Acta Phytopathologica et Entomologica Hungarica 50 (1), pp. 115–125 (2015)
DOI: 10.1556/038.50.2015.1.11

Virulence Determination of *Beauveria bassiana* Isolates on a Predatory Hemipteran, *Andrallus spinidens* Fabricius (Hemiptera: Pentatomidae)

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(Received: 30 June 2014; accepted: 4 September 2014)

Virulence of the two isolates of *Beauveria bassiana*, BB_2 and AM-118, were evaluated on adults of a predatory hemipteran, *Andrallus spinidens* Fabricius by conidial bioassay and enzymatic activities. Results of the bioassay revealed LC_{50} of 37×10^4 and 15×10^3 spore/ml for isolates BB_2 and AM-118, respectively. Activities of chitinase, lipase and ALP showed the higher activity in the media inoculated by AM-118 while no statistical differences were observed in activity of ACP. Although no statistical differences were found in general protease and Pr_1 activities but activity of Pr_2 in AM-118 was significantly higher than that of BB_2. Activity of general esterases demonstrated no statistical differences when α- and β-naphtyl acetate were used as substrates but activity of glutathione *S*-transferase in AM-118 was higher than that of BB_2 by using CDNB and DCNB as specific reagents. Results of the current study indicated higher virulence of isolate AM-118 against adults of *A. spinidens* by lower LC_{50} value and higher activities of the enzymes involved in pathogenicity. Recruiting of these isolates against *C. suppressalis* must be considered by their adaptability of *A. spinidens*. Moreover, AM-118 has been isolated from rice fields of northern Iran, so it may somehow indicate a type of host-microorganism interaction.

Keywords: *Beauveria bassiana*, virulence, *Andrallus spinidens*, microbial enzyme.

Entomopathogenic fungi are one of the important microbial agents against insect pests. These microorganisms caused epizootics and significantly affect population dynamic of insects. Among several entomopathogenic fungi, *Beauveria bassiana* (Hypocreales) have shown virulence against different insect pests and it is commonly used in integrated pest management systems (Barbarin et al., 2012). *B. bassiana* grows naturally in soils, acts as a parasite on various arthropod species causing white muscardine disease (Barbarin et al., 2012). Infection process of *B. bassiana* and other entomopathogenic fungi is almost similar to each others. Conidia of enthomopathogenic fungi adhere to the cuticle of host by a non-specific adhesion mechanism mediated by the hydrophobicity of the cell wall using hydrophobin proteins (Wessels et al., 1991; Ramzi and Zibaee, 2014).

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Then, conidia germinate the germ tubes to penetrate cuticle and access to hemocoel of insects. Finally, host is killed due to consumption of food reserves, physically disruption of tissues and producing toxins by blastospores produced in hemolymph (Ramzi and Zibaee, 2014). Since, entomopathogenic fungi have narrow host range, they might be the appropriate candidates to be used as biopesticides in integrated pest management (Grund and Hirsch, 2010). There are several steps to achieve the above mentioned purposes. First, fungal species and isolates must be obtained then identified from host insects or environment. Second, their virulence must be evaluated in laboratory conditions. If fungi have appropriate virulence on insects, then those must be mass produced and formulated to be used in field (Butt and Goettel, 2000).

Chilo suppressalis Walker (Lepidoptera: Crambidae) is the key constraint on rice production in northern Iran. The pest annually causes severe damages to the host plant leading to significant yield loss. Wide spraying of a synthetic chemical, diazinon, was the main control procedure against *C. suppressalis*. It has been now determined that some populations of *C. suppressalis* have shown resistance to diazinon in the area. Moreover, three natural enemies are present in rice fields of northern Iran including *Trichogramma brassicae* Westwood (Hymenoptera: Trichogrammatidae), *Andrallus spinidens* Fabricius (Hemiptera: Pentatomidae) and *B. bassiana*. The first one is an egg parasitoid that is reared on insectariums and commercially applied in rice fields. Although the second and third ones are naturally present in rice fields but *A. spinidens* prey larval stages of *C. suppressalis*. Recently, several studies have been made to find virulence of *B. bassiana* and efficiency of *A. spinidens* against *C. suppressalis* (Sorkhabi-Abdolmaleki et al., 2013a, b; Ramzi and Zibaee, 2014; Zibaee and Malagoli, 2014). Ramzi and Zibaee (2014) determined virulence of several entomopathogenic fungi including *B. bassiana senso latto* (three isolates), *Metrhizium anisopliae senso latto*, *Isaria fumosoroseus* and *Lecanicilium lecanni*. Results revealed the highest virulence of isolate BB_2[1] from *B. bassiana s.l.* in both bioassay and biochemical production. Currently, we collected another isolate of *B. bassiana* from Amol region (Northern Iran) that had been seriously infected larvae of *C. suppressalis* (AM-118)[2]. Meanwhile, feeding of *A. spinidens* on several preys revealed the highest performance on *C. suppressalis*. Since, it is required to combine all procedures to have an efficient pest control. So integrated usage of *B. bassiana* and *A. spinidens* might be of interest regarding their appropriate effects on *C. suppressalis*. Before handling this combination in the field, it must be elucidated the effects of *B. bassiana* isolates on *A. spinidens*. So, two isolates of *B. bassiana*; BB_2 showing the highest virulence on *C. suppressalis* and AM-118, currently collected isolate, were studied on adults of *A. spinidens* by spore bioassay and biochemical compositions produced in liquid media containing cuticle of the adults. These isolates were chosen due to results of our previous results (Ramzi and Zibaee, 2014; Zibaee and Malagoli, 2014). In those references, the authors were determined pathogenicity of various isolates of *B. bassiana* on larvae of *Chilo suppressalis* in addition to larval immunity. Now in the current study, we want to further determine the isolates' efficiency under IPM system. In details, we want to choose the most effective isolates' on larvae of *C. suppressalis* by

[1] EU-106 is a commercially provided fungi that now has been kept in Iranian Institute of Plant Protection.

[2] The isolate was recognized by Dr. A. Khodaparast in our department and now it has been kept in herbarium. It was isolated form rice fields of Amol and kept in herbarium of Department of Plant Portection. University of Guilan.

the lowest effect on the predator of larvae, *A. spinidens* using bioassay and enzymatic indicators. These results will help us to combined usage of fungi and predator in IPM system against *C. suppressalis*. These experiments will clear us potential interactions between the entomopathogenic fungus and the predator against *C. suppressalis*.

Materials and Methods

Entomopathogenic fungal cultures

The isolates of *B. bassiana*, BB_2 and AM-118 (Fig. 1), were cultured at 25 ± 1 °C on Potato Dextrose Agar (PDA) (pH 5.6) amended with 1% of yeast extract. After 20 days, conidia were washed off with a 0.01% aqueous solution of Tween 80 and 10^8 spore/mL as stock concentration of spores.

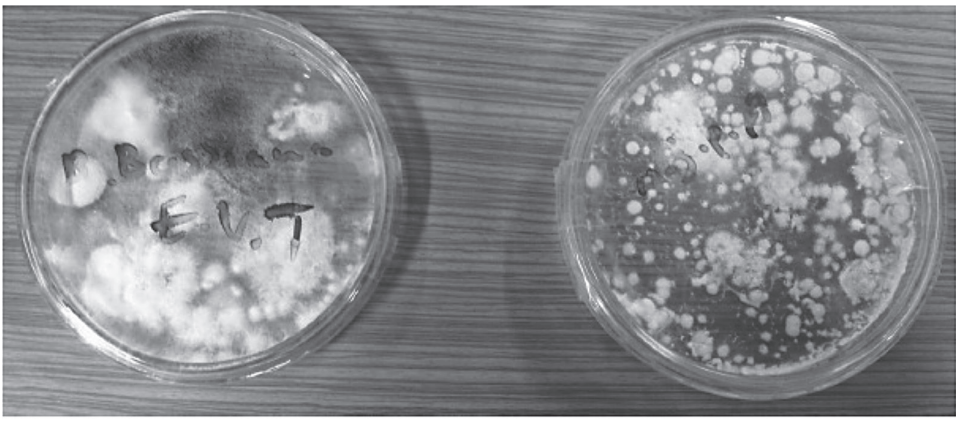

Fig. 1. *B. bassiana* isolates used against *A. spinidens*. These isolates now have been kept in fungal herbarium at the University of Guilan

Insect rearing and cuticle preparation

A colony of *A. spinidens* was established from harvested rice fields in Amol Institute of Rice Research (Mazandaran, North of Iran). Insects were reared on the fifth instars of *C. suppressalis* L. (Lepidoptera: Crambidae) as a food source. Also, those were provided with wet cotton plugs fitted into small plastic dishes (2.5 cm diameter) to serve as moisture sources. Rearing conditions were 25 ± 2 °C, 80% relative humidity and 16L:8D as photoperiod.

Bioassay

Spores of 14-day-old PDA cultures were removed from media and serial concentrations were prepared from 10^2 to 10^8 spores/ml. Adults of *A. spinidens* were separately immersed for five seconds in each concentration and a solution containing Tween 80 (0.01%) was used for control treatment. After inoculation, adults were divided into four groups including five adults and maintained at 28 ± 2 ˚C, 80% of relative humidity and 16L:8D. Values of lethal concentration were calculated by POLO-PC software (Finney, 1971).

Liquid culture for enzyme production

The liquid media used for enzymatic production contained; $CaCl_2$, 0.01%; KH_2PO_4 0.02%; Na_2HPO_4 0.02%; $MgSO_4$ 0.01%; $ZnCl_2$ 0.01% (Merck Co. Germany) (Zibaee and Bandani, 2009). Culture media were augmented with cuticle of adults (5% of weight) to obtain protease, chitinase, lipase, general esterase, glutathione *S*-transferase, acid- and alkaline phosphatases. The media were separately inoculated with 10^7 spores/mL of isolates incubated on a rotatory shaker (50 rev/min) for 8 days at 25 ± 1 °C (Zibaee and Bandani, 2009) and darkness.

Sample preparations for enzymatic assays

After 8 days of incubation, each mixture was separately harvested by centrifugation at 5000 rpm for 30 min and washed in ice-cold 25 mM Tris-HCL pH 8. Weighed mycelia were ground to a fine powder, suspended in distilled water, homogenized and centrifuged at 13000 rpm for 30 min to obtain supernatant.

Chitinase assay

For chitinase, the reaction mixture contained 20 μl of enzyme, 50 μl of 0.5% colloidal chitin and 100 μl of Tris-HCl buffer (20 mM, pH 7) based on the estimation of reducing sugars released during the hydrolysis of colloidal chitin (Sigma-Aldrich, Co., Austria). The mixture was kept in a water bath at 50 °C for 60 min. The amount of reducing sugar liberated was estimated by Miller (1959) method using dinitrosalysilic acid (DNS). At the end, 100 μl of DNS was added, samples were incubated for 10 min at boiling water prior to reading absorbance at 545 nm (Microplate reader; Awareness: Statfax:2100; USA). One unit (U) of activity was defined as the amount of enzyme which catalyzed the release of 1 μl of reducing sugar per ml per minute under the reaction conditions. The tubes containing the negative control were placed in a boiling water bath for 15 min to destroy the enzyme activity and then cooled prior to being added to the substrate. The experiment was conducted in three replicates.

Lipase assay

The enzyme assay was carried out as described by Tsujita et al. (1989). Twenty microliters of the enzyme, 100 μl of Tris-HCl buffer (20 mM, pH 7) and 50 μl of *p*-nitrophenyl butyrate (27 mM, Sigma-Aldrich, Co., Austria) were incorporated, thoroughly mixed and incubated at 37 °C. After 5 min, 100 μl of NaOH (1 N) was added to each tube (control and experimental samples) and absorbance was read at 405 nm (Microplate reader; Awareness: Statfax:2100; USA). One unit of the enzyme releases 1.0 nm of *p*-nitrophenol per minute at pH 7.2 at 37 °C using *p*-nitrophenyl butyrate as substrate. The tubes containing the negative control were placed in a boiling water bath for 15 min to destroy the enzyme activity and then cooled prior to being added to the substrate. The experiment was conducted in three replicates.

General proteolytic assay

General proteolytic activities of isolates were measured by using 2% azocasein (Sigma-Aldrich, A2765) as substrate described by Elpidina et al. (2001). The reaction mixture consisted of 80 µl of Tris–HCl buffer (20 mM, pH 7), 40 µl of azocasein (2%) and 20 µl of enzyme. After incubation at 37 °C for 60 min, proteolysis was stopped by the addition of 100 µl trichloroacetic acid (TCA) (30%). Precipitation was achieved by cooling at 4 °C for 120 min and it was centrifuged at 13,000 rpm for 10 min. An equal volume of NaOH (2 N) was added to the supernatant and the absorbance was recorded at 454 nm (Microplate reader; Awareness: Statfax:2100; USA). The blank consisted of all mentioned portions except for the enzyme solution.

Specific proteolytic assay

Subtilisin-like and trypsin-like activities were assayed using succinyl-(alanine) 2-proline-phenylalanine-*p*-nitroanilide and benzoyl-phenylalanine-valinearginine-*p*-nitroanilide as substrates (Sigma-Aldrich, Co., Austria), respectively. The reaction mixture consisted of 30 μl substrate (5 mM), 100 μl of Tris-HCl buffer (20 mM, pH 7) and 20 μl of enzyme. The mixture was incubated for 10 min at 25 °C and the reaction was terminated by adding 100 μl of 30% trichloroacetic acid (Zibaee and Bandani, 2009). Absorbance was read at 405 nm (Microplate reader; Awareness: Statfax:2100; USA).

General esterase assay

General esterase activity was determined by the method of Han et al. (1998). Thirty microliters of α-naphthyl acetate (10 mM) and β-naphthyl acetate (10 mM) as well as 30 μl of fast blue RR salt (1 mM) (Sigma-Aldrich, Co., Austria) were added into each microplate well. The reaction was initiated by addition of 20 μl of enzyme solution. Temperature of the experiment was 25 °C for 5 min. Absorbance was read at 450 nm (Microplate reader; Awareness: Statfax:2100; USA). The experiment was conducted in three replicates.

Glutathion S-transferase activity

For glutathione S-transferase activity, the method reported by Oppenorth (1985) was adopted. Thirty microliters of CDNB (1-chloro, 2,4-dinitrobenzene; 20 mM) or DCNB (2,4-dinitrochlorobenzene; 20 mM) were pipetted into the microplate wells (Sigma-Aldrich, Co., Austria), then 30 μl of glutathione (20 mM) and 20 μl of enzyme solution were added. Temperature of the experiment was 25 °C for 5 min. Absorbance was read at 340 nm (Microplate reader; Awareness: Statfax:2100; USA). The experiment was conducted in three replicates.

Acid and alkaline phosphatase assay

The enzyme assays were carried out as described by Bessey et al. (1946). The buffered substrates (*p*-nitrophenol phosphate Tris-HCl, 20 mM, pH 5 for acid phosphatase and pH 8 for alkaline phosphatase, Sigma-Aldrich, Co., Austria) were incubated with samples for 30 min. Alkali were added to stop the reaction and adjust the pH for the determination of concentration of the product formed. The spectral absorbance of *p*-nitrophenolate was read at 340 nm (Microplate reader; Awareness: Statfax:2100; USA). The experiment was conducted in three replicates.

Protein assay

Protein concentration was evaluated by the method of Lowry et al. (1951) using bovine serum albumin (Ziest Chem Co., Tehran) as the standard.

Statistical analysis

Data of bioassay were analyzed using POLO-PC software to obtain LC_{50} values. Data of enzymatic assays were analyzed using *t*-test and statistical differences were marked by asterisks when probability was found less than 0.05.

Results and Discussion

Bioassay of B. bassiana *isolates on adults of* A. spinidens

Results of *B. bassiana* bioassay using isolates BB_2 and AM-118 on adults of *A. spinidens* are shown in Table 1. Calculation of lethal concentrations showed $LC_{10–50}$ of 77, 11×10^3 and 37×10^4 spore/mL for BB_2 and 22, 10×10^2 and 15×10^3 spore/mL, respectively (Table 1). These results clearly indicate that required spores of Am-118 isolate for killing of *A. spinidens* adults is lower than that of BB_2 isolate (Table 1). It must come to attention that BB_2 is a commercially isolate that is used for majority of pests but Am-118 specifically has been isolated from *C. suppressalis* larvae. These differences might be attributed to fungal physiological differences, mainly production of extracellular en-

Table 1
LC values (spore/ml) of *B. bassian* isolates on adults of *A. spinidens*

Isolates	LC_{10}	LC_{30}	LC_{50}	Slope ± SE	X^2	df
BB_2	77	11×10^3	37×10^4	0.348 ± 0.115	0.2196	2
AM-118	22	10×10^2	15×10^3	0.451 ± 0.113	0.9175	2

Data were calculated by POLO-PC software.
"LC" stands for Lethal concentration. "X^{2}" refers as chi square and "df" refers to degree of freedom

zymes, recognition by host immune system and producing secondary metabolites (Zibaee and Malagoli, 2014).

Extracellular enzymes produced by B. bassiana *isolates*

Insect integument is a multi-layered structure including four functional regions: epicuticle, procuticle, epidermis, and basement membrane (Chapman, 2012). The epidermis is a secretory tissue formed by a single layer of epithelial cells that has been lay on a basal membrane and produces procuticle (Chapman, 2012). Major components of this part are microfibers of chitin surrounded by a matrix of protein. There is an oriented monolayer of wax molecules lying above the cuticulin layer. Also, a cement layer covers the wax and protects it from abrasion (Chapman, 2012). The major components of these two layers are different types of lipids. Because of unique composition of integument, it is very often involved in defensive strategies of insects towards predators and pathogenic agents (St. Leger, 1991). In fact, integument serves as a physical and biochemical barrier because of its hardness and chemical composition to achieve an efficient protection. Because of these properties, an efficient entomopathogenic fungi must overcome the integument to access present nutrients in hemocoel. So, entomopathogenic fungi produce several enzymes to break-down integument layers. Proteases and chitinases are the main extracellular enzymes although entomopathogenic fungi utilize lipases and general esterases to hydrolase biochemical bonds of wax and cement (St. Leger, 1991; Ramzi and Zibaee, 2014). On the other hands, pathogenicity of an entomopathogenic fungi is a complex mechanism using mechanical pressure of spore's germ tube and production of extracellular enzymes. Chitinases, proteases and lipases are the three enzymes helping fungi to penetrate via insect cuticle. Fungi containing higher amount (= higher activity) of these enzymes do have higher efficiency to kill host. These days, determining of fungi pathogenicity just indicating LC values is not acceptable and determining these biochemical capabilities even in molecular level would be interesting.

Activities of chitinase, lipase and alkaline phosphatase demonstrated significant differences in the liquid media inoculated by isolates BB_2 and AM-118 but no significant differences were found in case of acid phosphatase (Table 2). Chitinases are complex and important enzymes in biological systems that hydrolyze b-(1,4)-linked polymer of N-acetyl-D-glucosamine of chitin. These enzymes have been distributed in various organisms including bacteria, fungi, plants, insects, mammals, and viruses (Matsumoto, 2006). Besides the role of chitinases during infection processes of entomopathogenic fungi, it is

Table 2
Comparison of chitinase, lipase and phosphatases activities (U/mg protein) in the two isolates of *B. bassiana* reared on *A. spinidens* cuticle

Enzyme	B_2	AM-118	*p*
Chitinase	3.67 ± 0.38	10.63 ± 0.99*	≤ 0.018
Lipase	0.02 ± 0.006	0.09 ± 0.022*	≤ 0.026
ACP	0.025 ± 0.006	0.033 ± 0.008	≤ 0.379
ALP	0.058 ± 0.017	0.100 ± 0.045*	≤ 0.040

* Statistical differences have been shown by asterisks (*T*-test, $p \leq 0.05$)

also involved in several functions of fungal biology such as conidial germination, hyphal growth and morphogenesis (St. Leger, 1991). Charnley and St. Leger (1991) reported that chitinases were activated during later steps of penetration. It means that the enzyme might have a minor role in cuticle penetration (Charnley and St. Leger, 1991). In our previous study, activity of the enzyme varied among various entomopathogenic fungi mainly *B. bassiana* isolates when those were cultured in the media containing *C. suppressalis* cuticle (Ramzi and Zibaee, 2014). Higher activity of chitinase by an isolate might increase efficiency of its virulence via feasible passing through integument.

Significant differences of lipases and phosphatase activities were found in isolates BB_2 and AM-118 (Table 2). Lipases hydrolyze carboxyl ester bonds that lead to liberate fatty acids and glycerol but alkaline phosphatase (ALP, E.C.3.1.3.1) and acid phosphatase (ACP, E.C.3.1.3.2) hydrolyze phosphomonoesters under alkaline or acid conditions, respectively. (Bessy et al., 1946; Tsujita et al., 1989). ALP and ACP are mainly involved in the transphosphorylation reactions (Sakharov et al., 1989). Since lipids are another components in the insect integuments mainly in wax and cement layers, production of these enzymes by entomopathogenic fungi will facilitate penetration process through integument. Varela and Morales (1996) reported that high virulent isolates of *B. bassiana* show higher production and activity of lipases. It was found that cuticular lipids significantly affect germination of *B. bassiana* and *Isaria fumosorosea* when exposed to silverleaf whitefly (James et al., 2003). These compounds might be toxic or inhibitory activities against conidia so lipases do have crucial role in infection process of entomopathogenic fungi. Moreover, presence of both ACP and ALP in these isolates indicate their ability to survive in both acid and alkali pH values since the enzymes help the fungi to utilize nutrient compounds and release remaining components (mainly toxic materials) as glutathione conjugates (Ramzi and Zibaee, 2014).

Proteins are another main molecules in insect integument that their presence increase hardness of the cuticle. Fungi utilize two main groups of proteases to hydrolase these molecules. The first one is subtilisin-like serine protease (Pr1) that synthesized in the host cuticle during the early stages of penetration (Braga et al., 1999; Dias et al., 2008). The second one is trypsin-like enzyme (Pr2) that has critical role in degrading extracellular proteins complementary to that of Pr1 (Dias et al., 2008). It means that Pr2 synthesized after activity of Pr1 to complete protein hydrolysis during penetration process. Although no statistical differences were found in activities of general protease and

Pr1 between two *B. bassiana* isolates but Pr2 showed significantly higher activity in isolate AM-118 (Table 3). These results show proteolytic capability of the two isolates to hydrolase cuticular proteins of *A. spinidens* by indicating higher efficiency of Pr2 enzyme in AM-118. Although, St. Leger et al. (1987) showed the importance of both proteases and chitinases during infection of *M. anisopliae* on *Calliphora vomitoria* L. (Diptera: Calliphoridae) and *Manduca sexta* L. (Lepidoptera: Sphingidae), but Charnley and St. Leger (1991) reported that proteases facilitated cuticle penetration by initial degradation of proteins in early time of infection similar to findings of Guoliang et al. (2009). Also, Gupta et al. (1994) reported that Pr1-like proteases of *B. bassiana* had the highest activity in early onset of mortality of *Galleria mellonella* L. (Lepidoptera: Gelechidae) larvae. Ramzi and Zibaee (2014) found statistical differences of Pr1 and Pr2 in the entomopathogenic fungi cultured in the media containing *C. suppressalis* cuticle. In details, the highest activities of these proteases were found to be Pr1 of BB_1 *(B. bassiana)* and Pr2 of *L. lecanii.*

Activity of the two detoxifying enzymes were evaluated in the media cultured by isolates BB_2 and AM-118. It was found that no statistical activity of general esterase produced by the two isolates (Table 4). Activity of glutathione *S*-transferase in AM-118 was statistically higher than that of BB_2 by using both reagents (Table 3). General esterases (ESTs) and glutathione *S*-transferases are the two key detoxifying enzymes that catalyze hydrolysis of the esteric bonds in synthetic chemicals and conjugation of electrophile molecules with reduced glutathione (GSH) to increase solubility of toxic materials in water (Grant and Matsumura, 1989; Hemingway and Karunatne, 1998). Similar to lipases that hydrolyze esteric bonds of lipids, general esterases have a broader activity and degrade the relevant bonds in all molecules. Their importance is going to be more crucial when a toxic

Table 3

Comparison of total (OD/min) and specific protease (U/mg protein) activities in the two isolates of *B. bassiana* reared on *A. spinidens* cuticle

Enzyme	B_2	AM-118	*p*
Total protease	0.65 ± 0.005	0.65 ± 0.001	≤ 0.453
Pr_1	0.08 ± 0.046	0.08 ± 0.006	≤ 0.403
Pr_2	0.004 ± 0.003	0.024 ± 0.003*	≤ 0.041

* Statistical differences have been shown by asterisks (*T*-test, $p \leq 0.05$)

Table 4

Comparison of general esterase (OD/min) and glutathione *S*-transferase (OD/min) activities in the two isolates of *B. bassiana* reared on *A. spinidens* cuticle

Enzyme	B^2	AM-118	*p*
EST (α-naphtyl acetate)	0.061 ± 0.010	0.058 ± 0.007	≤ 0.425
EST (β-naphtyl acetate)	0.021 ± 0.003	0.018 ± 0.004	≤ 0.299
GST (CDNB)	0.034 ± 0.007	0.052 ± 0.007*	≤ 0.047
GST (DCNB)	0.032 ± 0.017	0.025 ± 0.009*	≤ 0.354

* Statistical differences have been shown by asterisks (*T*-test, $p \leq 0.05$)

molecule is present in the cuticle as both basic compound or processed one. Similar activities of general esterases in isolates BB_2 and AM-118 might be attributed their equal potential to hydrolase molecules containing esteric bonds. Also, it was previously mentioned that acid and alkaline phosphatases liberate phosphoric functional groups from cuticle of insects and release remaining components as glutathione conjugates (Ramzi and Zibaee, 2014). So, glutathione *S*-transferase is important during infection process by inactivating the detoxified intermediary molecules as glutathione conjugates. Higher activity of the enzyme in Am-118 show higher efficiency to remove toxic materials from the penetration site.

In the current study, virulence of the two isolates of *B. bassiana* (one commercial and another isolated from rice fields) was evaluated on adults of a predatory hemipteran, *A. spinidens*. Results of bioassay indicated higher virulence of isolate AM-118 by lower amount of exposed conidia. Moreover, the isolate had higher activity of the enzymes during penetration process. Previous studies showed great potential of *A. spinidens* against larval stages of *C. suppressalis*. Moreover, all isolates of *B. bassiana* caused significant mortality on population of the pest. So, it seems that combination of these two natural agents will simultaneously have appropriate impact on the pest and decrease economic loss. There is an incomplete understanding of potential effects of *B. bassiana* on *A. spinidens*. The current study and upcoming ones on immunity of the hemipteran to *B. bassiana* will clear us the possibility of their integration in pest control procedures.

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