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


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 quatuordecimpunctata (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 behavior probably allows the coccinellids to escape from fungal infection in natural conditions.

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

Entomopathogenic 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 (PENG et al. 1988). In Slovakia, the highest number of O. nubilalis egg masses was observed usually in the first decade of July (CAGÁN & BARABÁS 1996b). The highest efficiency of B. bassiana strains in field conditions was found at the same time (UHLÍK 1999). But, larger populations of coccinellid larvae and adults were observed on the maize plants in July (CAGÁN 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 (4th 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, 5th 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 x 10^6 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 quatuordecimpunctata*, 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 containing maize leaves infested by larvae and aphid 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% ethylalcohol 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

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### 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)

<table>
<thead>
<tr>
<th>Strain</th>
<th>24 h</th>
<th>48 h</th>
<th>72 h</th>
<th>96 h</th>
<th>120 h</th>
<th>144 h</th>
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<td>47.5cd</td>
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<td>90.0</td>
<td>100f</td>
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<td>15.0cde</td>
<td>35.0b</td>
<td>48.5b</td>
<td>52.5</td>
<td>87.5</td>
<td>97.5ef</td>
</tr>
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<td>5.0b</td>
<td>42.5bc</td>
<td>48.5b</td>
<td>55.0</td>
<td>78.5</td>
<td>92.5de</td>
</tr>
<tr>
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<td>7.5bc</td>
<td>47.5cd</td>
<td>52.0bc</td>
<td>57.5</td>
<td>75.0</td>
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### 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)

<table>
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<th>Strain</th>
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<th>72 h</th>
<th>96 h</th>
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<td>51.0bc</td>
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<td>82.5</td>
<td>97.5ef</td>
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<tr>
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Table 3. Efficiency of Beauveria bassiana spore suspensions against the adults of Coccinella septempunctata, adults of Propylea quatuordecimpunctata and larvae of Coccinella septempunctata. 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)

<table>
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<th>Predator</th>
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<td>0a</td>
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</tbody>
</table>

*mortality caused by cannibalism

adults of C. septempunctata and P. quatuordecimpunctata 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<sub>50</sub> = 7.1). According to VANDENBERG (1996), LT<sub>50</sub> ranged from 4.6 days to 12.2 days for B. bassiana applied against the Russian wheat aphid. Mortality rates of Diatrea saccharalis (F.) infected by B. bassiana ranged from 50 to 90% with a LT<sub>50</sub> 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 instar of O. nubilalis are considered to be to 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 its exoskeleton at the time of melting (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; AREGGGER-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 soils without a significant negative impact on the forest-dwelling invertebrate population. But, according to JAYANTI and PADMAVATHAMMA (1996), B. bassiana was infective to groundnut pests and coccinellid predators. Temperature, starvation, and nutrition stresses significantly affected the susceptibility of Chrysopeira 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 possess 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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References


QUINTELA E. D., LORIO J. C., WRAIGTH S. P., ALEVS S. B., ROBERTS D. W. (1990): Pathogenicity of Beauveria bassiana (Hypomyces: Moniliae) to larval and adult Chal-


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Súhrn


Kmene Beauveria bassiana, ktoré boli izolované z Ostrinia nubilalis, sa testovali proti larvám O. nubilalis a lienkam v laboratórnych podmienkach (25 °C). Prvé mŕtvé larvy O. nubilalis sa pozorovali 48 hodín po aplikácii. Počas prvého piatich dní po aplikácii bola účinnosť suspenzie spôr preukazována vyššia ako jej výsledok formulácia so suchými spórami. Suspenzia spôr zabila viac ako 50 % lariev počas 72 alebo 96 hodín. Ak sa používala formulácia so suchými spórami, viac ako 50 % lariev zahynulo za 96 alebo 120 hodín. B. bassiana zabila 50 % lariev len počas 48 hodín. Po ďalších 24 hodinách zahynulo 83,3 % (kmeň SK78) alebo 100 % (kmeň SK99) lariev lenok. 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 zabíja imág a larvy z čeľade Coccinellidae. Rozdielne správanie (pohyby na povrchu rastlín v relatívne suchom prostredí) pravdepodobne umožňuje liénam uniknúť infekcii hubami v prirodzených podmienkach.

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

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