https://doi.org/10.17221/45/2017-PPS

Pathogenicity of *Beauveria bassiana* Strain 202 against Sap-sucking Insect Pests

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Abstract

Bugti G.A., Bin W., Na C., Feng L.H. (2018): Pathogenicity of *Beauveria bassiana* strain 202 against sap-sucking insect pests. Plant Protect. Sci., 54: 111–117.

An experimental trial was conducted to determine the pathogenicity of *Beauveria bassiana* strain 202 (Bb-202) against multiple targeted sucking insect species that are serious pests of crops and ornamental plants. The insect species, *Myzus persicae* Sulzer (Hemiptera: Aphididae), *Jacobiasca formosana* Paoli (Hemiptera: Cicadellidae), *Bemisia tabaci* Gennadius (Hemiptera: Aleyrodidae), and *Stephanitis nashi* (Hemiptera: Tingidae) were exposed to conidia of *B. bassiana* at rates of 1.0×10^2, 3.5×10^3, 5×10^4, and 6.75×10^5 conidia/mm^2 to determine the pathogenicity of *B. bassiana*. The fungal strain Bb-202 showed the highest pathogenicity to *M. persicae* and 100% corrected mortality observed in the treatments over 1.0×10^2 conidia/mm^2, followed by the *J. formosana* with the final corrected mortality of 86.6, 94.4, and 97.4% after 10 days of fungal inoculation with 3.5×10^3, 5×10^4, and 6.75×10^5 conidia/mm^2, respectively. The strain Bb-202 also showed good pathogenicity to *B. tabaci* and corrected mortalities of 77.9 and 81.1% were recorded when exposed to 5×10^4 and 6.75×10^5 conidia/mm^2. Relatively weak pathogenicity was observed in *S. nashi*, in which the highest corrected mortality of 63.7% occurred at 6.75×10^5 conidia/mm^2. Accordingly, the LC_{50} and LT_{50} values of concentrations 1×10^5, 1×10^6, 1×10^7, and 1×10^8 conidia/ml were calculated for *M. persicae, J. formosana, B. tabaci*, and S. *nashi* that were determined as 6.7×10^4, 1.3×10^6, 3.6×10^6, and 1.2×10^7 conidia/ml and LT_{50} was observed as 5.2~8.24, 5.1~6.6, 7.2~9.0, and 7.9~9.6 in days, respectively.

Keywords: entomopathogenic fungi; *Beauveria bassiana*; plant sucking insects

More than one million phytophagous insect species feed on plants and obtain food from different parts of plants either by sucking sap or chewing from aerial or belowground plant parts (Mithöfer & Boland 2008). Among these aphids *Myzus persicae* Sulzer (Homoptera: Aphididae), leafhopper *Jacobiasca formosana* Paoli (Hemiptera: Cicadellidae), whitefly *Bemisia tabaci* Gennadius (Hemiptera: Aleyrodidae), and lace bug *Stephanitis nashi* (Hemiptera: Tingidae) are serious pests of field crops and garden plants. Both adults and nymphs suck the plant saps during vegetative growth (Balakrishnan *et al.* 2007; Alves *et al.* 2008; Fu *et al.* 2014; Cao *et al.* 2015; Hou *et al.* 2015). A wide range of insecticides, acetamiprid, organophosphates, thiamethoxam, imidacloprid, synthetic pyrethroids, and neonicotinoids, were used for the control of insects different. These insecticides are expensive and their efficiency is often low (Elnaggar & Zidan 2013;

Supported by the National Natural Foundation Project of China, Grant No. 31471822 and National key, Project of China No. 2017YFD02009 "Crop immunization and physical control techniques and product development".

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https://doi.org/10.17221/45/2017-PPS

Varghese & Mathew 2013; Schmidtjeffris & Nault 2016; Regan *et al.* 2017). Due to the overuse of insecticides, the most of the serious vegetable and crop insect pests have developed resistance to many insecticides (El & Devine 2003; Ma *et al.* 2007). Furthermore, chemical insecticides have adverse effects on non-target insects and humans (Decourtye & Devillers 2010; Godfray *et al.* 2014).

Biological control of insect pests with entomopathogenic fungi is an alternative to conventional insecticides, safe to plants, humans, animals (Khetan 2001) as well as non-targeted insects (Loc *et al.* 2002; Wu *et al.* 2014). Approximately 1000 entomopathogenic fungal species are known to kill insects (Shang *et al.* 2015) and about 100 mycoinsecticides are commercially registered worldwide (Jaronski 2010). Among these pathogenic fungi, *B. bassiana* (Balsamo Crivelli) is one of the promising biocontrol agents (Biswas *et al.* 2015). *Beauveria bassiana* based mycopesticides affect 700 insect species (Glare & Milner 1991; Humber 1991; Goettel *et al.* 2000; Lacey *et al.* 2001; Zimmermann 2007) and are used in the control of agricultural, veterinary, and medical pests throughout the world (Todorova *et al.* 2002; Askary & Yarmand 2007; Mahmoud 2009; Elif *et al.* 2010). A mycopesticide kills insects as a result of the insect coming into contact with the spores either by spray droplets or by walking on a treated surface. Once the fungal spores attach to the insect's cuticle, the fungus spores penetrate into the insect's body; it takes several days for infected insects to die. The cadaver of the insect acts as a source of spores for secondary spread of the fungus (Long *et al.* 2000; Sevim *et al.* 2012). The fungal pathogens are convenient for controlling sucking insect pests (Leger & Roberts 1997; Roy *et al.* 2008).

Host specificity for entomopathogenic fungi is well known, but less information is available compared to viruses so far, hence there is dire need to explore the entomopathogenicity of fungi on multiple insect pests in the field conditions. Therefore we designed the present study to evaluate the pathogenicity of *B. bassiana* against multiple sap-sucking insect pests under laboratory conditions.

MATERIAL AND METHODS

Preparation of the entomopathogenic fungus. The entomopathogenic fungi *B. bassiana* strain 202 (Bb-202) used in the experiment originated from a Cerambycidae (Coleoptera) beetle species and was provided by Research Center on Entomogenous Fungi (RCEF, WDCM1031), Anhui Agricultural University, Hefei (31°N and 117°E), China. The strain was preserved at –70°C prior to use. Amount of 200 µl of the spore suspension were inoculated to Sabouraud dextrose agar SDA (agar 20 g, peptone 10 g, dextrose 40 g, potassium 0.5 mg, cycloheximide 40 g, and penicillin/streptomycin 2.5 ml in 1000 ml distilled water) medium using Petri dishes (9 cm diameter) and kept at 24 ± 1°C in an incubation chamber for 12 days. Fully grown conidia were harvested from the upper surface of the culture by scraping and diluted in a 200-ml conical flask containing 150 ml 0.05% Tween 80. The flask containing conidia was subjected to the vortex for 5 min for homogenisation. The diluted conidia were filtered through a sterile 30-ml syringe with cotton into a sterile small beaker. Then suspensions were adjusted to defined concentrations using a haemocytometer and a microscope. Conidial suspensions were standardised at 1×10^5, 1×10^6, and 1×10^7, and 1×10^8 conidia/ml.

Insect collection and bioassay procedures. The insects green peach aphid *Myzus persicae* Sulzer (Hemiptera: Aphididae), tea green leafhopper *Jacobiasca formosana* Paoli (Hemiptera: Cicadellidae), sweat potato whitefly *Bemisia tabaci* Gennadius (Hemiptera: Aleyrodidae), and Japanese pear lace bug *Stephanitis nashi* (Hemiptera: Tingidae) were collected in plant gardens of Anhui Agricultural University, Hefei, China from *Pittosporum tobira, Prunus cerasifera*, *Gardenia jasmiodides,* and *Malus halliana*, respectively. *B. tabaci* and *J. formosana* were collected manually with a small aspirator, *M. persicae* and *S. nashi* by picking infested leaves of host plants. About 700 individuals of each species were collected.

Insects were brought to the laboratory and fed with host plant twigs until adult eclosion, then used for the fungal bioassay. *B. tabaci* and *J. formosana* were placed cooled for 5 min at 5°C temperature to avoid escaping and make inoculation easy. Conidial suspensions at four different concentrations of 1×10^5, 1×10^6, 1×10^7, and 1×10^8 conidia/ml were used in the bioassay.

The 2-ml conidial suspension from each concentration was sprayed on the tested insects with the Potter Precision Laboratory Spray Tower (UK) having a droplet spray nozzle (0.7 mm internal diameter). A glass slip (20 × 20 mm) was placed in Petri dish along with leaves on the bucket bottom to collect the number of sprayed conidia while insects were exposed. The conidia on the slip were randomly

https://doi.org/10.17221/45/2017-PPS

counted from 5 fields (0.01 mm^2/field) under the electric microscope at 400× magnification and an average number of conidia per mm^2 was calculated. After spraying with conidial concentrations 1×10^5, 1×10^6, 1×10^7, and 1×10^8 the numbers of conidia per unit area were calculated as 1.0×10^2, 3.5×10^3, 5×10^4, and 6.75×10^5 conidia/mm^2, respectively. The control insects which were sprayed with 0.05% Tween-80, both treated and control insects were then transferred into plastic boxes (17 × 12 × 5.5 cm) with host plant twigs and placed in an insect rearing chamber at 21 ± 1°C and RH 78 ± 5%. Fresh twigs were changed daily through a hole (6 cm diameter) on the top of the plastic box covered with nylon fabric to prevent adults to escape. Each treatment was repeated 4 times (20 insects of each species/replicate). Daily observations were continued for up to 10 days. The dead insects were removed from the boxes and placed in Petri dishes with wet filter paper providing moisture for mycelial growth.

Data analysis. Insect mortality percentage was corrected by Abbott's formula: $[1 - (nt/nc)] \times 100$; where: n – insect population; t – treatment; c – control. The differences were tested by one-way ANOVA followed by Tukey's test. Data were further subjected to probit analysis, generating a concentration-mortality relationship for the estimates of LC_{50} and associated 95% confidence limits for each concentration. LT_{50} test was performed to check the efficiency of concentrations within a period of time. All the statistical analyses were performed using SPSS v 21.0. (2012). The significance level of $P \leq 0.05$ was accepted. Pictures were taken with Microscope Model Olympus 1X-71 with DP.26 camera, lenses U Plan FL N.4x0.13 phL and 10x0.25 RC1.

RESULTS

B. bassiana strain 202 was pathogenic to all target insect pests (Figure 1). The final corrected mortalities ranged from 73.6% to 100% for the *M. persicae* population, 82.6–97.7% for the *J. formosana* population, 53.9–81.1% for *B. tabaci*, and 6.1–63.7% for *S. nashi* (Figure 2). Mortality increased as the conidial concentration increased, especially in *S. nashi* and *B. tabaci*, when it was observed that the development of fungal disease depends on conidial density (Figure 3).

Myzus persicae. Strong virulence of the fungal strain against the *M. persicae* population was observed in all treatments. The final corrected mortality 73.6, 100, 100, and 100% was observed after exposure to 1.0×10^2 conidia/mm^2, 3.5×10^3, 5×10^4, and 6.75×10^5 conidia/mm^2, respectively (Figure 2) while mortality in the control was < 10%, significantly less than in all fungal treatments ($df = 4$, $F = 5.15$, $P = 0.001$). The LT_{50} varied with the fungal concentrations. The highest LT_{50} of 8.2 days was observed in the lowest concentration of 1×10^5 conidia/ml. No significant difference was observed in LT_{50} between the other three concentrations of 1×10^6, 1×10^7, and 1×10^8 conidia/ml. The high pathogenicity of the fungal strain to *M. persicae* resulted in the lowest LC_{50} of 6.70×10^4 conidia/ml (Table 1).

Jacobiasca formosana. *B. bassiana* strain 202 was also highly virulent for *J. formosana*. The final corrected mortality of 82.6, 86.6, 94.4, and 97.4% was wererecorded when the insect was treated with 1.0×10^2, 3.5×10^3, 5×10^4, and 6.75×10^5 conidia/mm^2, respectively (Figure 2) while mortality in the control was 7.5 %. All treatments had a significant effect on the *J. formosana* population ($df = 4$, $F = 3.96$ $P = 0.004$).

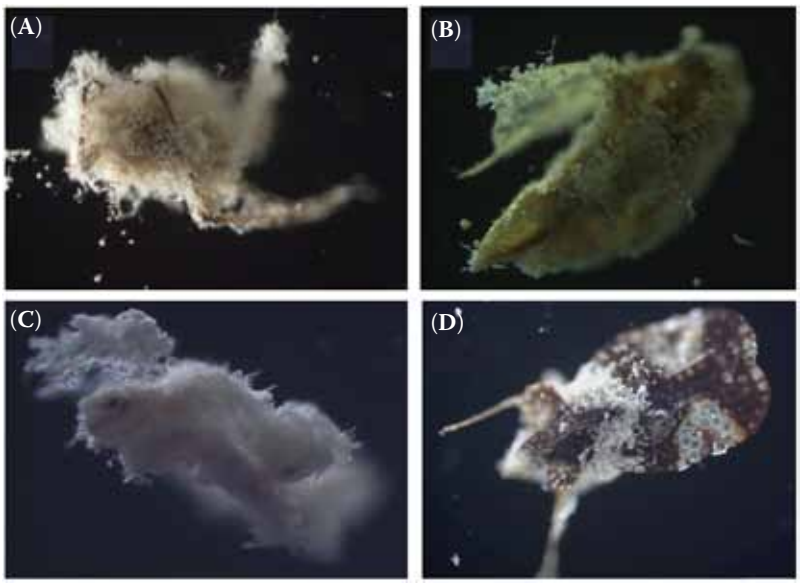

Figure 1. Insects infected by the entomopathogenic fungus *B. bassiana* strain 202: (**A**) *M. persicae*, (**B**) *J. formosana*, (**C**) *B. tabaci*, and (**D**) *S. nashi*

https://doi.org/10.17221/45/2017-PPS

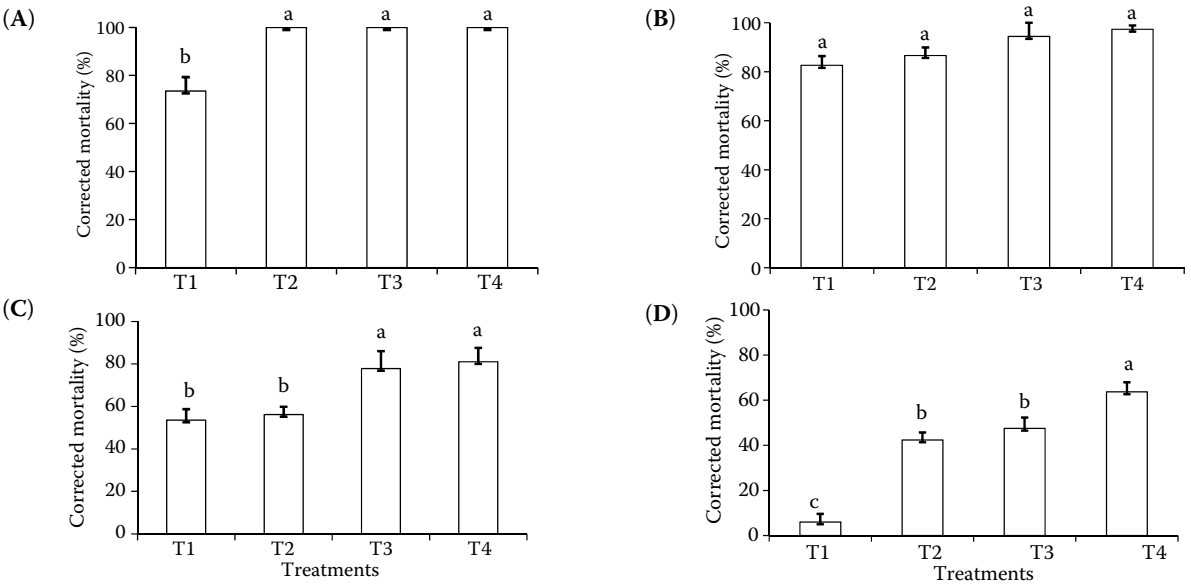

Figure 2. Corrected mortality (%) of different nsect pests caused by different conidial density of *Beauveria bassiana*: (**A**) *M. persicae,* (**B**) *J. formosana*, *(***C**) *B. tabaci*, and (**D**) *S. nashi*

T1 = 1.0 × 10^2 conidia/mm^2; T2 = 3.5 × 10^3conidia/mm^2; T3 = 5 × 10^4 conidia/mm^2; T4 = 6.75 × 10^5 conidia/mm^2; data with different lowercase letters indicate significant differences at 0.05 level

The LT_{50} was 6.3, 6.6, 5.7, and 5.1 days at concentrations of 1 × 10^5, 1 × 10^6, 1 × 10^7, and 1 × 10^8 conidia/ml, respectively. The LC_{50} was 1.33 × 10^6 conidia/ml, slightly higher than in *M. persicae* (Table 1).

Bemisia tabaci. After the exposure of *B. tabaci* to fungal conidia at a rate of 1.0 × 10^2, 3.5 × 10^3, 5 × 10^4, and 6.75 × 10^5mm^2 the corrected mortalities 53.6–81.1% were recorded as in the lowest to the highest treatment. The higher rates, 3.5 × 10^3 and 5 × 10^4 conidia/mm^2, were more efficient than the lowest rate of conidia/mm^2. The mortality of the control was 8.8%. All conidial concentrations had a significant effect (*df* = 4, *F* = 4.96, *P* = 0.001). However, average mortality at the higher rate of conidia was 25% higher than at a lower conidial rate applied to the insect (Figure 2). *B. bassiana* thus showed

Table 1. Estimated lethal time and lethal concentration dose mortality (LT_{50} and LC_{50}) of *Beauveria bassiana* against different insect pests

Insect	Concentrations	Estimated (LT_{50})	Lower bound	Upper bound	LC_{50}
M. persicae	1 × 10^5	8.2	7.5	9.2	6.7 × 10^4
	1 × 10^6	5.2	4.8	5.9	
	1 × 10^7	6.2	6.0	6.5	
	1 × 10^8	5.6	5.1	6.5	
J. formosana	1 × 10^5	6.3	5.6	7.7	1.3 × 10^6
	1 × 10^6	6.6	6.4	6.8	
	1 × 10^7	5.7	5.2	6.5	
	1 × 10^8	5.1	4.3	6.7	
B. tabaci	1 × 10^5	9.0	8.7	9.3	3.6 × 10^6
	1 × 10^6	8.4	8.2	8.7	
	1 × 10^7	7.6	7.0	8.2	
	1 × 10^8	7.2	6.8	7.7	
S. nashi	1 × 10^5	–	–	–	1.2 × 10^7
	1 × 10^6	9.5	8.4	11.5	
	1 × 10^7	9.6	8.6	11.3	
	1 × 10^8	7.9	7.5	8.3	

https://doi.org/10.17221/45/2017-PPS

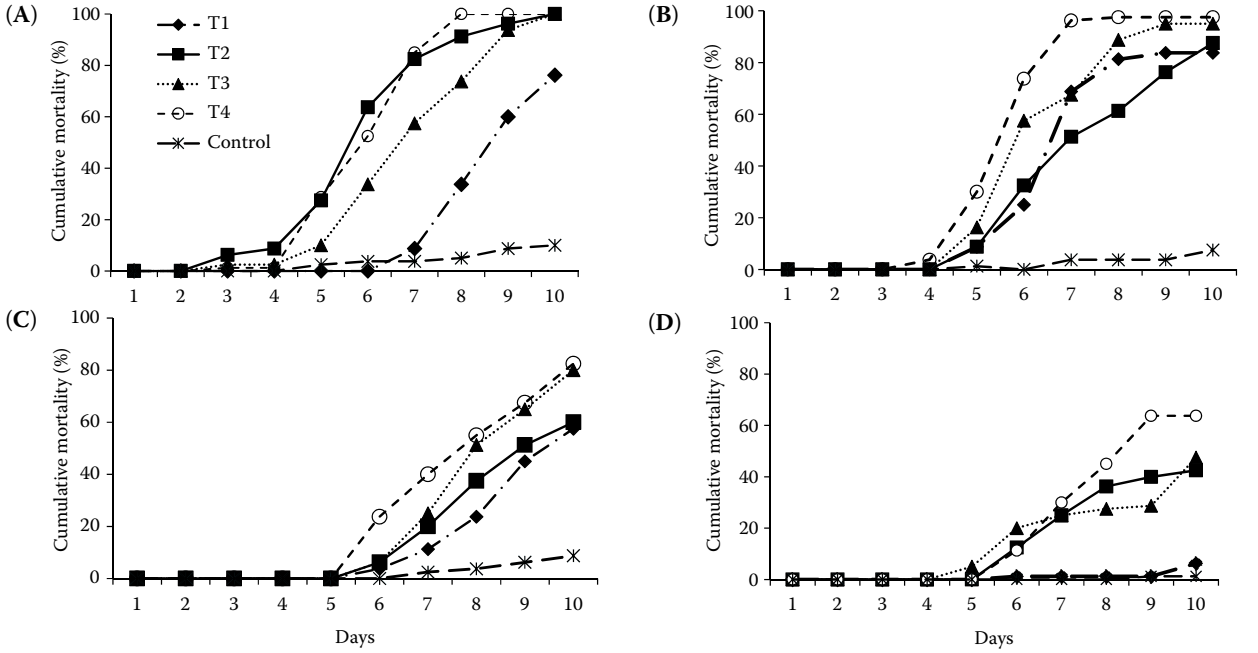

Figure 3. Cumulative mortality (%) of different insect pests caused by different conidial density of *Beauveria bassiana*: (**A**) *M. persicae*, (**B**) *J. formosana*, (**C**) *B. tabaci*, and (**D**) *S. nashi*

T1 = 1.0 × 10^2 conidia/mm^2; T2 = 3.5 × 10^3 conidia/mm^2; T3 = 5 × 10^4 conidia/mm^2; T4 = 6.75 × 10^5 conidia/mm^2

some pathogenicity, but not so strong as in the case of *M. persicae* and *J. formosana*. The LT_{50} at the four concentrations ranged from 9.0 to 7.2 days and was nearly 2 days longer than in *J. formosana*. The LC_{50} value was estimated 3.6 × 10^6 conidia/ml (Table 1).

Stephanitis nashi. The *B. bassiana* strain 202 was the least virulent for *S. nashi*. A maximum final corrected mortality of 63.7% was observed at 6.75 × 10^5 conidia/mm^2, while low mortality of 6.1% was observed at 1.0 × 10^2 conidia/mm^2 (Figure 2). The mortality of the control was 1.3%. There was no significant difference between the rate of 1.0 × 10^2 conidia/mm^2 and the control, while other treatments were significantly different from the control (df =4, F =8.7, P = 0.001). The LT_{50} was 9.5, 9.6, and 7.9 days for conidial concentrations of 1 × 10^6, 1 × 10^7 and 1 × 10^8, respectively (Table 1). The LC_{50} was 1.2 × 10^7 conidia/ml.

DISCUSSION

Entomopathogenic fungi play an important role in the control of sap-sucking insect pests. The pathogenicity of *B. bassiana* was observed on *Nilaparvata lugens* where mortality ranged from 17.2% to 79.1% after 10 days of inoculation (Li *et al.* 2014). Similarly, Zafar *et al.* (2016) found that *B. bassiana* induced mortality of 65.30% and 88.82% on eggs and nymphs of *Bemisia tabaci*, respectively. Wu *et al.* (2016) demonstrated the control efficiency of *B. bassiana* and predatory mite *Neoseiulus barkeri* as biological control against *Frankliniella occidentalis*, furthermore they stated that *B. bassiana* caused 77.5% mortality and showed rapid control against *Frankliniella occidentalis* as compared to the predatory mite. In our experiment the *B. bassiana* strain 202 showed good pathogenicity to both *M. persicae* and *J. formosana*, medium virulence on *B. tabaci*, and weak virulence on *S. nashi*. It could be speculated that the fungal strain Bb-202 has a great potential of the efficient control of *M. persicae*, *J. formosana* and even of the *B. tabaci* population, but was not efficient against the *S. nashi* population.

Development of a fungal disease in an insect pest population is normally considered as dosage-dependent and insect mortality usually highly varies with conidial concentration. Selvaraj *et al.* (2012) observed that the pathogenicity of *B. bassiana* to *Aphis craccivora* was closely related to application dosage. Mortality of 94.98% occurred in the treatment with 1× 10^{10} conidia/ml while it decreased to 58.56% in the treatment with 1 × 10^4 conidia/ml. Similarly, Poprawski *et al.* (1999) mentioned that the dosage of fungal conidia highly affected the insect control, they observed 79.8 and 94.4% mortality when they applied 2.5 × 10^{13} and 5 × 10^{13} conidia/ha mycocides, respectively.

https://doi.org/10.17221/45/2017-PPS

The present study revealed that *B. bassiana* is highly effective to control the pest population of target species; however, the pathogenicity level varies from species to species. Several authors described the pathogenicity level of fungal species in different insect pests. Wu *et al.* (2014) demonstrated the host specificity of *B. bassiana* (isolate SZ-26) on various insect species in field conditions. They observed the maximum 96% mortality of *Frankliniella occidentalis,* whereas the lowest pathogenicity to predatory species (mites). Similarly, Riosvelasco *et al.* (2009) reported that *B. bassiana* and *M. anisopliae* strains are highly pathogenic to psyllids, thrips, and whiteflies, further, the pathogenicity level of these strains ranged from 80% to 100%, respectively. Moreover, Mohammadbeigi and Port (2013) compared the pathogenicity level of *B. bassiana* and *M. anisopliae* and concluded that *B. bassiana* suppressed 100% population of horned grasshopper compared to *M. anisopliae* 47%. Likewise, Sevim *et al.* (2013) investigated the entomopathogenicity of thirteen fungal isolates, they found the *B. bassiana* isolate KTU.24 more lethal against lace bug, nymphs 83% and adults 80% compared to all other fungal isolates.

In the present study, we concluded that the Bb-202 strain has a strong potential for the control of sap-sucking insect pests. It was highly efficient against *M. persicae* and *M. persicae*. Effective control of *B. tabaci* was recorded only in high dosage treatments. A weak pathogenicity was observed in the *S. nashi* population. The biological control of multiple insect pests by one pathogenic fungal strain is thus possible and helpful in IPM strategies.

Acknowledgements. The authors would like to thank the Anhui Provincial Key Laboratory of Microbial Control, Anhui Agricultural University, Hefei, China for providing the fungal strain.

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Received: 2017–03–27
Accepted after corrections: 2017–07–12
Published online: 2017–09–18