larvae and to Ladybirds (Coleoptera: Coccinellidae)

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Abstract

CAGÁŇ Ľ., UHLÍK V. (1999): **Pathogenicity of** *Beauveria bassiana* **strains isolated from** *Ostrinia nubilalis* Hbn. (Lepidoptera: Pyralidae) **to original host and to ladybirds** (Coleoptera: Coccinelidae). Pl. Protect. Sci., **35**: 108–112.

B. bassiana strains isolated from *O. nubilalis* were tested against the larvae of *O. nubilalis* and coccinellid beetles in laboratory conditions (25°C). The first dead *O. nubilalis* larvae were observed 48 hours after the application. During the first five days after the application, the efficiency of spore suspension was significantly higher than the efficiency of dry spore formulation. Spore suspension killed more than 50% of larvae after 72, or 96 hours. After dry spore formulation was used, more than 50% of larvae were killed during 96, or 120 hours. *B. bassiana* killed 50% of coccinellid larvae during 48 hours. After another 24 hours 83.3% (strain SK78), or 100% (strain SK99) coccinellid larvae were killed by fungus. More than 50% of dead adults of *Coccinella septempunctata* L. and *Propylea quattuordecimpunctata* (L.) was found 72–120 hours after application of fungus. This means that *B. bassiana* was not adapted specifically to original host and killed effectively the adults and larvae of Coccinellidae. Different behaviour probably allows the coccinellids to escape from fungal infection in natural conditions.

Key words: *Beauveria bassiana*; *Ostrinia nubilalis*; *Coccinella septempunctata*; *Propylea quattuordecimpunctata*; pathogenicity of *Beauveria* strains

Entomopatogenic fungus *Beauveria bassiana* (Balsamo) Vuillemin is a pathogen reported to be capable of infecting over 100 different insect species belonging to a variety of insect orders (MCCOY *et al.* 1988). On the other hand, a high degree of host specificity was revealed from bioassay data obtained from various *B. bassiana* isolates (FARGUES 1976). According to FERRON (1978) or MCCOY (1990), different *B. bassiana* strains can possess different host-ranges. Similarly, different strains of *Metarrhizium anisopliae*, another fungal entomopathogen, showed that there was a very strict adaptation to the original host (FERRON *et al.* 1972).

Field experiments with *B. bassiana* against *O. nubilalis* showed that it is necessary to spread fungal conidia at the time of hatching of larvae. When conidia were placed on plants prior to *O. nubilalis* larvae, mortality decreased with time (FENG *et al.* 1988). In Slovakia, the highest number of *O. nubilalis* egg masses was observed usually in the first decade of July (CAGÁŇ & BARABÁS 1996b). The highest efficiency of *B. bassiana* strains in field conditions was found at the same time (UHLÍK 1999). But, larger populations of coccinelid larvae and adults were observed on the maize plants in July (CAGÁŇ 1993).

During 1995–1998, *B. bassiana* strains were isolated from European corn borer, *O. nubilalis* Hbn. larvae collected in Slovakia. The present work shows how specific is the virulence of such isolates to predators from the family Coccinellidae.

MATERIAL AND METHODS

The efficiency of entomopathogenic fungus *Beauveria bassiana* (Bals.) Vuill. was tested against the larvae of *O. nubilalis* and coccinellid beetles in laboratory conditions (25°C, 100% RH, 16L : 8D photoperiod).

The strains of *B. bassiana* used in experiments were isolated from dead *O. nubilalis* larvae collected during the autumn in maize plants at various localities of Slovakia. Strain SK67 originated from locality Kráľovský Chlmec (48°26′ N, 21°59′ E), SK78 from locality Nitra-Malanta (48°19′ N, 18°09′ E) and both SK99 and SK100 from locality Komjatice (48°09′ N, 18°08′ E).

Larvae of *O. nubilalis* originated from Slovakian population bred in laboratory for more than three generations on semi-artificial diet (NAGY 1974). Coccinellid adults and larvae (4^{th} instar) were collected in maize fields at locality Nitra–Malanta during the end of June and the beginning of July 1998.

In the first experiment, *O. nubilalis* larvae (last, 5^{th} instar) were placed on the *B. bassiana* culture (Sabouraud dextrose agar) two weeks old which produced dry spores on the surface. After 5 minutes, the larvae were removed and placed in a Petri dish containing maize leaves.

In the second experiment, a water spore suspension was prepared from conidia on agar cultures of *B. bassiana*. Suspension concentration was 5×10^8 spores per ml. Spore suspension was applied with the help of a brush, individually, to each larva.

The strains SK67, SK78, SK99 a SK100 were used in both experiments with *O. nubilalis* larvae. Each strain was tested in 4 replications (Petri dishes). Each Petri dish contained 10 larvae. Control variant was organised in the same way.

Two strains of *B. bassiana* were applied against the adults of *Coccinella septempunctata* and *Propylea quattuordecimpunctata*, and the larvae of *C. septempunctata*. Spore suspension was used as in the experiment with *O. nubilalis* larvae (4 replications, 10 animals in each replication). Coccinellid larvae and adults were bred in Petri dishes contained maize leaves infested by larvae and apterous females of *Metopolophium dirhodum* (Walker).

Mortality of *O. nubilalis* and coccinellids was checked every 24 hour during 7 days. Dead animals were surface sterilised by dipping to 70% ethylacohol for 2 minutes and incubated in a high-humidity environment (wet filter paper in Petri dish) to allow growth of surface mycelia and sporulation of *B. bassiana*.

RESULTS

Tables 1 and 2 show the efficiency of *Beauveria bassiana* dry spore and suspension against *O. nubilalis* larvae during seven days. The first dead larvae were found 48 hours after the application.

During the first five days after the application, the efficiency of spore suspension was significantly higher than the efficiency of dry spore formulation. Spore suspension killed more than 50% of larvae after 72, or 96 hours. After dry spore formulation was used, more than 50% of larvae were killed during 96, or 120 hours.

By 168 hours post-exposure, mortalities of more than 90% were obtained both by dry spore or suspension and all were significantly higher ($p < 0.05$) than in controls (Tables 1 and 2). The efficiency of some of strains achieved 100%.

Larvae of *O. nubilalis* bred without presence of *B. bassiana* all survived during seven days and started their pupation.

The efficiency of two *B. bassiana* strains against coccinellid adults and larvae is shown in Table 3. The first dead animals were observed 48 hours after the spore application. Both strains of *B. bassiana* killed 50% of coccinellid larvae during 48 hours. After another 24 hours, 83.3% (strain SK78), or 100% (strain SK99) coccinellid larvae were killed by fungus. More than 50% of dead

Table 1. Efficiency of dry spore formulation of *Beauveria bassiana* against *O. nubilalis* larvae. Means marked with the same letter are not significantly different ($P = 0.05$, Tukey's multiple range test where data from the same hour in Tables 1–3 were compared)

	% of dead larvae after						
Strain	24 h	48 h	72 h	96 h	120 h	144 h	168 h
SK67	0	12.5bcd	47.5cd	50.0bc	75.5	90.0	100f
SK78	0	15.0cde	35.0b	48.5b	52.5	87.5	97.5ef
SK99	0	5.0b	42.5bc	48.5b	55.0	78.5	92.5de
SK100	0	7.5bc	47.5cd	52.0bc	57.5	75.0	87.5cd
Control	0	0a	0a	0a	0	0	0a

Table 2. Efficiency of spore suspension formulation of *Beauveria bassiana* against *O. nubilalis* larvae. Means marked with the same letter are not significantly different ($P = 0.05$, Tukey's multiple range test where data from the same hour in Tables 1–3 were compared)

	% of dead larvae after						
Strain	24 h	48 h	72 h	96 h	120 h	144 h	168 h
SK67	0	22.5ef	47.5cd	51.0bc	75.0	82.5	97.5ef
SK78	0	22.5ef	47.5cd	50.0bc	80.0	97.5	100f
SK99	0	25.0fg	50.0cd	56.5c	85.5	97.0	100f
SK100	0	20.0def	52.5d	57.0c	85.0	85.0	92.5de
Control	0	0a	0a	0a	0	0	0a

Table 3. Efficiency of *Beauveria bassiana* spore suspensions against the adults of *Coccinella septempunctata*, adults of *Propylea quattuordecimpunctata* and larvae of *Coccinella septempunctata*. Means marked with the same letter are not significantly different (P = 0.05, Tuckey's multiple range test where data from the same hour in Tables 1–3 were compared)

Predator	Strain	% of dead animals after						
		24 h	48 h	72 h	96 h	120 h	144 h	168 h
C. 7-punctata adults	SK 78	0	17.5cdef	42.5bcd	82.5d	93.0	100.0	100.0f
	SK99	0	32.5gh	42.5bcd	50.0bc	62.0	70.0	75.0b
	Control	0	0a	0a	0a	0	0	20.0a
P. 14-punctata adults	SK78	0	43.3hi	43.3bc	43.3b	50.0	55.0	66.7b
	SK99	0	50.0i	56.6d	56.6bc	77.7	77.7	77.7c
	Control	0	0a	0a	0a	0	0	0a
C. 7-punctata larvae	SK78	0	50.0h	83.3e	83.3d	100.0	100.0	100.0f
	SK99	0	50.0h	100.0f	100.0e	100.0	100.0	100.0f
	Control	0	0a	0a	0a	0	20.0*	20.0a*

*mortality caused by cannibalism

adults of *C. septempunctata* and *P. quattuordecimpunctata* was found 72–120 hours after application of fungus (Table 3).

Adults of both coccinellid species survived in Petri dishes without the *B. bassiana* spores. Some larvae were killed due to cannibalism. Coccinellid larvae did not pupate during the time of the experiment.

The efficiency of various *B. bassiana* strains was not significantly different during the experiments with *O. nubilalis* or coccinellid larvae and adults.

When incubated at high humidity, dead *O. nubilalis* and coccinellids exposed to *B. bassiana*, developed surface mycelia.

DISCUSSION

B. bassiana caused the host mortality to be higher than 80% during its strongest development, usually on the seventh day after spore application (DORSCHNER *et al.* 1991; STIMAC *et al.* 1993). QUINTELA *et al.* (1990) used the *B. bassiana* spores against the last larval instar of *Chalcodermus bimaculatus* Fiedler and they found the mortality 80% only after 14 days (LT_{50} = 7.1). According to VANDENBERG (1996), LT_{50} ranged from 4.6 days to 12.2 days for *B. bassiana* applied against the Russian wheat aphid. Mortality rates of *Diatraea saccharalis* (F.) infected by *B. bassiana* ranged from 50 to 90 % with a LT_{50} of 2.1 to 8.4 days (LEUCONA *et al.* 1996). After seven days, the mortality of *O. nubilalis* larvae in our experiments achieved minimally 87%. Three days after the spore application, the strains SK99 and SK100 killed more than 50% of host larvae. The results show very high virulence of our strains. But, TIMONIN *et al.* (1980) showed that after repeated reisolation and reinfection, the strains of *B. bassiana* or *M. anisopliae* killed 100% of target pest individuals during 48–56 hours.

Developmental stage of the host influences efficiency of the fungus. Usually, larvae are the most susceptible and adults are the most resistant (MARKOVA 1992; MIKLOŠ 1983). The differences were found also among the larval instars. The first and the fifth intar of *O. nubilalis* are considered to be to be the most susceptible to infection caused by *B. bassiana* (RIBA *et al.* 1983; FENG *et al.* 1985). An insect may escape *B. bassiana* infection by casting the infectious inoculum with their exosceleton at the time of molting (VEY & FARGUES 1977). This was the reason why we used the last instar larvae in our experiments.

Many researchers recorded the important role of high relative humidity, or the presence of free water for the germination of fungus spores (TENG 1962; WILDING 1969; WALSTAD *et al.* 1970; RIBA & GOUSSARD 1984; KUBERAPPA & JAYARAMAIAH 1987; AREGGER-ZAVADIL 1992). Our results showed that development of infections with *B. bassiana* spores, applied in suspension, was faster than the development with dry spore formulation. Water in suspension probably activated the germination of spores also when larvae were bred in optimal conditions for fungus development (100% RH, 25°C).

Studies with Scarabaeidae larvae infected by different strains of *Metarhizium anisopliae* showed that there was a very strict adaptation to the original host (FERRON *et al.* 1972). Similarly, bioassay data, obtained from various selected *B. bassiana* isolates, revealed a high degree of host specificity (FARGUES 1976; FERRON 1978; MCCOY *et al.* 1990). When thirty-eight strains of *B. bassiana* were examined by RFLP and RAPD analyses (MAURER *et al.* 1997), strains isolated from members of the

Pyralidae were recovered as two main groups, one group consisted of all strains isolated from *Ostrinia* irrespective of their origin. Other studies (PARKER *et al.* 1997) suggested that *B. bassiana* could be applied to forest soil without a significant negative impact on the forest-dwelling invertebrate population. But, according to JAYANTHI and PADMAVATHAMMA (1996), *B. bassiana* was infective to groundnut pests and cocinellid predators. Temperature, starvation, and nutrition stresses significantly affected the susceptibility of *Chrysoperla carnea* (Stephens) to *B. bassiana* (DONEGAN & LEIGHTHART 1989). Our results show that *B. bassiana* strains isolated from *O. nubilalis* killed the adults and larvae of Coccinellidae in a very short time, in some cases shorter than those necessary to kill the *O. nubilalis* larvae. This result suggests that isolates did not posses strong host specificity and had very negative effect on beneficial arthropods. In natural conditions, the effect of the fungus should be different. Larvae of *O. nubilalis* usually occur at very wet places of the plant such as the whorl, leaf sheaths and stem. On the other hand, coccinellid larvae and adults usually move onto the leaves or stems. Thus they could escape fungal infection, which requires high relative humidity for its development.

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Súhrn

CAGÁŇ Ľ., UHLÍK V. (1999): **Účinok kmeňov** *Beauveria bassiana* **izolovaných z** *Ostrinia nubilalis* Hbn. (Lepidoptera: Pyralidae) **na pôvodného hostiteľa a na lienky** (Coleoptera: Coccinelidae). Pl. Protect. Sci., **35**: 108–112.

Kmene *Beauveria bassiana*, ktoré boli izolované z *Ostrinia nubilalis*, sa testovali proti larvám *O. nubilalis* a lienkam v laboratórnych podmienkach (25 °C). Prvé mŕtve larvy *O. nubilalis* sa pozorovali 48 hodín po aplikácii. Počas prvých piatich dní po aplikácii bola účinnosť suspenzie spór preukazne vyššia ako účinnosť formulácie so suchými spórami. Suspenzia spór zabila viac ako 50 % lariev počas 72 alebo 96 hodín. Ak sa použila formulácia so suchými spórami, viac ako 50 % lariev zahynulo za 96 alebo 120 hodín. *B. bassiana* zabila 50 % lariev lienok počas 48 hodín. Po ďalších 24 hodinách zahynulo 83,3 % (kmeň SK78) alebo 100 % (kmeň SK99) lariev lienok. Viac ako 50 % mŕtvych imág *Coccinella septempunctata* L. a *Propylea quattuordecimpunctata* (L.) sa zistilo 72–120 hodín po aplikácii huby. To znamená, že *B. bassiana* nebola prispôsobená iba pôvodnému hostiteľovi a zabíjala imága a larvy z čeľade Coccinellidae. Rozdielne správanie (pohyb na povrchu rastlín v relatívne suchom prostredí) pravdepodobne umožňuje lienkam uniknúť infekcii hubami v prírodných podmienkach.

Kľúčové slová: *Beauveria bassiana*; *Ostrinia nubilalis*; *Coccinella septempunctata*; *Propylea quattuordecimpunctata*; patogenita kmenů *Beauveria*

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