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Research Article

Relatedness of proteolytic potency and virulence in entomopathogenic fungus *Beauveria bassiana* isolates

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Abstract: Entomopathogenic fungi produce a variety of degrading enzymes, including proteases, chitinases and lipases, to facilitate their entry through the massive barriers of insect cuticle. Isolates of the entomopathogenic fungi vary considerably in their proteolytic activity and virulence. The proteolytic activity of different isolates has been hypothesized to reflect their virulence toward the host. In this study, we evaluated the virulence and proteolytic activity of 17 *Beauveria bassiana sensu lato* isolates collected from different geographical regions in Iran. The selective medium D0C2 was used for isolating *B. bassiana* from soil samples. Casein substrate was used for protease assay. Total mortalities caused by different *B. bassiana* isolates through the dipping method, ranged from 25 to 60% with the highest and lowest rates for isolates BA and MITE, respectively. Our results revealed a wide variation in both proteolytic activity and virulence among the studied isolates. Additionally, we found a strong positive correlation between the proteolytic activity on Casein substrate and virulence of the isolates against the Khapra beetle, *Trogoderma granarium*. This finding will facilitate the screening and selection process of virulent fungal isolates as efficient agents for use in biological control programs of insect pests.

Keywords: entomopathogenic fungus, protease activity, *Trogoderma granarium*, insect, *Beauveria bassiana*

Introduction

In recent decades, with the increasing concerns related to pesticide residues in agricultural products and environment, insect resistance to insecticides as well as the adverse effects of chemical pesticides on beneficial organisms (Fitt, 1994; Gatehouse *et al*., 1994; Haq *et al*., 2004), efforts for development of alternative non-chemical strategies have experienced greater attention (Scholte *et al*., 2004). The use of biological control agents including predators, parasitoids and entomopathogenic agents (bacteria, nematodes, viruses, fungi, etc.) has been promising candidate for control of insect pests. Among these agents, entomopathogenic fungi have been reported to infect a very wide variety of insects that are of great economic importance in agriculture worldwide (Roberts and Humber, 1981). Although, the precise number of entomopathogenic fungi is unclear, it has been estimated to be more than 700 species across the world (Onofre *et al*., 2001).

Soil is the main center for establishment and growth of entomopathogenic fungi because it protects the fungi against desiccation and ultra-violet radiation (Klingen

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and Haukeland, 2006). On the other hand, about 95 percent of insect species have been estimated to spend, at least part or all of their life within the soil (Kuhnelt, 1963). This makes the soil an ideal environment for natural control of insect populations by entomopathogenic fungi.

Entomopathogenic fungi enter the insect body mainly through their integument (Scholte *et al*., 2004; Balachander *et al*., 2012). This entry through the relatively massive barriers of the insect cuticle occurs by a combination of mechanical pressure and enzymatic degradation (St-Leger *et al*., 1986). Insect cuticle comprises up to 70% protein and it is not surprising that extracellular fungal proteases appear to be particularly important in the penetration process (Charnley, 2003). For example, the three common entomopathogenic fungi, *Metarhizium anisopliae* (Mets.) Sorokin, *Beauveria bassiana* (Bals.) Vuill. and *Lecanicillium muscarium* (Petch) Zare and Gams (formerly known as *Verticillium lecanii* (Zimm.) Viegas) produce a variety of extracellular hydrolytic enzymes in liquid cultures containing locust cuticle as sole carbon source (St-Leger *et al*. 1986). These enzymes are believed to play important role in penetration into host body because they act against the major components of insect cuticle i.e. proteins and less importantly, lipids, and chitin (Raymond *et al*., 1986; St-Leger *et al*. 1986; Bidochka and Khachatourians, 1994; Clarkson and Charnley, 1996). The production of cuticle-degrading enzymes has been proposed as an important attribute determining the virulence of the entomopathogenic fungi toward their hosts (St-Leger *et al*., 1995; Pinto *et al*., 2002). For example, high level of proteases produced by *B. bassiana* has been shown to be directly related to early onset of mortality in the larvae of the wax moth, *Galleria mellonella* (Lep.: Pyralidae) (Gupta *et al*., 1994). The resemblance of genes encoding for degrading protease in *B. bassiana* and *M. anisopliae* (Joshi *et al*., 1995) suggests that similar proteases may be widespread among different entomopathogenic fungi. However, recent evidence indicates that different species and even isolates of the entomopathogenic fungi may exhibit some degrees of variation in production of cuticle degrading proteases (Clarkson and Charnley, 1996; Pinto *et al*., 2002; Boldo *et al*., 2009; Dhar and Kaur, 2010; Revathi *et al*., 2011). These variations may reflect the differences observed in the virulence of different isolates and species such that, isolates with higher proteolytic activity are expected to represent higher virulence toward their host. In this study, we evaluated the virulence as well as the proteolytic activity of 17 native isolates of the entomopathogenic fungus *B. bassiana sensu lato* (Hypocreales: Cordycipitaceae) collected from different geographical regions in Iran.

Materials and Methods

Fungal isolate, culture conditions and purification

To obtain the fungal isolates needed for this study (Table 1), samplings were carried out from different sources including soil, infected individuals of the striped rice borer, *Chilo suppressalis* (Lep.: Crambidae), and the two-spotted spider mite, *Tetranychus urticae* (Acari: Tetranychidae) located at three Northern provinces of Iran (Mazandaran, Guilan, and Alborz). The samples containing fungus were placed in plastic bags and transferred to the Biological Control Laboratory, University of Tehran. To exclude any non-target organism, one g of each soil sample was mixed with 200 ml of sterile distilled water containing 0.02% (v/v) Tween 80. After shaking for approximately 30 min on a shaker at 150 rpm, 200 µl of each soil suspension was plated onto Petri dishes (9 cm in diameter) containing the D0C2 selective medium for *B. bassiana* (Shimazu and Sato, 1996). The Petri dishes were incubated in continuous dark condition at 25 °C for 14 days until the fungal colonies were formed. Colonies with fruiting structures were transferred to fresh SDAY (Sabouraud's dextrose agar with yeast) culture medium

(Merck, Germany). To isolate the fungi grown on insect body surface, the insects were superficially sterilized by 70% ethanol for one min and 5% sodium hypochlorite for 3 min and rinsed three times in sterile distilled water for one min. After secondary conidiogenesis of the fungus, they were transferred to SDAY medium, incubated in continuous dark condition at 25 °C for 14 days. Identification of fungal species (Humber, 2005) was performed by mounting the conidia and conidiophores produced on SDAY medium onto microscopic slides using the lacto-phenol cotton blue staining method. The fungi were single spored according to the method of Wang-Ching and Wen-Hsiung (1997).

Table 1 Native *Beauveria bassiana* isolates used in this study.

Isolate	Accession No.	Source/Host[1]	Locality
LAHI	EUT201	Soil	Lahijan
SHALI	EUT202	Soil	Amol/Rice Research Institute
MITE	EUT203	*T. urticae*	Karaj/College of Agriculture
JB1	EUT204	*C. suppressalis*	Babol/Rice field
JB2	EUT205	*C. suppressalis*	Babol/Rice field
JF1	EUT206	*C. suppressalis*	Fereydunkenar/Rice field
JF2	EUT207	*C. suppressalis*	Fereydunkenar/Rice field
JM1	EUT208	*C. suppressalis*	Mahmoudabad/Rice field
JM2	EUT209	*C. suppressalis*	Mahmoudabad/Rice field
RI1	EUT210	*C. suppressalis*	Amol/Rice Research Institute
RI2	EUT211	*C. suppressalis*	Amol/Rice Research Institute
DD	EUT212	*C. suppressalis*	Dabudasht/Rice field
BA	EUT213	*C. suppressalis*	Amol/Rice field
BB	EUT214	*C. suppressalis*	Amol/Rice field
BC	EUT215	*C. suppressalis*	Noshahr/Rice field
BD	EUT216	*C. suppressalis*	Babolsar/Rice field
BF	EUT217	*C. suppressalis*	Nour/Rice field

[1] *T: Tetranychus, C. Chilo.*

Insect

The Khapra beetle, *Trogoderma granarium* (Col.: Dermestidae) was used as host for the entomopathogenic fungus. The larvae and adults of *T. granarium* were obtained from the laboratory of Insect Physiology and Toxicology at the University of Tehran, and a stock colony was established in ventilated jars (24 × 18 × 9 cm) at 33 ± 2 ºC, 50% R. H. and continuous dark condition. Comminuted wheat grain was used as diet for the beetle. To avoid the over accumulation of excrements and larval exuviae, the growing media were refreshed three times weekly.

Virulence assays

A preliminary bioassay was performed to determine LC_{50} for one of the fungal isolates (JB2, see Table 1) selected randomly. The conidia of this isolate, which developed on SDAY medium, were harvested directly from the 10 day-old fungal cultures by scraping the sporulated colony and suspending in 20 ml suspension solution containing 0.2% Tween 80 and 2 g glass beads. The concentration of conidia was estimated using an improved neubauer haemocytometer under a light microscope (Zeiss) at × 40 magnification. Eight serial concentrations of conidial suspension, including 10^3-10^8 conidia ml^{-1}, were used in the preliminary bioassay.

Ten ml of the prepared conidial suspensions were poured in Petri dishes (9 cm in diameter) and 15 *T. granarium* 2nd instar larvae were released in the Petri dishes where they were exposed to the conidial suspensions for 5 sec. The larvae were then wiped by a filter paper and transferred to new Petri dishes containing comminuted wheat grain where they were incubated for 8 days. The Petri dishes were checked daily for larval mortality and the dead larvae were removed from the Petri dishes. The control larvae were treated only with distilled water containing 0.02% Tween 80. This assay was carried out with three replicates. After determination of LC_{50} for this isolate, the virulence of all isolates on *T. granarium* larvae was evaluated only at this concentration. The dead larvae were sterilized superficially and incubated at 25 ºC for verification of pathogen growth. Whole experiment was repeated twice and pooled data was used in statistical analysis.

Protease assay

To induce the production of proteases in the entomopathogenic isolates, a suspension of the conidia (10^7 conidia per ml) was prepared. One ml of the suspension was transferred to Petri dishes containing SDY liquid medium and the Petri dishes were incubated at 28 ± 1 ºC, for 5 days. The mixture was then centrifuged at 4000 rpm for 5 min and the supernatant was collected as protease source. The protease assay was done according to the method described by Kunitz (1947). Casein substrate was prepared for enzyme assay by dissolving 2% casein (Sigma) in 0.01 M Tris HCl (pH 8.0) containing 10 mM CaCl2 (pH 8.0) and 50 ml distilled water. Four hundreds µl of casein substrate was added to 200 µl of culture extract in 0.01 M Tris HCl pH 8, 10 mM CaCl2. The reaction mixture was incubated at 35 ºC for 10 min and the reaction was terminated by adding 500 µl trichloroacetic acid (TCA). The reaction mixture was centrifuged at 8000 rpm for 5 min and the absorbance of the resultant supernatant was observed at 280 nm (Ultrospec II, LKB Biochrom, UK). Two controls were considered for this assay, one containing 600 µl of pure SDY medium and the other containing 600 µl buffer (0.01 M Tris HCl pH 8, 10 mM CaCl2) in 50 ml distilled water. Three replicates were considered for this experiment.

One unit of protease activity was defined as the amount of enzyme that produced 1 mM of Tyrosine per minute under the above conditions (St-Leger *et al*., 1987; Gupta *et al*., 1992; Revathi *et al*., 2011). This was calculated using the Beer-Lambert law as follow:

$A = \varepsilon b c$

Where A is absorbance, ε is the molar absorptivity with units of L mol^{-1} cm^{-1} (Tyrosine = 1280), b is the path length of the sample (cuvette) and c is the concentration of the compound in solution (mol L^{-1}).

Statistical analysis

POLO-PLUS software was used for LC_{50} estimation of the fungal isolate JB2. Analysis of variance (ANOVA) was performed to compare the virulence and protease activity among different isolates of the fungus. The relationship between virulence and proteolytic activity in isolates was tested using Pearson correlation coefficient. Bonferroni test was applied to test this coefficient statistically. These analyses were done using computer software SYSTAT 12.

Results and Discussion

Our preliminary bioassay revealed that a concentration of 3.9×10^6 conidia per ml from the JB2 isolate of *B. bassiana sensu lato* caused an average mortality of 50% in larvae of *T. granarium*. Results of main bioassay showed that all isolates were pathogenic to *T. granarium* (Fig. 1) with isolate MITE of *B. bassiana sensu lato* causing the highest rate of larval mortality (59%). Isolate BA caused the lowest larval mortality (only 25%). The larval mortalities caused by the 17 isolates are shown in Fig. 1. Analysis of variance revealed that the 17 isolates significantly differed in their virulence against *T. granarium* ($F_{16,40} = 2.72$, $P < 0.01$). The onset of larval mortality was recorded at 4^{th} day after treatment, although it varied among different isolates.

We found a significant difference in proteolytic activity of the 17 different isolates of *B. bassiana* ($F_{16,40} = 5213.47$, $P < 0.00001$) on Casein substrate. The highest proteolytic activity was detected in MITE isolate (1.028 U/ml), while the BB isolate showed the lowest enzyme activity (0.157 U/ml).

The relationship between the virulence and proteolytic activity of the 17 isolates was evaluated using correlation analysis. A significant correlation was observed between virulence of isolates and their proteolytic activities (Bonferoni test: $r = 0.84$, $P < 0.001$).

The entomopathogenic fungus *B. bassiana* is an important biological control agent on a very wide variety of arthropods, including some of economically important pests of agriculture, horticulture, and forestry. It has been estimated to naturally occur in more than 700 species of arthropods (Inglis *et al*., 2001). In the current study, a total of 17 isolates belonging to *B. bassiana* were collected from three Northern provinces of Iran (Mazandaran, Guilan, and Alborz). The majority of these isolates were detected from infected larvae of the striped rice borer, *C. suppressalis* (Table 1), an important pest of rice in Northern Iran, indicating that *B. bassiana* is a common natural regulation agent of *C. suppressalis* in these regions.

All isolates obtained from soil, *C. suppressalis* (a moth), and *T. urticae* (a mite) showed significant virulence against Khapra beetle, *T. granarium,* implying that *B. bassiana* isolates are capable of invading a wide range of arthropods belonging to taxa with different evolutionary origins. The mortality of larvae initiated from the 4th day after treatment with the fungus. This delay is probably related to the time needed for adhesion, penetration, germination and growth of the fungus. Such effect of entomopathogenic fungi has been previously reported in different studies (Thomas *et al*., 1997; Ekesi, 2001).

We found a wide variation in virulence of *B. bassiana sensu lato* isolates against *T. granarium* (Fig. 1). The larval mortality of *T. granarium* ranged from 25 to 59% as a result of treatment with isolates BA and MITE, respectively (Fig. 1). Such variations in virulence of different isolates of entomopathogenic fungi have been well documented in different species and seems to be caused by a number of factors such as insect host, host plant properties, host food, environmental conditions, etc. (Todorova *et al*., 1994; Vandenberg *et al*., 1998; Santiago-Alvarez *et al*., 2006; Talai-Hassanloui *et al*., 2006; Safavi *et al*., 2007; Carneiro *et al*., 2008; Ngumbi *et al*., 2011; Sanchez-Pena *et al*., 2011).

In encountering with insect cuticle, the entomopathogenic fungi produce a variety of degrading enzymes, including proteases, chitinases, and lipases, against the major components of the cuticle, i.e. proteins, chitin, and lipids, respectively (Raymond *et al*., 1986). As proteins constitute the majority of insects' cuticle (about 70%) (Hepburn, 1985; Charnley, 2003), the degrading activities of proteases are expected to play much more important role in penetration of the entomopathogenic fungi through insect cuticle compared to enzymes which catalyze other components such as chitins and lipids. Therefore, the proteolytic activities are expected to reflect the virulence of the entomopathogenic fungi, a hypothesis that has been received great attention during the last decades (Paris and Segretain, 1978; Bidochka and Khachatourians, 1990; Charnley and St. Leger, 1991). We found significant differences in proteolytic activities of the 17 studied isolates of *B. bassiana* (Fig. 2). Such variations in proteolytic activity of different isolates of *Beauveria bassiana* and some other fungi have been demonstrated in previous studies and seem to be widespread among entomopathogenic fungi (Rosato *et al*., 1981; Kaur and Padmaja, 2009; Dhar and Kaur, 2010). Strong evidences have uncovered the existence of a wide genetic variability in production of cuticle degrading enzymes in different isolates of entomopathogenic fungi (Braga *et al*. 1994; Pinto *et al*., 2002). Interestinglyand as expected, the differences in proteolytic activities of our 17 studied isolates were in accordance with the changes in their virulence, such that the higher the proteolytic activity of a given isolate, the higher the virulence of that isolate. A relatively strong correlation was found between the virulence and proteolytic activity of the 17 isolates (r = 0.84).

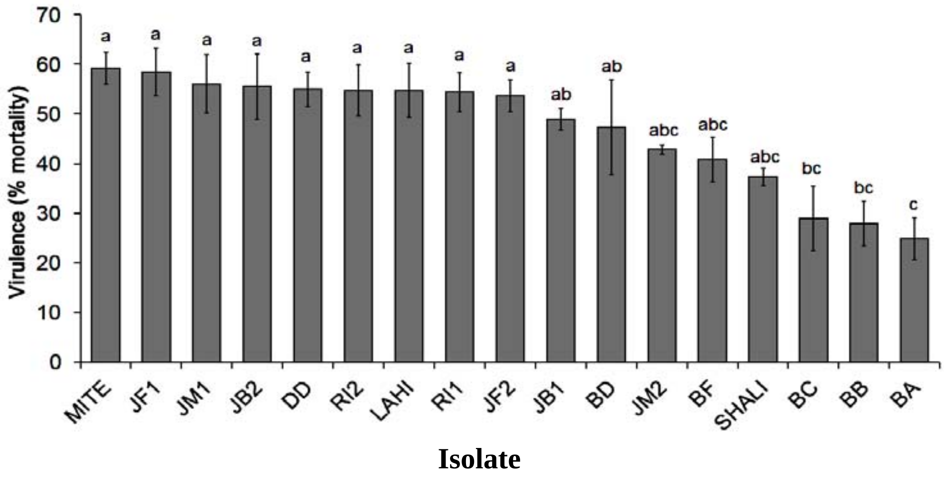

Figure 1 Mean (± SE) percentage mortalities caused by different native *Beauveria bassiana* isolates on the Khapra beetle, *Trogoderma granarium* larvae at 3.9×10^6 conidia per ml, a LC_{50} level of JB2, Means followed by the different letters are significantly different (F-LSD, $P < 0.05$).

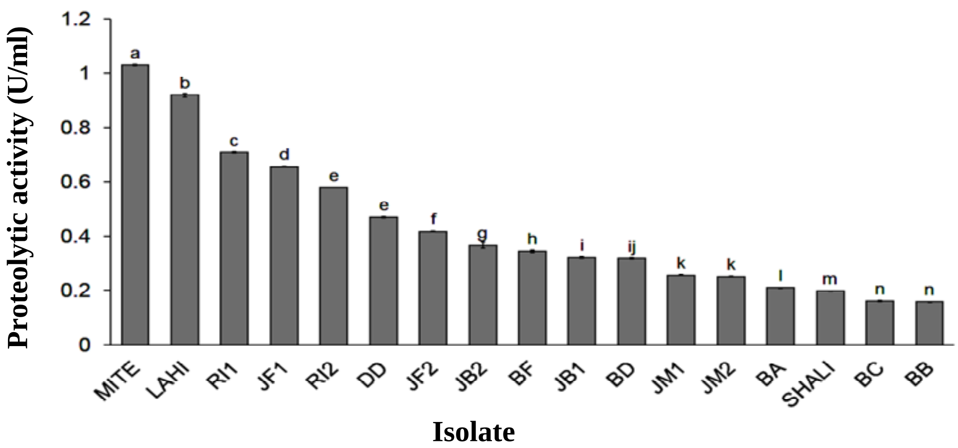

Figure 2 Mean (± SE) proteolytic activity of different native *Beauveria bassiana* isolates on Casein substrate. Means followed by the different letters are significantly different (F-LSD, $P < 0.05$).

Altogether, our current study highlights that convergent variability exists in proteolytic activity and virulence among different isolates of *B. bassiana sensu lato*. Several authors have considered that protease activity can determine the virulence of entomopathogenic fungi to some degree, thus can be used as a virulence index (St-Leger *et al*., 1987; St-Leger *et al*., 1996; Feng, 1998; Gillespie *et al*., 1998; Castellanos-Moguel *et al*., 2008). However, there are other studies in which, no reliable relationship between the protease activity and virulence of studied entomopathogenic fungi has been established (Gillespie *et al*., 1998; Vargas *et al*., 2003; Dias *et al*., 2008).

It is proposed that virulent isolates of *B. bassiana sensu lato,* for using as effective biological control agents, could be screened and selected based on their degrading protease production pattern which is consistent with the findings of Rosato *et al*. (1981) and Castellanos-

Moguel *et al*. (2008). Additionally, recent advances in biotechnology provide new insights into the more efficient control of insect pests using entomopathogenic fungi. For example, overproduction of the endochitinase, Bbchit1, through cloning of *Bbchit1* gene has been suggested to increase the virulence of *B. bassiana* against aphids (Fang *et al*., 2005). Continued studies in these areas will undoubtedly improve the potential of these biological control agents for use in insect population management.

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References

Balachander, M., Remadevi, O. K., Sasidharan, T. O. and Sapna, B. N. 2012. Virulence and mycotoxic effects of *Metarhizium anisopliae* on Mahogany shoot borer, *Hypsipyla robusta* (Lepidoptera: Pyralidae). Journal of Forestry Research, 23-651-659.

Bidochka, M. J. and Khachatourians, G. G. 1990. Identification of *Beauveria bassiana* extracellular protease as virulence factor in pathogenicity towards the migratory grasshopper *Melanoplus sanguinipes*. Journal of Invertebrate Pathology, 56: 362-370.

Bidochka, M. J. and Khachatourians, G. G. 1994. Protein hydrolysis in grasshopper cuticles by entomopathogenic fungal extracellular proteases. Journal of Invertebrate Pathology, 63: 7-13.

Boldo, J. T., Junges, A., Amaral, K. B., Staats, C. C., Vainstein, M. H. and Schrank, A. 2009. Endochitinase CHI2 of the biocontrol fungus *Metarhizium anisopliae* affects its virulence toward the cotton stainer bug *Dysdercus peruvianus*. Current Genetics, 55: 551-560.

Braga, G. L., Messias, C. L. and Vencorsky, R. 1994. Estimates of genetic parameters related to protease production by *Metarhizium anisopliae*. Journal of Invertebrate Pathology, 64: 6-12.

Carneiro, A. A., Gomes, E. A., Guimaraes, C. T., Fernandes, F. T., Carneiro, N. P. and Cruz, I. 2008. Molecular characterization and pathogenicity of isolates of *Beauveria* spp. to fall armyworm. Pesquisa Agropecuária Brasileira, 43: 513-520.

Castellanos–Moguel, J., Cruz–Camarillo, R., Aranda, E., Mier, T. and Toriello, C. 2008. Relationship between protease and chitinase activity and the virulence of *Paecilomyces fumosoroseus* in *Trialeurodes vaporariorum* (Hemiptera: Aleyrodidae). Revista Mexicana de Micologia, 28: 73-80.

Charnley, A. K. and St-Leger, R. J. 1991. The role of cuticle degrading enzymes in fungal pathogenesis in insects. In: The Fungal Spore and Disease Initiation in Plants and Animals (G. T. Cole and H. C. Hoch, eds.), Plenum New York, pp. 267-286.

Charnley, A. K. 2003. Fungal pathogens of insects: Cuticle degrading enzymes and toxins. Advances in Botanical Research, 40: 241-321.

Clarkson, J. M. and Charnley, A. K. 1996. New insights into mechanisms of fungal pathogenesis in insect. Trends in Microbiology, 4: 197-203.

Dhar, P. and Kaur, G. 2010. Cuticle-degrading proteases produced by *Metarhizium anisopliae* and their induction in different media. Indian Journal of Microbiology, 50: 449-455.

Dias, B. A. Neves, P. M., Furlaneto-Maia L. and Furlaneto, M. C. 2008. Cuticle-degrading proteases produced by the entomopathogenic fungus *Beauveria bassiana* in the presence ofcoffee berry borer cuticle. Brazilian Journal of Microbiology, 39: 301-306.

Ekesi, S. 2001. Pathogenecity and antifeedant activity of entomopathogenic hyphomycetes to the cowpea leaf beefle, *Ootheca mutabilis* Shalbery. Insect Science and its Application, 21: 55-60.

Fang, W., Leng, B., Xiao, Y., Yin, K., Ma, J., Fan, Y., Feng, Y., Yang, X., Zhang, Y. and Pei, Y. 2005. Cloning of *Beauveria bassiana* chitinase gene Bbchit1 and its application to improve fungal strain virulence. Applied and Environmental Microbiology, 71: 363-370.

Gatehous, A. M., Hilder, V. A., Powell, K. S., Wang, M., Davison, G. M., Gatehouse, L. N., Down, R. E., Edmonds, H. S., Boulter, D. and Newell, C. A. 1994. Insect-resistant transgenic plants: choosing the gene to do the 'job'. Biochemical Society Transactions, 22: 944-949.

Gupta, S. C., Leaters, T. D., El-Sayed, G. N. and Ignoffo, C. M. 1992. Insect cuticle-degrading enzymes from the entomogenous fungus *Beauveria bassiana*. Experimental Mycology, 16: 132-137.

Gupta, S. C., Leathers, T. D., El-Sayed, G. N. and Ignoffo, C. M. 1994. Relationships among enzyme activities and virulence parameters in *Beauveria basiana* infections of *Galleria mellonella* and *Trichoplusia ni*. Journal of Invertebrate Pathology, 64: 13-17.

Haq, S. K., Atif, S. M. and Khan, R. H. 2004. Protein proteinase inhibitor genes in combat against insects, pests, and pathogens: natural and engineered phytoprotection. Archives of Biochemistry and Biophysics, 431: 145-159.

Hepburn, H. R. 1985. Structure of the integument. In: Comprehensive Insect Physiology, Biochemistry and Pharmacology (G. A. Kerkut and L. I. Gilbert, eds.), Vol. 3, Pergamon Oxford, pp. 1-58.

Humber, R. A. 2005. Entomopathogenic fungal identification, key to major genera. Available on: *www.ars.usda.gov/SP2UserFiles/Place/19070510/APSwkshoprev.pdf*

Inglis, G. D., Goettel, M. S., Butt, T. M. and Strasser, H. 2001. Use of hyphomycetous fungi for managing insect pests. In: Butt, T. M., Jackson, C. W., and Magan, N. (Eds.), Fungi as Biocontrol agents: progress, problems and potential. CABI Publishing, Wallingford, UK, pp. 23-55.

Joshi, L., St. Leger, R. J. and Bidochka, M. J. 1995. Cloning of a cuticle-degrading protease from the entomopathogenic fungus, *Beauveria bassiana*. FEMS Microbiology Letters, 125: 211-218.

Kaur, K. and Padmaja, V. 2009. Relationships among activities of extracellular enzyme production and virulence against *Helicoverpa armigera* in *Beauveria bassiana*. Journal of Basic Microbiology, 49: 264-274.

Kunitz, M. 1947. Isolation of a crystalline protein compound of trypsin and of soybean trypsin-inhibitor. Journal of General Physiology, 30:311-320.

Feng, M. G. 1998. Reliability of extracellular protease and lipase activities of *Beauveria bassiana* isolates used as their virulence indices. Acta Microbiologica Sinica, 38: 461-467.

Fitt, G. P. 1994. Cotton pest management: Part 3. An Australian perspective. Annual Review of Entomology, 39: 543-562.

Klingen, I. and Haukeland, S. 2006. The soil as a reservoir for natural enemies of pest insects mites with emphasis on fungi and nematodes. In: Eilenberg J, Hokkanen HMT (Eds.), An Ecological and Societal Approach to Biological Control. Springer, Dordrecht, The Netherlands, pp. 145-211.

Kuhnelt, W. 1963. Soil-inhabiting Arthropoda. Annual Review of Entomology, 8: 115-136.

Ngumbi, P. M., Irungu, L. W., Ndegwa, P. N. and Maniania, N. K. 2011. Pathogenicity of *Metarhizium anisopliae* (Metch.) Sorok. and *Beauveria bassiana* (Bals.) Vuill. to adult *Phlebotomus duboscqi* (Neveu-Lemaire) in the laboratory. Journal of Vector Borne Diseases, 48: 37-40.

Onofre, S. B., Miniuk, C. M., De Barros, N. M. and Azevedo, J. L. 2001. Growth and sporulation of *Metarhizium flavoviride* var. *flavoviride* on culture media and lighting regimes. Scientia Agricola, 58: 613-616.

Gillespie, J. P., Bateman, R. and Charnley, A. K. 1998. Role of cuticle-degrading protease in the virulence of *Metarhizium spp*. for the desert locust, Schistocerca gregaria. Journal of Invertebrate Pathology 71: 128-137.

Paris, S. and Segretain, G. 1978. Etude de l'activite lipasique–esterasique intra cellulaire de Beauveria tenella. Annales de l'Institut Pasteur Microbiology, 1293: 133-145.

Pinto, F. G. S., Fungaro, M. H. P., Ferreira, J. M., Valadares-Inglis, M. C. and Furlaneto, M. C. 2002. Genetic variation in the cuticle-degrading protease activity of the entomopathogen *Metarhizium flavoviride*. Genetics and Molecular Biology, 25: 231-234.

Revathi, N., Ravikumar, G., Kalaiselvi, M., Gomathi, D. and Uma, C. 2011. Pathogenicity of three entomopathogenic fungi against *Helicoverpa armigera*. Plant Pathology and Microbiology, 2: 1-4.

Roberts, D. W. and Humber, R. A. 1981. Entomogenous fungi. In: Cole GT, Kendrick B, (Eds.), Biology of conidial fungi. Academic Press, New York, pp. 201-236.

Rosato, Y. B., Messias, C. L. and Azevedo, J. L. 1981. Production of extracellular enzymes by isolates of *Metarhizium anisopliae*. Journal of Invertebrate Pathology 38: 1-3.

Safavi, S. A., Shah, F. A., Pakdel, A., Rasoulian, G. R., Bandani, A. R. and Butt, T. M. 2007. Effect of nutrition on growth and virulence of the entomopathogenic fungus *Beauveria bassiana*. FEMS Microbiology Letters, 270: 116-123.

Sanchez-Pena, S. R., Lara, J. S. and Medina, R. F. 2011. Occurrence of entomopathogenic fungi from agricultural and natural ecosystems in Saltillo, Mexico, and their virulence towards thrips and whiteflies. Journal of Insect Science, 11: 1.

Santiago-Alvarez, C., Maranhao, E. A., Maranhao, E. and Quesada-Moraga, E. 2006. Host plant influences pathogenicity of *Beauveria bassiana* to *Bemisia tabaci* and its sporulation on cadavers. BioControl, 51: 519-532.

Scholte, E. J., Knols, B. J., Samson, R. A. and Takken, W. 2004. Entomopathogenic fungi for mosquito control: A review. 24 pp. Journal of Insect Science, 4: 19, Available online: insectscience.org/4.19.

St-Leger, R. J., Cooper, R. M. and Charnley, A. K. 1986. Cuticle-degrading enzymes of entomopathogenic fungi: regulation of production of chitinolytic enzymes. Journal of General Microbiology, 132: 1509-1517.

St-Leger R. J., Charnley, A. K. and Cooper, R. M. 1987. Characterization of Cuticle-Degrading Proteases Produced by the Entomopathogen *Metarhizium anisopliae*. Archives of Biochemistry and Biophysics, 253: 221-232.

St-Leger, R. J., Joshi, L., Bidochka, M. J. and Roberts, D. W. 1995. Protein synthesis in *Metarhizium anisopliae* growing on host cuticle. Mycological Research, 99: 1034-1040.

St-Leger, R. J., Joshi, L., Bidochka, M. J., Rizzo, N. and Roberts, D. W. 1996. Biochemical characterization and ultrastructural localization of two extracellular trypsins produced by *Metarhizium anisopliae* in infected insect cuticles. Applied and Environmental Microbiology, 62: 1257 1264.

Talaei-Hassanloui, R., Kharazi-Pakdel, A., Goettel, M. and Mozaffari, J. 2006. Variation in virulence of *Beauveria bassiana* isolates and its relatedness to some morphological characteristics. Biocontrol Science and Technology, 16: 525-534.

Thomas, M. B., Blanford, S. and Lomer, C. J. 1997. Reduction of feeding by the variegated grasshopper, *Zonocerus Variegatus*, following infection by the fungal pathogen, *Metarhizium flavoviride*. Biocontrol Science and Technology, 7: 327-334.

Todorova, S. I., Cote, J. C., Martel, P. and Coderre, D. 1994. Heterogeneity of two *Beauveria bassiana* strains revealed by biochemical tests, protein profiles and bio-assays on *Leptinotarsa decemlineata* (Col.: Chrysomelidae) and *Coleomegilla maculate lengi* (Col.: Coccinellidae) larvae. Entomophaga, 39: 159-169.

Vandenberg, J. D., Ramos, M. and Altre, J. A. 1998. Dose-response and age- and temperature-related susceptibility of the diamondback moth (Lepidoptera: Plutellidae) to two isolates of *Beauveria bassiana* (Hyphomycetes: Moniliaceae). Environmental Entomology, 27: 1017-1021.

Vargas, L. B., Rossato, M., Ribeir, R. S. and de Barros, N. M. 2003. Characterization of *Nomuraea rileyi* strains using polymorphic DNA, virulence and enzyme activity. Brazilian Archives of Biology and Technology, 46: 13-18.

Wang-Ching, H. and Wen-Hsiung, K. 1997. A simple method for obtaining single-spore isolates of fungi. Botanical Bulletin of Academia Sinica, 38: 41-44.

ارتباط توانایی پروتئولیتیکی و زهرآگینی در جدایه‌های قارچ بیمارگر حشرات *Beauveria bassiana*

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چکیده: قارچ‌های بیمارگر حشرات برای تسهیل ورود خود از میان موانع عظیم کوتیکول به داخل بدن حشره، آنزیم‌های تجزیه‌گر متنوعی ازجمله پروتئازها، کیتینازها و لیپازها را تولید می‌کنند. جدایه‌های قارچ‌های بیمارگر حشرات از نظر فعالیت پروتئولیتیک و زهرآگینی، تفاوت قابل‌توجهی دارند. این‌که فعالیت پروتئولیتیک جدایه‌های مختلف می‌تواند نشانه‌ای از زهرآگینی آنها روی میزبان باشد، به‌عنوان یک پیش‌فرض ارائه شده است. در این مطالعه، زهرآگینی و فعالیت پروتئولیتیک ۱۷ جدایه از قارچ *Beauveria bassiana sensu lato* جمع‌آوری شده از مناطق جغرافیایی مختلف در ایران مورد ارزیابی قرار گرفته است. محیط کشت D0C2 برای جداسازی جدایه‌ها از نمونه‌های خاک مورد استفاده قرار گرفت. سنجش فعالیت پروتئازی روی سوبسترای کازئین انجام شد. مرگ‌ومیر کل ایجاد شده به‌وسیله‌ی جدایه‌ها با روش غوطه‌وری روی لاروهای *Trogoderma granarium*، از ۲۵ تا۶۰ درصد متغیر بود که این مقادیر پایین و بالای مرگ، به‌ترتیب مربوط به جدایه‌های BA و MITE بود. نتایج ما نشان داد که بین جدایه‌های مورد مطالعه، هم در فعالیت پروتئولیتیک و هم در زهرآگینی علیه شپشه *T. granarium* تنوع زیادی وجود دارد. علاوه بر این، یک همبستگی مثبت قوی بین فعالیت پروتئولیتیک روی کازئین و زهرآگینی جدایه‌ها علیه این میزبان مشاهده شد. این یافته، غربال‌گری و روند انتخاب جدایه‌های زهرآگین قارچی به‌عنوان عوامل کارآمد برای استفاده در برنامه‌های کنترل بیولوژیک آفات را تسهیل می‌نماید.

واژگان کلیدی: قارچ بیمارگر حشرات، فعالیت پروتئازی، حشره، *Trogoderma granarium*، *Beauveria bassiana*