Egyptian Journal of Biological Pest Control

RESEARCH

Open Access

Biological control potential of two *Beauveria bassiana* isolates against the stink bugs *Nezara viridula* L. and *Piezodorus guildinii* Westwood (Hemiptera: Pentatomidae) in common bean

Yordanys Ramos[1,2*], Orelvis Portal[3,4], Nicolai V. Meyling[5,6] and Ingeborg Klingen[6]

Abstract

Background The stink bugs, *Nezara viridula* L. and *Piezodorus guildinii* Westwood (Hemiptera: Pentatomidae) are the most important and widespread species of polyphagous stink bugs in the tropical and subtropical regions of Latin America, which affect the quality and yield of the common bean (*Phaseolus vulgaris* L.). The use of synthetic chemical insecticides is the major control strategy to manage stink bugs in common beans and alternatives are needed. In this study, mortality and median Lethal Time (LT_{50}) of two Cuban isolates of the entomopathogenic fungus *Beauveria bassiana* (18 S-O-R and 96 P-O-E), as well as one commercial Cuban isolate (Bb-18), at a concentration of 1×10^8 conidia/ml were evaluated. These evaluations were conducted against both stink bug species using Petri dish bioassays and a semi-field experiment in common beans.

Results In Petri dish bioassays, the isolates 18 S-O-R and 96 P-O-E caused 100% mortality of both *N. viridula* and *P. guildinii*. This was significantly higher than for isolate Bb-18, which caused 86.3% *N. viridula* and 81.3% *P. guildinii* mortality. In the semi-field experiment, when pooling both stink bug species, total mortality after 14 days was 91.3% for 18 S-O-R, 80.0% for 96 P-O-E and 73.8% for Bb-18 isolates. LT_{50} value for isolate 18 S-O-R tested under laboratory conditions was 6.04 ± 0.18 days for *N. viridula* and 5.32 ± 0.14 days for *P. guildinii* at the same concentration of 1×10^8 conidia/ml. LT_{50} value for isolate 18 S-O-R in semi field was 6.79 ± 0.37 days for *N. viridula* and 7.71 ± 0.32 days for *P. guildinii* at 1×10^8 conidia/ml.

Conclusion The study highlights the potential of *B. bassiana* 18 S-O-R as a promising candidate for control of stink bugs in common bean under tropical conditions as an alternative to conventional chemical insecticides in integrated pest management (IPM) programs. Moving forward, further research should focus on validating the efficacy under diverse field conditions and integrating application methods into practical IPM approaches. Future use of *B. bassiana* will enhance sustainability and reduce environmental impacts associated with pesticide use.

Keywords *Nezara viridula*, *Piezodorus guildinii*, Entomopathogenic fungi, Hypocreales, Biological control, Common bean

*Correspondence:
Yordanys Ramos
yordanys.ramos@umich.mx
Full list of author information is available at the end of the article

Background

Common bean (*Phaseolus vulgaris* L.) is the most important edible legume for direct consumption in the world (Suárez-Martínez et al. 2015) and it is part of the daily diet of many Cubans (Morales-Soto et al. 2022) and elsewhere in Latin America (Lopes et al. 2015a). Attack by stink bugs (Hemiptera: Pentatomidae) during the pod stage affects the quality and quantity of the common bean crop (Ramos et al. 2017a). The most important and widespread species of polyphagous stink bugs in the tropical and subtropical regions of Latin America are the red banded stink bug, *Piezodorus guildinii* Westwood and the southern green stink bug *Nezara viridula* L. (Souza et al. 2015). The two pests introduce their piercing-sucking mouthparts through the pods and inject salivary secretions to feed on the seed nutrient contents (Depieri and Panizzi 2011). This feeding activity can cause seed abortion or deformation and the feeding injury facilitates plant pathogen infection (Zerbino and Panizzi 2019).

Use of synthetic chemical insecticides is the major control strategy to manage stink bugs in soybeans (Marques et al. 2019) and insecticide resistance in stink bug populations is now a big challenge (Ademokoya et al. 2022). When pesticide efficacy is reduced, application rates often increase. World-wide there is, however, an increasing focus on sustainable use of chemical pesticides to reduce the risks and impacts on human health and the environment. This has led to the promotion of Integrated Pest Management (IPM) and of alternative approaches or techniques that may be used as alternatives for chemical pesticides. Many countries have established guidelines for this e.g. the EU Directive 2009/128/EC. Improved IPM strategies are continuously being developed and several have proved to be successful also against sink bugs e.g. use of synthetic insecticides when needed (Brown et al. 2012), use of botanical insecticides (Werdin et al. 2013), use of beneficial insects such as predators and parasitoids (Cingolani et al. 2013) as well as entomopathogenic fungi (EPF) (Oliveira et al. 2016).

Beauveria spp. (Ascomycota: Hypocreales) are among the most widely used entomopathogenic fungal taxa for microbial control (Mascarin and Jaronski 2016). The effect of *B. bassiana* sensu lato isolates against different species of stink bugs under controlled conditions have been the subject of various studies (Silva-Santana et al. 2022). However, the potential of *B. bassiana* isolates as a microbial control agent against *N. viridula* and *P. guildinii* in common beans in the tropics and the effect against stink bugs in semi-field conditions has not been evaluated. *B. bassiana* was widespread in common bean fields in Cuba and isolates could be obtained from soil, as endophytes of common bean plants and as natural infections in stink bugs (Ramos et al. 2017b). However, the potential of these isolates for inundation or inoculation biological control of stink bugs has not been evaluated.

The aim of this study was to evaluate the biocontrol potential of three Cuban *B. bassiana* isolates, Bb-18 (formulated and sold commercially in Cuba), and two naturally occurring isolates (18 S-O-R and 96 P-O-E) against *N. viridula* and *P. guildinii* compared to the control potential of the synthetic insecticide Methamidophos under laboratory and semi-field conditions.

Methods

Cultivation of common bean plants

Seeds of the common bean cultivar 'Chévere' were sown separately inside polyethylene bags containing 500 g of an Inceptisol soil (Soil taxonomy; USDA) collected at the Experimental Station "Álvaro Barba Machado" belonging to Universidad Central "Marta Abreu" de Las Villas, Villa Clara, Cuba. The soil was sterilized by heating it to 121 °C three times before being used in the experiment. Bags with soil and seed were then placed in a climatic chamber at 20 ± 1 °C, 50% relative humidity (RH), 14-h of light and 10-h of darkness (L14:D10) and fluorescent light of 40 W/m^2 (Dobben et al. 1981) and watered daily. After six days, when seedlings had unfolded leaves, they were placed in a greenhouse with day temperatures of 30 °C and night temperatures of 20 °C. Plants were irrigated twice per day, at 9.00 am and 5.00 pm.

Rearing of *N. viridula* and *P. guildinii*

Nezara viridula and *P. guildinii* were collected from a common bean field (cv. 'Chévere' in 2016) located in the Sandino orchard (22°57′56"N, 79°28′34"W), Remedios, Cuba. Sixty adults *N. viridula* and sixty adults *P. guildinii* (sex ratio 1:1) were distributed in three glass containers (30 × 18 cm, covered with lids perforated with 20 holes for aeration), separately for each species (20 stink bugs per container), and reared in a climatic chamber (Memmert, Germany) at 26 ± 2 °C, 65 ± 10% RH, 14L:10D as described by Silva et al. (2013). The insects were fed on a diet of fresh pods of common bean and soybean (*Glycine max* L.) obtained from an indoor plant culture. Food was replaced every two days. As described by Bentivenha et al. (2018), cotton pieces (3 × 3 cm) moistened with distilled water were placed in the containers to obtain appropriate humidity. Cotton pieces were replaced every two days. To facilitate oviposition, paper towels were placed inside each container. Egg masses were collected daily and transferred to plastic cages with moistened filter paper for hatching, and emerging nymphs were transferred to glass containers (30 × 18 cm) with pods of bean and soybean and covered with a mesh (1.35 µm).

Fungal isolates and synthetic insecticide

Two *B. bassiana* isolates, 18 S-O-R (isolated from soil in a common bean field in Remedios, Cuba at 22° 57′ 56″ N, 79° 28′ 34″ W) and 96 P-O-E (isolated from common bean plant tissue in a common bean field in Encrucijada, Cuba at 22° 37′ 01″ N, 79° 51′ 58″ W) (Ramos et al. 2017b), were used in the experiments. The isolates were stored at 4 °C in 40 ml test tubes with Sabouraud Dextrose Agar (SDA) (BioCen, Cuba) at the Universidad Central "Marta Abreu" de Las Villas collection of EPF, Santa Clara, Cuba. In addition, the *B. bassiana* isolate Bb-18 was supplied by the Plant Health Laboratory in Santa Clara belonging to the Cuban Ministry of Agriculture. This commercial strain (commercialized in Cuba) was originally isolated from *Hypothenemus hampei* Ferrari (Coleoptera: Curculionidae) in Manicaragua, Cuba and it is the most used mycoinsecticide in Cuba (Baldiviezo et al. 2020). All three isolates used were confirmed by molecular methods in earlier studies to be *B. bassiana* sensu stricto (Ramos et al. 2017b). Mycelia from each of the three *B. bassiana* isolates were obtained by inoculation of 100 ml Sabouraud Dextrose Broth (SDB) (BioCen, Cuba) with 5 mm plugs of 14-days-old fungal cultures grown on SDA at 25 °C. The fungal cultures were grown on an orbital shaker (120 rpm) for five days at 25 °C and in the dark as described in Ramos et al. (2017b). Sixty milliliters of mycelium were then harvested from the SDB fungal culture and used to inoculate 300 g autoclaved (30 min at 120 °C) rice (*Oryza sativa* L.) in polypropylene bags (35 × 22 cm). Afterwards, bags were sealed and incubated at 25 °C, 75% RH in the dark for 14 days. Rice with fungi was then air-dried in a laminar flow hood to allow harvesting of dry conidia. Dry conidia were manually harvested by adding 100 g of rice to a 0.25 m^2 sieve with a 5 mm mesh and shake for 10 min. Finally, conidia from each *B. bassiana* isolate were suspended in sterile distilled water with 0.01% Tween 80 and adjusted to a concentration of 1×10^8 conidia/ml by using a Neubauer hemocytometer chamber (Brand, Germany). Before using this concentration in the experiment, it was checked for conidial viability. This was done by counting viable and non-viable conidia under a microscope (400×) 22 h after fungal inoculation on an SDA plate, following the procedure described by Oliveira et al. (2015). Conidia were considered viable when germ tubes were longer than the conidial diameter; all suspensions used showed germination rates ≥ 95%.

The chemical insecticide Tamaron 60 Suspension Concentrate (SC) with the active ingredient Methamidophos (1.5 g/l a.i.) was used at the concentration recommended on the label by the supplier (Bayer Crop Science, Germany). Tamaron 60 SC is an organophosphate used against piercing-sucking insects and it is reported to be effective against stink bugs (Sosa-Gómez et al. 2001). Sterile distilled water was used as the control treatment in all experiments.

Experimental set up: bioassays

Twenty adults (34-days-old) *N. viridula* 10 females/10 males and 20 adults (31-days-old) *P. guildinii* 10 females/10 males were individually dipped for 15 s in 10 ml of each of the five treatments shown in Table 1, using the method described by Raafat et al. (2015). This procedure was repeated at separate occasions, using new conidial suspensions. Each stink bug was then placed separately in a sterile Petri dish (12.5 cm) with a moistened filter paper and a fully-grown common bean pod of the cultivar 'Chévere' collected from plants in growth stage BBCH 75 (Meier 2001) from the indoor plant culture described above. Common bean pods were replaced every two days. Each pod was superficially sterilized before use by dipping it for 3 min in 1.5% sodium hypochlorite, 2 min in 70% ethanol and then rinsed three times in sterile distilled water (Parsa et al. 2013). Petri dishes with stink bugs and common bean pods were then placed at 25 ± 1 °C, 85% RH, 14L:10D and observed daily

Table 1 List of treatments, origin of *Beauveria bassiana* isolates and concentration rates used in Petri dish bioassays and semi-field experiments with *Nezara viridula* and *Piezodorus guildinii*

No	Treatments	Origin of isolate	Application concentration
1	Sterile distilled water + 0.01% Tween 80 (control)	–	–
2	Tamaron 60 SC (a.i. Methamidophos)*	–	1.5 g/l a.i
3	*Beauveria bassiana* (Bb-18 strain) + 0.01% Tween 80	*Hypothenemus hampei* (Coleoptera: Cucurlionidae) from Manicaragua, Cuba	1×10^8 conidia/ml
4	*Beauveria bassiana* 18 S-O-R + 0.01% Tween 80	Soil from common bean field in Remedios, Cuba (22° 57′ 56″ N, 79° 28′ 34″ W) (Ramos et al. 2017b)	1×10^8 conidia/ml
5	*Beauveria bassiana* 96 P-O-E + 0.01% Tween 80	Bean plant tissue from common bean field in Encrucijada, Cuba (22° 37′ 01″ N, 79° 51′ 58″ W) (Ramos et al. 2017b)	1×10^8 conidia/ml

SC Soluble concentrate, *a.i.* Active ingredient

*The synthetic insecticide Tamaron 60 SC was used in both Petri dish bioassays and semi field experiments

for 11 days to evaluate stink bug mortality. Dead stink bugs from the *B. bassiana* treatments were surface sterilized as described before. Disinfected dead stink bugs were placed individually in a sterile plastic cup (40 ml) containing 1.5% water agar (BioCen, Cuba) and a moistened filter paper piece (2×2 cm) and sealed with a lid. These were incubated at 25 ± 1 °C, 85% RH in the dark for five days to observe for fungal growth. Presence of *Beauveria* was confirmed by morphological identification of fungal structure under microscope, according to Humber (2012). The experiments were repeated four times over time.

Experimental set up: semi-field experiment

Bean plants from the commercial common bean cv. 'Chévere' from the indoor plant culture described above were used. When half of the plants had reached growth stage BBCH 75 and its foliage had 15 cm in diameter, the five different treatments (Table 1) were applied to each of five bean plants at a total rate of 10 ml per plant according to the recommended concentration and as described by Afandhi et al. (2019). Each plant was sprayed independently by the use of a backpack sprayer (Hudson, USA; 10 l capacity). The backpack sprayer was disinfected with tap water, ethanol 70%, acetone 99% and washed three times with distilled water between treatments to avoid contamination among treatments (Castro et al. 2015). After spraying, plants were allowed to air dry before they were placed into a cage (30×30×30 cm) covered with a mesh (3350 BT Bionete 50 mesh; Technical Protection Textiles, Arrigoni 1936®, Italy). Four replicate cages per treatment with five plants in each were prepared for each stink bug species, placed outside and hence exposed to ambient weather conditions. Twenty adults (10 females/10 males) of either *N. viridula* (34-days-old) or *P. guildinii* (31-days-old) from the insect rearing were released into each of the four separate cages for each of the five treatments. Bean plants were irrigated twice a week. Stink bugs were checked on the plants every day for 14 days for mortality. Dead stink bugs from the *B. bassiana* treatments were surface sterilized, placed into a moist chamber as described above and observed for fungal growth for five days. Presence of *Beauveria* was confirmed by morphological identification of fungal structure under microscope, according to Humber (2012). The experiments were repeated at four separate occasions using different generations of reared stink bugs and new suspensions of *B. bassiana.*

Data analysis

Survival data for stink bugs in Petri dish bioassays and semi-field experiments were analyzed separately for each species with the PROC LIFETEST procedure in SAS 9.3, SAS Institute 2009 (Littell et al. 1996) using the log-rank test to evaluate equality among treatments with post-hoc comparisons by the Sidak adjustment. Proportions of dead stink bugs with fungal outgrowth identified to *Beauveria* as described above were analyzed by logistic regression using the GLIMMIX procedure in SAS including fungal isolate as fixed effect and experimental repetition as random effect. If significant effects were found ($P < 0.05$), means were separated by LS-means using the Tukey adjustment.

Results

Bioassays

In the Petri dish bioassays, Tamaron 60 SC (a.i. Methamidophos) caused 100% mortality for both stink bug species the first day after exposure and data of this treatment were therefore not included in survivorship analyses. Survival curves of *N. viridula* adults over time after exposure to the three *B. bassiana* isolates and the control were significantly different (Log-rank test: $\chi^2 = 258.65$, $df = 3$, $P < 0.0001$) (Fig. 1a). *N. viridula* (n = 80) had LT_{50} values of 6.04 ± 0.18 (SE) days when exposed to *B. bassiana* isolate 18 S-O-R, 6.75 ± 0.22 (SE) days when exposed to 96 P-O-E and 8.33 ± 0.23 (SE) days when exposed to the commercial isolate Bb-18 at the same concentration of 1×10^8 conidia/ml. All (100%) *N. viridula* were dead within 8 days (isolate 18 S-O-R) and 10 days (isolate 96 P-O-E) after exposure. This was significantly higher than for the commercial isolate Bb-18 that gave 86.3% *N. viridula* mortality at day 11 (Fig. 1a). No mortality was observed among control treated stink bugs after 11 days (Fig. 1a). All *B. bassiana* isolates led to fungal growth and sporulation of cadavers, and the highest proportion of cadavers developing mycosis was observed for isolate 18 S-O-R (Table 2).

Survivorship curves of *P. guildinii* stink bugs were also significantly different among treatments when excluding Tamaron 60 SC (a.i. Methamidophos) (Log-rank test: $\chi^2 = 252.05$, $df = 3$, $P < 0.0001$) (Fig. 1b). *P. guildinii* had LT_{50} values of 5.32 ± 0.14 (SE) days when exposed to 18 S-O-R, 5.63 ± 0.12 (SE) days when exposed to 96 P-O-E and 7.11 ± 0.21 (SE) days when exposed to the commercial isolate Bb-18 at the same concentration of 1×10^8 conidia/ml. All (100%) *P. guildinii* were dead after 7 days when exposed to 18 S-O-R and 96 P-O-E while the commercial isolate Bb-18 resulted in a maximum mortality of 81.3% at day 9. No control mortality was detected among *P. guildinii* (Fig. 1b). The proportion of cadavers developing mycosis was not significantly different between *B. bassiana* isolates (Table 2).

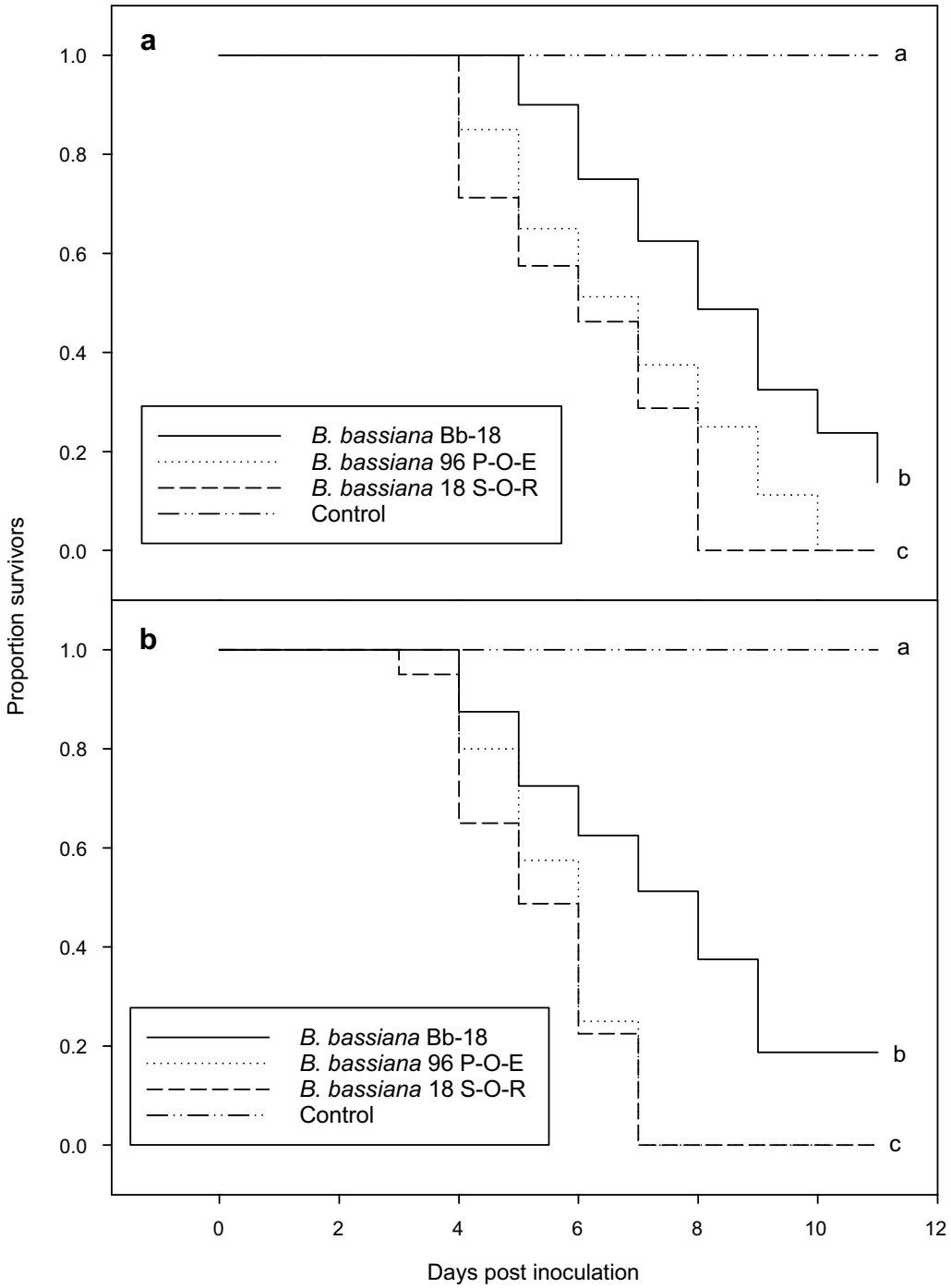

Fig. 1 Survivorship curves over 11 days for *Nezara viridula* (**a**) and *Piezodorus guildinii* (**b**) exposed to three different *Beauveria bassiana* isolates (Bb-18, 96 P-O-E, 18 S-O-R) at a concentration of 10^8 conidia/ml or a control in Petri dish bioassays (n = 80 stink bugs per treatment). Curves with different letters are significant different

Semi-field experiment

In the semi-field experiment, Tamaron 60 SC (a.i. Methamidophos) caused 100% mortality one day after application for both stink bug species and the survival data of this treatment were therefore not included in further analyses. Pooling both stink bug species, total mortality after 14 days, when observations ended, was 91.3% for 18 S-O-R, 80.0% for 96 P-O-E and 73.8% for

Table 2 Mean (± SE) proportion of cadavers developing mycosis in the two species of stink bugs exposed to three different *Beauveria bassiana* isolates in Petri dish bioassays

	Isolates			F-value (df)	*P* value
	18 S-O-R	96 P-O-E	Bb-18		
Nezara viridula	0.69 ± 0.03 a	0.46 ± 0.04 b	0.44 ± 0.04 b	5.80 (2.6)	0.0396
Piezodorus guildinii	0.73 ± 0.01	0.65 ± 0.04	0.51 ± 0.04	3.42 (2.6)	0.1020

Different letters within a row indicate significant different proportions. It was applied if tests showed significant differences between treatments on sporulation proportion

Bb-18. *N. viridula* survival curves are significantly different among the three *B. bassiana* isolates and the control treatment (Log-rank test: $\chi^2 = 168.62$, $df = 3$, $P < 0.0001$) (Fig. 2a). The only difference in survivorship curves among *B. bassiana* isolates was detected between 18 S-O-R and the commercial isolate Bb-18 (Fig. 2a), although curves of 18 S-O-R and 96 P-O-E were close to being significantly different ($\chi^2 = 6.6156$, $df = 1$, $P = 0.0591$). The *B. bassiana* isolate sprayed on plants 18 S-O-R induced mortality in adults of *N. viridula* from day 4 after inoculation while Bb-18 and 96 P-O-E caused mortality from day 5 after inoculation. For the 18 S-O-R isolate sprayed on plants, 91% of *N. viridula* adults were dead after 12 days when observations ended and had LT_{50} value of 6.79 ± 0.37 (SE) days at concentration of 1×10^8 conidia/ml. For the 96 P-O-E isolate sprayed on plants, 80% of *N. viridula* adults were dead after 13 days when observations ended and had LT_{50} value of 8.28 ± 0.37 (SE) days, and for the commercial isolate Bb-18 sprayed on plants, 68% of *N. viridula* adults were dead after 14 days when observations ended and had LT_{50} of 10.05 ± 0.39 (SE) days at 1×10^8 conidia/ml. No mortality was observed in the control treatment. Proportions of dead *N. viridula* with mycosis were not significantly different between the three fungal isolates (Table 3).

Piezodorus guildinii survival curves were significantly different among the three *B. bassiana* isolates and control treatments (Log-rank test: $\chi^2 = 207.28$, $df = 3$, $P < 0.0001$) with no mortality observed in the control (Fig. 2b). The *B. bassiana* isolates 18 S-O-R and 96 P-O-E induced mortalities in adults of *P. guildinii* from day 4 after inoculation while Bb-18 caused mortality from day 5 after inoculation. All (100%) *P. guildinii* on plants sprayed with 18 S-O-R were dead 12 days after inoculation and had LT_{50} of 7.71 ± 0.32 (SE days) at 1×10^8 conidia/ml. For the 96 P-O-E isolate, 92.5% of *P. guildinii* adults were dead after 14 days when observations ended and had LT_{50} of 9.28 ± 0.31 (SE) days at concentration of 1×10^8 conidia/ml. For the commercial isolate Bb-18 isolate, 53% of *P. guildinii* adults were dead after 14 days when observations ended and had LT_{50} of 10.03 ± 0.27 (SE) days at 1×10^8 conidia/ml (Fig. 2b). Proportions of dead *P. guildinii* with mycosis were non-significantly different among the three fungal isolates (Table 3).

Discussion

The study results revealed that the stink bug species *N. viridula* and *P. guildinii* were susceptible to all three *B. bassiana* isolates tested in Petri-dish bioassays and semi-field experiments. These findings are consistent with previous reports that *B. bassiana* isolates can infect stink bugs. Lopes et al. (2015b) demonstrated in a laboratory study that the stink bug species *Diceraeus melacanthus* Dallas (Heteroptera: Pentatomidae), *Euschistus heros* Fabricius (Heteroptera: Pentatomidae) and *Chinavia ubica* Rolston (Heteroptera: Pentatomidae) were susceptible to the Brazilian *B. bassiana* isolate CG1105. Further, an Egyptian *B. bassiana* strain isolated from *N. viridula* and the strain *B. bassiana* ARSEF2011 were reported to have damaging effects on the epicuticle and procuticle of *N. viridula* 72 h after infection (Raafat et al. 2015). Also, Nora et al. (2021) reported that *B. bassiana* isolates (UFSM-1 and UFSM-2 from the stink bug *E. heros* in soybean fields in Brazil) caused a high mortality of stink bugs over 15 days at a concentration of 1×10^8 conidia/ml, under laboratory conditions. However, in the present study, it was further confirmed in a series of experiments, from Petri-dish bioassays and semi-field experiments, that *B. bassiana* isolates resulted in relatively high stink bug mortality, indicating potential for use in biological control of these pests in common bean.

In the laboratory study (at 25 ± 1 °C, 85% RH, 14L:10D), the *B. bassiana* isolate 18 S-O-R at a concentration of 1×10^8 conidia/ml caused *N. viridula* and *P. guildinii* mortality of 100% after 8 and 7 days, respectively. Silva-Santana et al. (2022) demonstrated in a laboratory study (at 26 ± 2 °C, 60% RH and 14L:10 D) that the isolate of *B. bassiana* Unioeste 76 at a concentration of 1×10^8 conidia/ml caused 96% mortality of *E. heros* 14 days after treatment. Parys and Portilla (2020) showed through a laboratory assay at 27 °C that the strains of *B. bassiana* BotaniGard® and NI8 at 5×10^7 conidia/ml caused 87 and 88% mortality of *P. guildinii*, respectively. This suggested that the Cuban *B. bassiana* isolate 18 S-O-R used against stink bug species (*N. viridula* and *P. guildinii*) in common

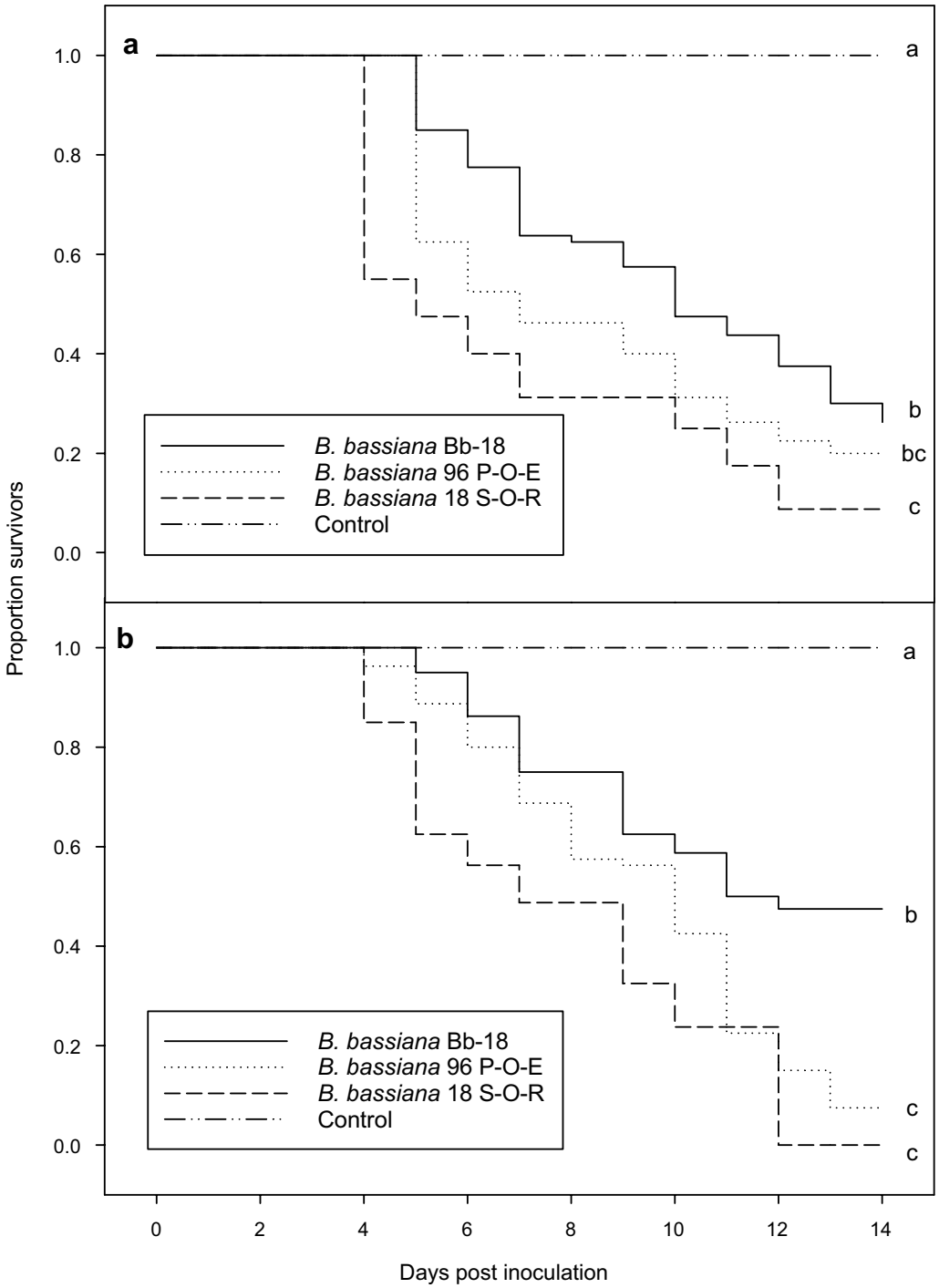

Fig. 2 Survivorship curves over 14 days for *Nezara viridula* (**a**) and *Piezodorus guildinii* (**b**) exposed to three different *Beauveria bassiana* isolates (Bb-18, 96 P-O-E, 18 S-O-R) at a concentration of 10^8 conidia/ml or a control in semi-field experiments with whole common bean plants (n = 20 per treatment and per stink bug specie). Curves with different letters are significant different

beans was comparable to BotaniGard® and maybe better than the *B. bassiana* isolate NI8 tested against and *P. guildinii*, in laboratory studies.

Further, our semi field experiment showed that the most promising *B. bassiana* isolate 18 S-O-R (at 1×10^8 conidia/ml) resulted in 91.3% mortality (both stink bug species pooled) and was comparable to the chemical

Table 3 Mean (±SE) proportion of cadavers developing mycosis in the two species of stink bugs exposed to three different *Beauveria bassiana* isolates in semi-field experiments

	Isolates			F-value (df)	*P* value
	18 S-O-R	96 P-O-E	Bb-18		
Nezara viridula	0.76 ± 0.06	0.74 ± 0.06	0.58 ± 0.05	2.72 (2.6)	0.1441
Piezodorus guildinii	0.74 ± 0.04	0.69 ± 0.02	0.83 ± 0.03	1.41 (2.6)	0.3146

insecticide Tamaron 60 SC (a.i. Methamidophos), which gave 100% mortality. Methamidophos caused death in less than a day while *B. bassiana* used longer time to kill the stink bug species (LT_{50}: 6.79 days 18 S-O-R against *N. viridula* at concentration of 1×10^8 conidia/ml) in present study.

Our results are consistent with those obtained by Silva-Santana et al. (2022) in a semi-field experiment on soybeans, where the isolate *B. bassiana* Unioeste 76, at concentrations of 1×10^8 conidia/ml and 1×10^9 conidia/ml, resulted in mortality rates of 80 and 90% on *E. heros*, respectively.

Patel et al. (2006) demonstrated that the soil-derived *B. bassiana* LRC28 isolate applied at a concentration of 4×10^7 conidia/ml exhibited higher virulence towards adult rice stink bugs (*Oebalus pugnax* Fabricius, Heteroptera: Pentatomidae) compared to *B. bassiana* isolated from *O. pugnax* applied at a concentration of 4×10^7 conidia/ml. The authors reported that a single application of *B. bassiana* LRC28 reduced 44% of the rice stink bug populations on six out of the nine sampling dates. Sosa-Gómez and Moscardi (1998) also demonstrated the susceptibility of *N. viridula* and *P. guildinii* to *B. bassiana* (CNPSo-Bb56) applied at a concentration of 1.5×10^{13} conidia/ml in field cage experiments with soybean, resulted in mortalities of 48% and 41%, respectively.

Conclusion

Obtained results indicated that the *B. bassiana* 18 S-O-R isolate had a potential as a biological control agent against stink bugs in common bean and suggested that it could be used as an alternative tool that may substitute traditional chemical insecticides in IPM programs. However, *B. bassiana* 18 S-O-R killed slower (higher LT_{50} values) than the chemical insecticide Tamaron 60 SC (a.i. Methamidophos) and therefore will not provide an immediate effect. This aspect needs to be emphasized when guiding farmers in effective application of this fungus as a microbial control agent. Further field studies are still needed to confirm the use of *B. bassiana* against the sting bugs in IPM programs.

Abbreviations

BBCH Phenological development stages of plants from Biologische Bundesanstalt Bundessortenamt und Chemische Industrie
EPF Entomopathogenic fungi
RH Relative humidity
SAS Statistical Analysis System
SDA Sabouraud Dextrose Agar
SDB Sabouraud Dextrose Broth
SC Suspension Concentrate
SE Standard Error
UCLV Universidad Central "Marta Abreu" de Las Villas
USDA United States Department of Agriculture

Acknowledgements

We thank Universidad Central "Marta Abreu" de Las Villas, Cuba for funding this study. Further, the Provincial Plant Health Laboratory in Villa Clara, Cuba is thanked for supplying the commercial *B. bassiana* strain Bb-18. Our gratitude goes to the Microbiology laboratory staff, including Annarella Chea and Anisley Blanco for preparing the culture media and inoculating the entomopathogenic fungi.

Author contributions

YR, OP and IK conceived the research. YR conducted the experiments. YR and NVM analyzed the data. YR, OP, NVM and IK wrote, reviewed and edited the manuscript. All authors read and approved the manuscript.

Funding

Not applicable.

Availability of data and materials

Not applicable.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Author details

[1]Instituto de Investigaciones Agropecuarias y Forestales, Universidad Michoacana de San Nicolás de Hidalgo, Morelia, Michoacán, México. [2]Departamento de Agronomía, Universidad Central "Marta Abreu" de Las Villas, Santa Clara, Cuba. [3]Departamento de Biología, Universidad Central "Marta Abreu" de Las Villas, Santa Clara, Cuba. [4]Centro de Investigaciones Agropecuarias, Facultad de Ciencias Agropecuarias, Universidad Central "Marta Abreu" de Las Villas, Santa Clara, Cuba. [5]Department of Plant and Environmental Sciences, University of Copenhagen, Frederiksberg C, Denmark. [6]Biotechnology and Plant Health Division, Norwegian Institute of Bioeconomy Research (NIBIO), Ås, Norway.

Received: 9 February 2024 Accepted: 17 April 2024
Published online: 23 April 2024

References

Ademokoya B, Athey K, Rubereson J (2022) Natural enemies and biological control of stink bugs (Hemiptera: Pentatomidae) in North America. Insects 13:932. https://doi.org/10.3390/insects13100932

Afandhi A, Widjayanti T, Emi AA, Tarno H, Afiyanti M, Handoko RN (2019) Endophytic fungi *Beauveria bassiana* Balsamo accelerates growth of common bean (*Phaseolus vulgaris* L.). Chem Biol Technol 6:11. https://doi.org/10.1186/s40538-019-0148-1

Baldiviezo LV, Pedrini N, Santana M, Mannino MC, Nieva LB, Gentile A, Cardoza RM (2020) Isolation of *Beauveria bassiana* from the chagas disease vector *Triatoma infestans* in the gran chaco region of Argentina: assessment of gene expression during host-pathogen interaction. J Fungi 6:219. https://doi.org/10.3390/jof6040219

Bentivenha JPF, Canassa V, Baldin ELL, Borguini M, Lima GPP, Lourenção AL (2018) Role of the rutin and genistein flavonoids in soybean resistance to *Piezodorus guildinii* (Hemiptera: Pentatomidae). Arthropod Plant Interact 12:311–320. https://doi.org/10.1007/s11829-017-9578-5

Brown SA, Davis JA, Ritcher AR (2012) Efficacy of foliar insecticides on eggs of *Nezara viridula* (Hemiptera: Pentatomidae). Fla Entomol 95:1182–1186. https://doi.org/10.1653/024.095.0449

Castro T, Roggia S, Wekesa VW, Andrade R, Demétrio C, Delalibera I, Klingen I (2015) The effect of synthetic pesticides and sulfur used in conventional and organically grown strawberry and soybean on *Neozygites floridiana*, a natural enemy of spider mites. Pest Manag Sci 79:1752–1757. https://doi.org/10.1002/ps.4204

Cingolani MF, Greco NM, Liljesthröm GG (2013) Multiparasitism of *Piezodorus guildinii* eggs by *Telenomus podisi* and *Trissolcus urichi*. Biocontrol 58:37–44. https://doi.org/10.1007/s10526-012-9484-9

Depieri RA, Panizzi AR (2011) Duration of feeding and superficial and in-depth damage to soybean seed by selected species of stink bugs (Heteroptera: Pentatomidae). Neotrop Entomol 40:197–203. https://doi.org/10.1590/S1519-566X2011000200007

Directive 2009/128/EC. https://eur-lex.europa.eu/legal-content/EN/ALL/?uri=celex%3A32009L0128. Accessed 30 August 2019

Dobben VHV, Ast AV, Corré WJ (1981) The influence of light intensity on morphology and growth rate of bean seedlings. Acta Bot Neerl 30:33–45

Humber RA (2012) Identification of entomopathogenic fungi. In: Lacey LA (ed) Manual of techniques in invertebrate pathology, 2nd edn. Academic Press, San Diego, pp 151–186

Littell RC, Milliken GA, Stroup WW, Wolfinger RD (1996) SAS system for mixed models. SAS Institute Inc., Cary

Lopes LM, Araújo AEF, Santos ACV, Santos VB, Sousa AH (2015a) Population development of *Zabrotes subfasciatus* (Coleoptera: Chrysomelidae) in landrace bean varieties occurring in Southwestern Amazonia. J Econ Entomol 109:467–471. https://doi.org/10.1093/jee/tov330

Lopes RB, Laumann RA, Blassioli-Moraes MC, Borges M, Faria M (2015b) The fungistatic and fungicidal effects of volatiles from metathoracic glands of soybean-attacking stink bugs (Heteroptera: Pentatomidae) on the entomopathogen *Beauveria bassiana*. J Invertebr Pathol 132:77–85. https://doi.org/10.1016/j.jip.2015.08.011

Marques RP, Cargnelutti Filho A, Melo AA, Guedes JVC, De Carli C, Rohrig A, Pozebon H, Perini CR, Ferreira DR, Bevilaqua JG, Patias LS, Forgiarini SE, Padilha G, Leitão JV, Moro D, Hahn L, Arnemann JA (2019) Managing stink bus on soybean fields: insights on chemical management. J Agric Sci 6:225–234. https://doi.org/10.5539/jas.v11n6p225

Mascarin GM, Jaronski ST (2016) The production and uses of *Beauveria bassiana* as microbial insecticide. World J Microbiol Biotechnol 32:177. https://doi.org/10.1007/s11274-016-2131-3

Meier U (2001) Growth stages of mono-and dicotyledonous plants. In: BBCH monograph, 2nd ed. Federal Biological Research Centre for Agriculture and Forestry, p 158

Morales-Soto A, Lamz-Piedra A, Chang-Sidorchuk L, Martínez-Zubiaur Y (2022) Genotipos de frijol común (*Phaseolus vulgaris* L.) con resistencia a BGYMV en Cuba. Agron Mesoam. https://doi.org/10.15517/am.v33i3.48436

Nora D, Piovesan B, Bellé C, Stacke R, Balardin R, Guedes J, Michaud JP, Jacques R (2021) Isolation and evaluation of entomopathogenic fungi against the neotropical brown stink bug *Euschistus heros* (F.) (Hemiptera: Pentatomidae) under laboratory conditions. Biocontrol Sci Technol 31:22–34. https://doi.org/10.1080/09583157.2020.1826904

Oliveira DGP, Pauli G, Mascarin GM, Delalibera I (2015) A protocol for determination of conidial viability of the fungal entomopathogens *Beauveria bassiana* and *Metarhizium anisopliae* from commercial products. J Microbiol Methods 119:44–52. https://doi.org/10.1016/j.mimet.2015.09.021

Oliveira DGP, Dudczak AC, Alves LFA, Sosa-Gómez DR (2016) Biological parameters of *Euschistus heros* (F.) (Heteroptera: Pentatomidae) and its susceptibility to entomopathogenic fungi when fed on different diets. Braz Arch Biol Technol 59:e16150141. https://doi.org/10.1590/1678-4324-2016150141

Parsa S, Ortiz V, Vega FE (2013) Establishing fungal entomopathogens as endophytes: towards endophytic biological control. J Vis Exp 74:1–5. https://doi.org/10.3791/50360

Parys KA, Portilla M (2020) Effectiveness of *Beauveria bassiana* against *Piezodorus guildinii* (Hemiptera: Pentatomidae), a key pest of soybeans in the neotropics. Biocontrol Sci Technol 30:451–461. https://doi.org/10.1080/09583157.2020.1731426

Patel DT, Fuxa JR, Stout MJ (2006) Evaluation of *Beauveria bassiana* for control of *Oebalus pugnax* (Hemiptera: Pentatomidae) in rice. J Entomol Sci 41:126–146. https://doi.org/10.18474/0749-8004-41.2.126

Raafat I, Meshrif WS, El Husseing EM, El-Hariry M, Seif AI (2015) *Nezara viridula* (Hemiptera: Pentatomidae) cuticule as a barrier for *Beauveria bassiana* and *Paecilomyces* sp. infection. African Entomol 23:75–87. https://doi.org/10.4001/003.023.0128

Ramos YG, Gómez JR, Klingen I (2017a) Seeding dates and cultivars effects on stink bugs population and damage on common bean *Phaseolus vulgaris* L. Neotrop Entomol 46:701–710. https://doi.org/10.1007/s13744-017-0512-4

Ramos Y, Portal O, Lysøe E, Meyling NV, Klingen I (2017b) Diversity and abundance of *Beauveria bassiana* in soils, stink bubs and plant tissues of common bean from organic and conventional fields. J Invertebr Pathol 150:114–120. https://doi.org/10.1016/j.jip.2017.10.003

Silva JPGF, Baldin EL, Souza ES, Canassa VF, Laurenção AL (2013) Characterization of antibiosis to the red banded stink bug *Piezodorus guildinii* (Hemiptera: Pentatomidae) in soybean entries. J Pest Sci 86:649–657. https://doi.org/10.1007/s10340-013-0527-5

Silva-Santana MF, Alves LF, Ferreira T, Bonini A (2022) Selection and characterization of *Beauveria bassiana* fungus and their potential to control the brown stink bug. Biocontrol Sci Technol 32:90–102. https://doi.org/10.1080/09583157.2021.1970716

Sosa-Gómez DR, Moscardi F (1998) Laboratory and field studies on the infection of the stink bugs, *Nezara viridula*, *Piezodorus guildinii*, and *Euschistus heros* (Hemiptera: Pentatomidae) with *Metarhizium anisopliae* and *Beauveria bassiana* in Brazil. J Invertebr Pathol 71:115–120. https://doi.org/10.1006/jipa.1997.4716

Sosa-Gómez DR, Corso IC, Morales L (2001) Insecticide resistance to endosulfan, monocrotophos and metamidophos in the neotropical brown stink bug, *Euschistus heros* (F.). Neotrop Entomol 30:317–320. https://doi.org/10.1590/S1519-566X2001000200017

Souza ES, Silva JPGF, Baldin ELL, Pierozzi CG, Cunha LS, Canassa VF, Pannuti LER, Lourenção AL (2015) Response of soybean genotypes challenged by a stink bug complex (Hemiptera: Pentatomidae). J Econ Entomol 109:898–906. https://doi.org/10.1093/jee/tov341

Suárez-Martínez SE, Ferriz-Martínez RA, Campos-Vega R, Elton-Puente JE, de la Torre K, García-Gasca T (2015) Bean seeds: leading nutraceutical source for human health. Cyta-J Food 14:131–137. https://doi.org/10.1080/19476337.2015.1063548

Werdin JOG, Laumann RA, da Silveira S, Blassioli MC, Borges M, Ferrero AA (2013) Lethal and sublethal effects of four essential oils on the egg parasitoids *Trissolcus basalis*. Chemosphere 92:608–615. https://doi.org/10.1016/j.chemosphere.2013.03.066

Zerbino MS, Panizzi AR (2019) The underestimated role of pest pentatomid parasitoids in Southern South America. Arthropod Plant Interact 13:703. https://doi.org/10.1007/s11829-019-09703-1

Publisher's Note