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Transmission possibility of the fungus *Beauveria bassiana* KCF102 by mating behavior between Sunn pest, *Eurygaster integriceps* (Hem.: Scutelleridae) adults

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Abstract

Horizontal transmission of an isolate of the etomopathogenic fungus, *Beauveria bassiana* KCF102, was examined by allowing inoculated *Eurygaster integriceps* Put. adults to mate with non-inoculated ones. In case of susceptibility to *B. bassiana*, there was no significant difference between the males and females. However, there was significant difference among treatments for mortality of non-inoculated adults. It meant horizontal transmission could be happened between the inoculated and non-inoculated adults. Recording no significant difference among the treatments of the inoculated males + non-inoculated males and treatment with inoculated females + non-inoculated males revealed that this kind of horizontal transmission was not merely due to the mating behavior, given that the same transmission could be existed between inoculated and non-inoculated males or between those of female adults. There was significant difference for mortality percentage of adults among the five body-part treatments, ranging from 16.6 to 48.9, with the highest mortality for total body treatment and the lowest one for the pronotum.
Key words: entomopathogenic fungus, *Beauveria bassiana*, horizontal transmission, mating behavior, *Eurygaster integriceps*

چکیده

در این پژوهش، امکان انتقال افقی جدایه‌ای از قارچ *Beauveria bassiana* KCF102 با رفتار جفت‌گیری بین افـراد نر و ماده‌ی سن گندم، *Eurygaster integriceps* Put.، بررسی شد. همچنین، برای تعیین میزان حساسیت افراد نر و ماده و بخش‌های مختلف بدن سن گندم به این قارچ؛ شاخک‌ها، پنجه‌ها، سطح شکمی شکم، پیش‌گرده و کل بدن به عنوان تیمارهای آزمایشی انتخاب شد. نتایج حاصله نشان داد که بین افراد نر و ماده از نظر حساسیت به قارچ *B. bassiana* اختلاف معنی‌دار وجود ندارد. بین تیمارهای مربوط به آزمایش انتقال افقی قارچ، اختلاف معنی‌دار وجود داشت و این نشان داد که انتقال افقی قارچ بین افراد بالغ می‌تواند صورت گیرد اما عدم وجود اختلاف معنی‌دار بین تیمارهای با اختلاط نر آلوده با نر غیرآلـوده و ماده‌ی آلوده با نر غیرآلوده، گویای این مطلب است که این نوع انتقال، با رفتار جفت‌گیری بین افراد نر وماده بروز نمـی‌کنـد بلکه صرفاً تماس فیزیکی بین افراد بدون وابستگی به جنس، موجب این انتقال است. بین تیمارهـای مربـوط بـه حـساسیت قسمت‌های مختلف بدن، اختلاف معنی‌داری مشاهده شد؛ تیمار پیش‌گرده با میانگین ۱٦/٦ و تیمار کل بدن با میـانگین ٤٨/٩ درصد مرگ و میر، به‌ترتیب کمترین و بیشترین حساسیت را در بین تیمارهای انتخاب شده داشتند.
واژگان کلیدی: قارچ بیمارگر، *Beauveria bassiana*، انتقال افقی، رفتار جفت‌گیری، *Eurygaster integriceps*

Introduction

Controlling crop pests with a foliar spray, be it chemical or biological, is often considered a discrete event with limited persistence. However, advantages of using fungal entomopathogens as microbial insecticides are the ability to infect their hosts primarily through the external cuticle and the potential for transmission to occur after the initial spray

(Long *et al*., 2000; Inglis *et al*., 2001). The cosmopolitan entomopathogenic fungus, *Beauveria bassiana* (Balsamo) Vuillemin (Ascomycota: Hypocreales) is an effective biological control agent for reducing densities of insects from most orders, including Sunn pest, *Eurygaster integriceps* Put. (Talaei-Hassanloui & Kharazi-Pakdel, 2002). Wind currents (Shimazu *et al*., 2002), rain splash from soil surfaces (Bruck & Lewis, 2002) and insect activities (Long *et al*., 2000; Meyling *et al*., 2006) could potentially contribute to the distribution of this fungus inoculum. Tsuda *et al*. (1997) examined transmission of *B. bassiana* by mating the behavior of *Plautia stali* Scott (Hem.: Pentatomidae) adults. Factors influencing horizontal transmission of this fungus into the populations of the Colorado potato beetle were examined through a series of laboratory studies (Long *et al*., 2000).

We sought to investigate the possibility of horizontal transmission of *B. bassiana* by mating behavior between Sunn pest adults by allowing inoculated adults to mate with non-inoculated ones. Since it is important to know which parts of body is more susceptible than the others and whether these parts contribute in mating behavior or contact with each other of males and females, so susceptibility to *B. bassiana* of different parts on Sunn pest adult body was examined too.

Materials and methods

Maintenance of insect and fungal isolate

Sunn pest adults were collected from their resting places under *Astragalus* shrubs environs Karaj and Varamin in autumn and transferred to the laboratory. They were maintained into the 10 × 20 × 30 cm plastic dishes under 27 ± 2°C, 60 ± 10% RH and 16L-8D photoperiod conditions. The adults were provided with the wheat grains (Sardari variety) and water into cotton-headed tubes.

A single-spored isolate of *B. bassiana* KCF102, which was originally isolated from soil in Karaj according to the method of Shimazu & Sato (1996), was cultured on Sabouraud's dextrose agar with yeast extract (SDAY) plates and incubated at 25 ± 1°C and 16L-8D photoperiod (Talaei-Hassanloui, 1999). Conidia were then harvested from the surface of two weeks old cultures by scraping and suspended in 0.02% Tween 80 in tubes. Conidial suspension was estimated with a hemocytometer under a light microscope. The viability of conidia was determined on SDAY plates.

Susceptibility of different sites on adult body

Susceptibility to *B. bassiana* of the different sites on adult body; antennae, tarsi, ventral abdomen, pronotum and total body were evaluated. Decreasing the mobility, Sunn pest adults were refrigerated (ca. 4°C) for half an hour. Each of these parts were topically treated with 10 µl of conidial suspension 1.4×10^8 co/ml. Whole body of Sunn pest adults was immersed into the conidial suspension for 4 seconds for the total body treatment. The experiment was carried out with three replicates and each one with 20 adults. The same numbers of adults were treated with 0.02% Tween 80 as the control for each treatment. Treated adults were maintained at the same conditions of insect rearing. Mortality data was daily recorded for 4 weeks and subjected to data transformation and Abbott formula correction. Tukey HSD test was used for mean comparisons among treatments.

Susceptibility of male and female adults

Determining the susceptibility of the adults to *B. bassiana*, the abdominal end of males and females was individually inoculated with 10 µl of conidial suspension 1.4×10^8 co/ml. This experiment was conducted with three replicates each one with 20 adults. Mortality data was daily recorded for 4 weeks and t-test was used to compare these 2 treatments.

Horizontal transmission through mating behavior

Collected Sunn pest adults from overwintering places were fed on wheat grains soaked in 0.05% Pyroproxifen (Admiral®, 10EC), separately for males and females. Abdominal end and antennae of both males and females were inoculated with 10 µl of conidial suspension 3.2×10^9 co/ml. Twenty four hours later, the males and females were mixed with the defined numbers of inoculated and non-inoculated adults for 48 hours and then males and females were segregated and monitored daily for 25 days. In total, 240 adults were used in 4 treatments.

Results and discussion

Susceptibility of different sites on adult body

There was a significant difference for mortality of adults among the 5 body-part treatments ($F_{4, 10} = 10.9$; $P < 0.001$). Angular-transformed percentage mortalities ranged between 16.6 to 48.9%, with the highest mortality for total body treatment and the lowest one for the pronotum treatment (fig. 1).

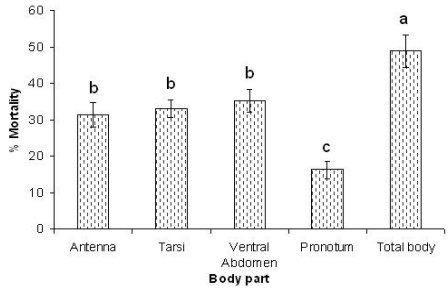

Figure 1. Mean percentage mortality of *E. integriceps* adults, inoculated with *B. bassiana* at different sites on their body. Mean ± SE followed by different letters are significantly different (Tukey HSD multiple comparisons, $P < 0.05$).

Susceptibility of male and female adults

There was no significant difference in susceptibility to *B. bassiana* of male and female adults ($t = 1.5$; $P = 0.196$), although different values were recorded for the longevity of the treated males (10-16 days) and females (6-14 days).

Regarding these different values, most likely it could be discussed that the existence of exceeding fat and more sutures at the genital portion of females comparing to the males provides simple entry routes and substrate for fungal penetration and infection.

Transmission through mating behavior

Analysis of variance indicated that there are significant differences among treatments for the mortality of non-inoculated adults ($F_{3, 8} = 65.5$; $P < 0.001$). This meant that horizontal transmission could be happened between inoculated and non-inoculated adults. However, significant difference between the treatments "inoculated males + non-inoculated males" and "inoculated females + non-inoculated males" (fig. 2) showed that this kind of horizontal transmission was not due to the mating behavior. Tsuda *et al*. (1997) indicated that inoculated adults of *P. stali* could transmit the fungus to the non-inoculated partners by mating behavior. They speculated that the antennae play an important role in the transmission of the fungus. Meyling *et al.* (2006) demonstrated that both the aphid *Microlophium carnosum* (Buckton) and its predator, *Anthocoris nemorum* (L.) were able to disperse inoculum from soil to nettle leaves. For *M. carnosum*, however, this was only found in Petri dish experiments and not when scaled up to microcosms. Furthermore, *A. nemorum* distributed *B. bassiana* conidia from secluded

cadavers within the upper nettle canopy. Our study showed that physical contact between individuals in mating behavior was responsible for the horizontal transmission of *B. bassiana* between those adults, as the same transmission could be existed between inoculated and non-inoculated males or between those of female adults. More probably, the type of mating behavior in Sunn pest adults with the least contact between susceptible sites of male and female is the reason for being no significant synergistic effect of mating behavior on horizontal transmission of this fungus.

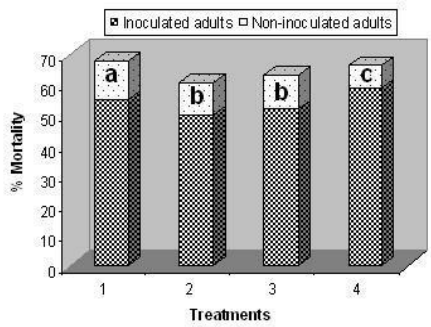

Figure 2. Mortality of Sunn pest adults caused by *B. bassiana* at different treatments as following arrangements: 1. inoculated ♂ + non-inoculated ♀, 2. inoculated ♂ + non-inoculated ♂, 3. inoculated ♀ + non-inoculated ♂, 4. inoculated ♀ + non-inoculated ♀.
Values followed by the same letter are not significantly different (Tukey HSD multiple comparisons, $P < 0.05$).

Acknowledgments

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References

Bruck, D. J. & Lewis, L. C. (2002) Rainfall and crop residue effects on soil dispersion and *Beauveria bassiana* spread to corn. *Applied Soil Ecology* 20, 183-190.

Inglis, G. D., Goettel, M. S., Butt, T. M. & Strasser, H. (2001) Use of hyphomycetous fungi for managing insect pests. pp. 23-69 in Butt, T. M., Jackson, C. & Magan. N.

(Eds) *Fungi as biocontrol agents*: *progress, problems and potential*. 390 pp. CAB International Publishing.

Long, D. W., Groden, E. & Drummond, A. (2000) Horizontal transmission of *Beauveria bassiana* (Bals.)Vuill. *Agricultural and Forest Entomology* 2, 11-17.

Meyling, N., Pell, J. & Eilenberg, J. (2006) Dispersal of *Beauveria bassiana* by the activity of nettle insects. *Journal of Invertebrate Pathology* 93, 121-126.

Shimazu, M. & Sato, H. (1996) Media for selective isolation of an entomogenous fungus, *Beauveria bassiana* (Deuteromycotina: Hyphomycetes). *Applied Entomology and Zoology* 31, 291-298.

Shimazu, M., Sato, H. & Maehara, N. (2002) Density of the entomopathogenic fungus, *Beauveria bassiana* Vuillemin (Deuteromycotina: Hyphomycetes) in forest air and soil. *Applied Entomology and Zoology* 37, 19-26.

Talaei-Hassanloui, R. (1999) Laboratory investigation on pathogenicity of the fungus *Beauveria bassiana* on Sunn pest, *Eurygaster integriceps* (Hem., Scutelleridae). M.Sc. Thesis. University of Tehran, Karaj. 99 pp. [In Persian with English summary].

Talaei-Hassanloui, R. & Kharazi-Pakdel, A. (2002) Evaluation of Sunn pest, *Eurygaster integriceps* susceptibility in different developmental stages to *Beauveria bassiana* (Bals.) Vuill. *2ème Conférence Internationale sur les moyens alternatifs de lutte contre les organismes nuisibles aux végétaux*, 588-592. [In English with French summary].

Tsuda, K., Tsutsumi, T. & Yamanaka, M. (1997) Transmission by mating behavior of entomogenous fungus, *Beauveria bassiana* E-9102, between brown-winged green bug, *Plautia stali* (Hemiptera: Pentatomidae) adults. *Japanese Journal of Applied Entomology and Zoology* 41, 99-101.

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