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Sublethal effects of *Beauveria bassiana sensu lato* isolate NJBb2101 on biological fitness and insecticide sensitivity of parental and offspring generations of brown planthopper, *Nilaparvata lugens*

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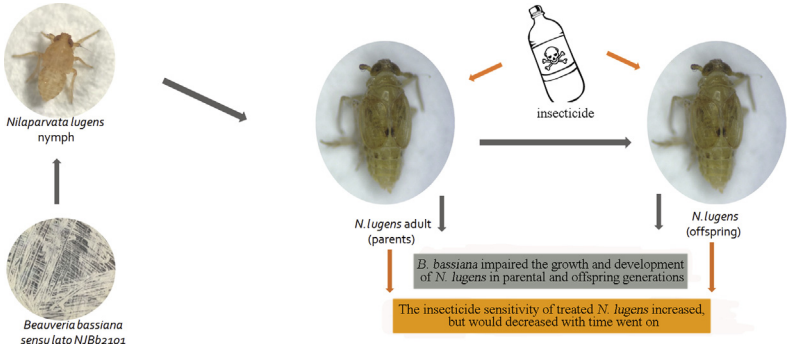
GRAPHICAL ABSTRACT



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ABSTRACT

Entomopathogenic fungi such as *Beauveria bassiana sensu lato* (*s. l.*) have been developed for biological control of sucking insect pests such as *Nilaparvata lugens*. However, the sublethal effects of *B. bassiana s. l.* on *N. lugens* remain unclear, and were investigated in the present study. *N. lugens* 3rd instar nymphs were inoculated with a sublethal concentration of *B. bassiana s. l.* isolate NJBb2101 conidia and then reared until they developed into adults. The resultant adults (parental generation) were paired to generate offspring. Life parameters in both the parental and the offspring generations were examined: nymph survival rate, emergence rate, nymph duration, sex ratio, copulation rate, fecundity, hatching rate and adult female longevity. Compared with the control, NJBb2101-treated *N. lugens* exhibited lower fitness indices such as low female ratio and fecundity, with the relative fitness of offspring being only 0.28. Both generations of NJBb2101-treated *N. lugens* were much more susceptible to three test insecticides with susceptibility increasing by 12.7–23.3-fold in parental females and 3.8–8.4-fold in the offspring (4th instar nymphs and females). The results suggest that sublethal effects of entomopathogenic fungi on *N. lugens* can reduce insect growth and development and increase insecticide susceptibility. The findings are of practical interest for pest insect management in terms of combining entomopathogenic fungi with chemical insecticides.

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1. Introduction

The brown planthopper (BPH), *Nilaparvata lugens* Stål (Hemiptera: Delphacidae), is one of the most economically significant rice pests in many parts of Asia. The insect reduces rice production either by directly sucking nourishment from stems or by transmitting plant viruses (Holt et al., 1996). At present, application of chemical insecticides is still the most common means of controlling *N. lugens* in China (Zhang et al., 2016). However, insecticide resistance in *N. lugens* has been widely reported and an integrated strategy combining biological and chemical pesticides has been recommended (Chen and Feng, 2003). Applied as a biological pesticide, entomopathogenic fungi have advantages such as a low probability of resistance development due to their complex modes of action (Thomas and Read, 2007) and no chemical residues. Different entomopathogenic fungi have been studied for their ability to infect *N. lugens* (Feng and Pu, 2005; Li et al., 2014; Li et al., 2012), amongst which *Beauveria bassiana sensu lato* (*s. l.*) has been developed commercially as an environmentally-friendly biological insecticide throughout the world (Zimmermann, 2007).

In addition to causing mortality, sublethal effects of entomopathogenic fungi on insects and spider mites have been reported (Jarrahi and Safavi, 2016; Seyed-Talebi et al., 2012; Torrado-León et al., 2006; Zhang et al., 2015). However, little research has been done on the sublethal effects of *B. bassiana s. l.* on *N. lugens* (Feng and Pu, 2005). Although *B. bassiana* has been considered as a promising biological control agent, its use has been limited due to its slow action; it is possible that combining *B. bassiana* with chemical insecticides may reduce time to kill. Two questions were considered in the present study. First, what are the sublethal effects of *B. bassiana s. l.* on the growth and development of inoculated *N. lugens* nymphs and are they also apparent in the offspring of those inoculated nymphs? Second, would sublethally-inoculated *N. lugens* and their offspring be more susceptible to chemical insecticides? Accordingly, we evaluated the sublethal effects of *B. bassiana s. l.* isolate NJBb2101 on the fitness and insecticide susceptibility of two generations of *N. lugens*, the parental generation exposed to NJBb2101 directly and the offspring generation arising from the parental generation.

2. Material and methods

2.1. Insects

N. lugens was obtained from the China National Rice Research Institute in September 2001 and had been reared on rice seedlings (about 10 cm high) at 26 ± 1 °C, 60%–80% relative humidity and a 16 h light/8h dark light regime in laboratory growth chambers (GXZ-500B-LED, Jiangnan Instrument Factory, Jiangsu, China). All experiments were performed under the same conditions.

2.2. Fungi and insecticides

B. bassiana s. l. isolate NJBb2101 was isolated from a muscardine weevil (Fig. 1) collected from Jiangsu Academy of Agricultural Science in July 2015; a BLAST search on the 5.8S ribosomal DNA (rDNA) sequence revealed 99% similarity with an isolate identified as *B. bassiana* IMI 356817 (GenBank: AJ560682.1 from NCBI). Therefore, isolate NJBb2101 was designated as *B. bassiana s.l.* and will be referred to as *B. bassiana* NJBb2101 in this paper. The presence of *B. bassiana s. l.* NJBb2101 in sublethally inoculated *N. lugens* in both parental and offspring generations was confirmed following Koch's postulates and 5.8s rDNA analysis.

The fungus was cultured on Potato Dextrose Agar (PDA) in plastic Petri dishes (90 mm diameter) at 25 ± 1 °C, in darkness for 13 ± 1 days. Ten mL 0.05% Tween-80 (Solarbio, China) was added to the medium and conidia were scraped from the medium surface. The conidial suspension was filtered through lens paper in order to remove hyphae. Conidial concentration was determined using a Burkard haemocytometer. Conidial viability was confirmed when ≥95% germinated after 24 h on nutrient medium (1g glucose, 0.5 g peptone, 20 g agar/1 L distilled water) at 25 °C.

Imidacloprid, chlorpyrifos and etofenprox, all at reagent grade, were purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.3. B. bassiana s. l. NJBb2101 treatment of N. lugens

In a preliminary experiment, applications of *B. bassiana s. l.* isolate NJBb2101 at concentrations of 5×10^5 and 5×10^7 conidia/mL to third instar nymphs of *N. lugens* caused corrected mortalities (Abbott's formula, Abbott, 1925) of 23.8 ± 4.3% and 73.2 ± 9.4%, respectively, seven days after inoculation (data not shown). Therefore 5×10^5 conidia/mL was selected as a sublethal concentration for subsequent experiments; conidial concentrations were adjusted to 5×10^5 conidia/mL with 0.05% Tween-80 solution. One hundred insects were anaesthetized with carbon dioxide and placed on the spray pan of a Potter spray tower (Burkad Manufacturing Co Ltd, Rickmansworth, UK) as one group. For each group, 2 mL of the conidia suspension was sprayed onto the 100 *N. lugens* 3rd nymphs by auto-load Potter spray tower. Nymphs sprayed with the same volume of 0.05% Tween-80 solution were used as controls. After spraying, the 100 insects were transferred to an insect rearing box containing fresh rice seedlings. Each treatment included 12 groups with 100 nymphs per group, and the experiment was repeated three times. Therefore, 72 groups of 100 nymphs were used in total with 36 groups for the treatment and 36 groups for the control. The nymph survival rate, emergence rate and other parameters (Table 1) were recorded following incubation.

2.4. Sublethal effects of B. bassiana s. l. NJBb2101 on the reproduction of N. lugens

Emerging *N. lugens* adults were collected every 12 h from the assay described in section 2.3. Adults emerging from the control nymphs were designated as untreated and the ones from the NJBb2101-treated nymphs were designated as treated. These adults were paired into four groups: untreated female and untreated male (CK), treated female and treated male (T1), treated female and untreated male (T2) and untreated female and treated male (T3), with 20 pairs per group and the assay was repeated independently three times corresponding to the three replicates in section 2.3. Each pair of insects was maintained in individual glass tubes; a piece of sponge was placed in the glass tube and a hole cut in its centre through which five rice seedlings were placed. Newly hatched neonates were counted on a daily basis and then removed into new glass tubes with fresh rice seedlings; female longevity was recorded. Fecundity (Fd) and hatchability (Ha) were calculated by counting the unhatched eggs on the rice shoots in the original glass tubes under a stereoscope once the female had died. Females that produced no neonates or eggs were considered to have failed in copulation, and the copulation rates were calculated accordingly (Liu and Han, 2006). Three independent assays were performed with adults from the three independent assays described in section 2.3.

2.5. Sublethal effects of B. bassiana s. l. NJBb2101 on fitness and life history parameters of N. lugens offspring

Life tables for the offspring were constructed as described previously (Liu and Han 2006). A hundred neonates (N_0) were collected randomly from the offspring of one pair of *N. lugens* in the CK and T1 treatments (5 repeats at least), and then transferred to new 1000 mL beakers with rice seedlings where they were maintained until they developed into adults. Survival rate (Sr) from neonate to 3rd instar nymph (Sr1) and from 3rd to 5th instar nymph (Sr2) were determined. Thereafter the emergence rate (Er) and female ratio (Fr) were

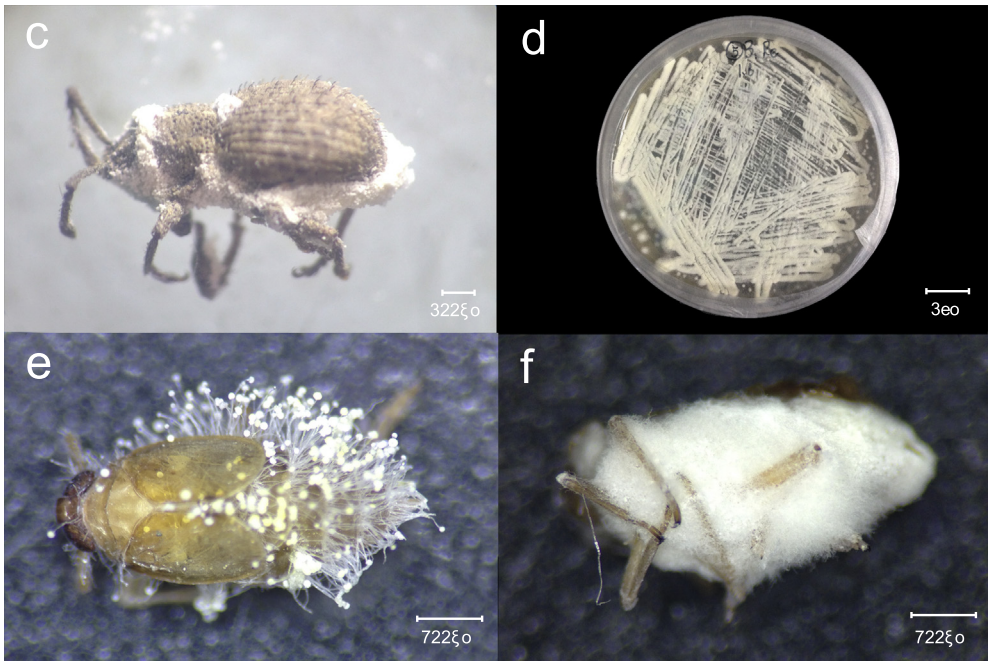

Fig. 1. The growth status of *Beauveria bassiana s. l.* NJBb2101 on PDA medium or host insects. (a) Muscardine weevil infected by *B. bassiana s. l.* found in a rice field. (b) *B. bassiana s. l.* NJBb2101 separated from the muscardine weevil in the laboratory. (c) Adult *N. lugens* (48 h) after death caused by NJBb2101. (d) Adult *N. lugens* (96 h) after death caused by NJBb2101.

Table 1
Sublethal effects of *Beauveria bassiana sensu lato* isolate NJBb2101 on 3rd instar *Nilarparva lugens* nymphs.

Parameter	Control (CK)[x]	Treatment 1 (T1)[x]	N	Sig. (2-tailed)
Nymph duration (d)	8.41 ± 0.73	8.94 ± 0.97	3	0.684
Nymph survival rate (%)	94.20 ± 3.36	72.56 ± 6.95*	3	0.049
Emergence rate (Er, %)	90.43 ± 5.11	71.60 ± 6.70	3	0.089
Female ratio (Fr, %)	50.30 ± 2.99	31.48 ± 3.22*	3	0.013

[x] Insects were sprayed with 5×10^5 conidia/mL suspension (T1) or 0.05% Tween-80 (CK). Data are presented as mean ± SEM from three independent replicates.

* Significant difference at level of $p < 0.05$ from student's *t* test.

calculated. Emerged males and females were paired into 'families', and the copulation rate (Cr), fecundity (Fd) and hatchability (Ha) were all assessed as described in 2.4. Three independent assays were performed with adults from the independent assays described in section 2.3. Population number of offspring (Nt), the population trend index (I) and relative fitness were also calculated as described previously (Liu and Han 2006). $Nt = N_0 \times Sr1 \times Sr2 \times Er \times Fr \times Cr \times Fd \times Ha$, with $N_0 = 100$; $I = Nt/N_0$ and relative fitness $= I_{T1}/I_{CK}$.

2.6. Effect of B. bassiana s. l. NJBb2101 treatment on insecticide susceptibility of N. lugens in two generations

Two-day-old females were collected from the control and NJBb2101-treated *N. lugens* from the assay described in section 2.3. The 4th instar nymphs and females were collected from the offspring of the CK and T1 treatments from the assay described in section 2.4. Therefore, *N. lugens* from two generations were collected with the females generated from 2.3 designated as the parental generation and the nymphs and females derived from 2.4 as the offspring generation. Insecticides (imidacloprid, chlorpyrifos and etofenprox) were diluted into a series of concentrations ranging from 2.50 to 750.00 ng/μL in acetone and topically applied onto the insect prothorax notum (20 nL/insect) after the insects had been anesthetized with carbon dioxide (Liu and Han, 2006). Each concentration was tested against thirty insects on each of the three occasions that the assay described in section 2.3 was run. 20 nL of acetone was used as control. The LD_{50} (lethal dose, 50%) values for each insecticide against both generations of *N. lugens* were determined.

2.7. Statistical analysis

Mortality was corrected according to Abbott's formula (Abbott, 1925). LD_{50} values, 95% confidence intervals and slope of the concentration-mortality lines were determined and analyzed in GraphPad Prism version 7.00 for Windows (GraphPad Software, La Jolla California USA). The normal distribution of each data set was verified using the one-sample Kolmogorov-Smirnov test in PASW Statistics version 18.0 (IBM Corp., 2009). Comparisons between two independent datasets were made using T-test and multiple comparisons were made using one-way ANOVA followed by Tukey's test in PASW Statistics version 18.0.

3. Results

3.1. B. bassiana s. l. NJBb2101 reduced survival and female ratio of N. lugens in the parental generation

A sublethal concentration (5×10^5 conidia/mL) of *B. bassiana s. l.* NJBb2101 significantly reduced nymph survival and female ratio in the treated *N. lugens* compared with the control (Table 1). Fewer treated *N. lugens* nymphs emerged as adults compared with the control.

3.2. B. bassiana s. l. NJBb2101 reduced the reproduction of N. lugens in the parental generation

Reproduction of both the T1 (both female and male treated with NJBb2101) and the T2 groups (only female treated with NJBb2101) were influenced by NJBb2101: copulation rate (Cr), fecundity (Fd) and hatchability (Ha) were significantly lower than in the CK group (Table 2). The T3 group (only male treated with NJBb2101) had significantly lower Ha than the CK group. Adult female longevity was similar amongst the T1, T2, T3 and CK groups (Table 2).

Table 2
Key reproduction parameters of the different pairs of *Beauveria bassiana sensu lato* isolate NJBb2101-treated and untreated *Nilaparvata lugens*.

Parameter[#]	Control (CK)[x]	Treatment 1 (T1)[x]	Treatment 2 (T2)[x]	Treatment 3 (T3)[x]	Sig.
	♀C×♂C	♀T×♂T	♀T×♂C	♀C×♂T	
Copulation rate (Cr, %)	88.72 ± 6.33a	53.66 ± 7.23b	57.24 ± 8.16b	85.20 ± 7.79a	0.002
Fecundity (Fd, eggs/female)	274.36 ± 28.06a	186.55 ± 24.28b	193.75 ± 22.81b	258.56 ± 27.13a	0.019
Hatchability (Ha, %)	87.15 ± 8.54a	65.82 ± 8.70a	68.24 ± 7.10a	37.30 ± 6.24b	0.001
Female adult longevity (d)	18.84 ± 2.38a	20.55 ± 3.16a	21.32 ± 3.70a	20.74 ± 2.63a	0.859

[#] Multiple comparisons amongst the four treatments (CK, T1, T2 and T3) in each row were made using one-way ANOVA with Tukey's tests in SPSS version 18. Different lower-case letters indicate significant differences at level of $p < 0.05$ amongst values in the same row.

[x] ♀C and ♂C represent females and males from control nymphs (CK) treated with 0.05% tween-80 (section 2.3 in the Material and Methods and Table 1); ♀T and ♂T represent females and males from nymphs treated (T1) with 5×10^5 conidia mL^{-1} suspension of *B. bassiana s. l.* NJBb2101 (section 2.3 in the Material and Methods and Table 1). Data are presented as mean ± SEM from three independent replicates.

Table 3
Growth parameters of the offspring of NJBb2101-treated and untreated *Nilaparvata lugens*.

Parameter[#]	Control (CK)[x]	Treatment (T1)[x]	df	Sig. (2-tailed)
Egg[#] duration (d)	7.24 ± 0.58	11.30 ± 0.87*	4	0.018
1st instar nymph duration (d)	2.58 ± 0.17	3.26 ± 0.24	4	0.086
2nd instar nymph duration (d)	2.72 ± 0.21	3.95 ± 0.30*	4	0.028
3rd instar nymph duration (d)	2.51 ± 0.22	3.61 ± 0.24*	4	0.027
4th instar nymph duration (d)	2.60 ± 0.20	3.02 ± 0.26	4	0.266
5th instar nymph duration (d)	3.27 ± 0.24	3.42 ± 0.27	4	0.702
Total nymph duration (d)	13.40 ± 1.05	17.71 ± 1.29	4	0.060
Female adult longevity (d)	18.92 ± 1.54	21.05 ± 1.72	4	0.409

[#] Eggs were laid by pairs of *N. lugens* adults from the CK (3rd instar nymphs treated with 0.05% Tween-80) and T1 (3rd instar nymphs treated with 5×10^5 conidia/mL suspension of *Beauveria bassiana s.l.* NJBb2101) groups (Section 2.3 and 2.4 in the Material and Methods).

[x] Data in each set were normally distributed and presented as mean ± SEM from three independent replicates.

* Significant difference at level of $p < 0.05$ from *t* test.

3.3. *B. bassiana s. l. NJBb2101 prolonged the immature stages of N. lugens offspring*

The development of offspring from the CK and T1 groups was assessed by recording the duration of each life stage (Table 3). The duration of the egg, 2nd instar and 3rd instar stages of the T1 group were all significantly longer than those in the CK group, while no significant difference was observed in the duration of the 4th and 5th instar stages between T1 and CK. The duration of the 1st instar and the overall total duration from egg to adult in the T1 group were both longer than in the CK group although not significantly so. Adult females lived for a similar number of days in both the CK and T1 groups.

3.4. *B. bassiana s. l. NJBb2101 negatively affected the life history parameters of N. lugens offspring*

The influence of a sublethal dose of *B. bassiana s. l.* NJBb2101 on *N. lugens* offspring was also examined by monitoring the life history parameters of the offspring derived from the CK and T1 groups (Table 4). Sr1 and Fd of the T1 group were both significantly lower compared with those of the CK group. The relative fitness of the T1 group was 0.28, which was much lower than the value for the CK group (1.00). However, no statistical difference was observed in Sr2, Er, Fr, Cr, or Ha between the T1 and CK groups although differences in Sr2 and Er between these groups were noticeable.

Table 4
Life tables of the offspring of NJBb2101-treat and untreated *Nilaparvata lugens*.

Parameter	Control (CK)[x]	Treatment (T1)[x]	df	Sig. (2-tailed)
Starting neonate[#] number per experiment	100	100	4	/
Survival rate from neonate to 3rd instar (Sr1, %)	90.52 ± 6.04	65.43 ± 6.45*	4	0.047
Survival rate from 3rd to 5th instar (Sr2, %)	92.78 ± 4.66	76.29 ± 5.87	4	0.093
Emergence rate (Er, %)	89.04 ± 5.05	70.52 ± 6.26	4	0.083
Female ratio (Fr, %)	49.40 ± 3.69	43.16 ± 4.54	4	0.346
Copulation rate (Cr, %)	89.12 ± 5.13	85.80 ± 6.63	4	0.712
Fecundity (Fd, eggs per female)	283.05 ± 4.86	222.74 ± 17.43*	4	0.029
Hatchability (Ha, %)	85.60 ± 5.64	76.33 ± 6.39	4	0.338
N, predicted number of offspring	7976.70	2216.25	/	/
I, population trend index	79.77	22.16	/	/
Relative fitness	1.00	0.28	/	/

[#] Neonates hatched from eggs laid by pairs of *N. lugens* adults from the CK (3rd instar nymphs treated with 0.05% Tween-80) and T1 (3rd instar treated with 5×10^5 conidia/mL suspension of *Beauveria bassiana s. l.* NJBb2101) groups. (Sections 2.3 and 2.4 in the Material and Methods).

[x] Data were presented as mean ± SEM from three independent replicates.

* Significant difference at level of $p < 0.05$ from *t* test.

3.5. *B. bassiana s. l. NJBb2101 increased the insecticide susceptibility of N. lugens in both generations*

LD_{50} values for the three test insecticides (imidacloprid, chlorpyrifos and etofenprox) against *N. lugens* females in the parental generation, the 4th instar offspring nymphs and adult offspring females are presented in Table 5. After treatment with a sublethal concentration of *B. bassiana s. l.* NJBb2101, *N. lugens* was more susceptible to the three insecticides, and the susceptibility decreased with generation and development. For example, in terms of etofenprox, the susceptibility ratio of NJBb2101-treated insects was 23.32 in the parental generation but 8.37 and 6.67 in the 4th instar nymphs and adult females in the offspring generation, respectively. A similar reduction in susceptibility was observed for the other two insecticides.

4. Discussion

When *N. lugens* nymphs were treated with a sublethal dose of *B. bassiana* NJBb2101, the insects development and reproduction were adversely affected, and they were more susceptible to three different pesticides, in both the parental and offspring generations. Researchers have documented various effects of inoculation with sublethal doses of entomopathogenic fungi on different pest species. Fitness costs associated with inoculation of entomopathogenic fungi have been reported in several studies (Nguyen et al., 2007; Sedaratian et al., 2013) while

Table 5
Insecticide susceptibility of NJBb2101-affected *Nilaparvata lugens* in parental and offspring generations.

Insects	Insecticide	Control (CK)		Treatment (T1)		Susceptibility ratio[η]
		Slope[δ,ψ]	LD_{50}[ζ] (ng/insect)	Slope[δ]	LD_{50}[ζ,ψ] (ng/insect)	
Female[#]	Imidacloprid	3.16 ± 0.23	7.13 (6.83–7.48)	1.72 ± 0.24	0.56 (0.40–0.92)**	12.73
	Chlorpyrifos	2.85 ± 0.26	4.32 (4.16–4.52)	1.65 ± 0.28	0.23 (0.15–0.38)**	18.78
	Etofenprox	3.04 ± 0.26	9.56 (9.03–9.97)	1.57 ± 0.19	0.41 (0.20–0.74)**	23.32
Offspring[x] 4th instar nymph	Imidacloprid	3.25 ± 0.31	1.42 (1.38–1.48)	2.03 ± 0.32	0.27 (0.22–0.37)**	5.26
	Chlorpyrifos	2.92 ± 0.34	1.08 (1.05–1.14)	2.35 ± 0.36	0.19 (0.16–0.25)**	5.68
	Etofenprox	2.76 ± 0.28	4.35 (4.06–4.69)	2.44 ± 0.41	0.52 (0.40–0.73)**	8.37
Offspring[x] female	Imidacloprid	2.91 ± 0.31	6.22 (5.93–6.56)	2.32 ± 0.38	1.63 (1.48–1.88)**	3.82
	Chlorpyrifos	2.60 ± 0.37	2.76 (2.58–3.12)	1.96 ± 0.46	0.71 (0.62–0.90)**	3.89
	Etofenprox	2.47 ± 0.42	7.60 (7.02–8.37)	2.51 ± 0.40	1.14 (0.97–1.32)**	6.67

[#] Insects developed from 3rd instar nymphs treated with 0.05% tween-80 (CK) or 5×10^5 conidia/mL NJBb2101 suspension (Section 2.3 in the Material and Methods).
[x] Insects were the offspring of adults from the 3rd instar nymphs treated with 0.05% Tween-80 (CK) or 5×10^5 conidia/mL NJBb2101 suspension (Sections 2.3 and 2.4 in the Material and Methods).
[δ] Data are presented as mean ± SEM from three independent replicates.
[ζ] Data are presented as mean (minimum-maximum value) from three independent replicates.
[ψ] LD_{50} values in the same row were analyzed using a *t* test in GraphPad Prism version 7.00 for Windows.
** Significant difference at level of $P < 0.01$ from *t* test.
[η] Susceptibility ratio = LD_{50} of control/LD_{50} of T1.

Wu et al. (2016) reported no significant sublethal effects of *B. bassiana* on the larval growth, adult longevity, oviposition, pupation or eclosion success of *Cyclocephala lurida*. The underlying mechanisms for sublethal effects have been speculated to relate to nutritional deficiency and insect humoral immunity (Torrado-León et al., 2006).

Female ratio was significantly reduced by about 20% in NJBb2101-treated *N. lugens* (Table 1), which is different from *Anopheles stephensi* in which the sex ratio was not changed after treatment with *B. bassiana* isolate IMI-391,510 (Chantal et al., 2014). The infection of female *N. lugens* with NJBb2101 also reduced copulation rate and fecundity (T2 in Table 2) significantly. We also noticed that hatchability of the T3 group (only male treated with NJBb2101) was far lower than any of the other three groups (Table 2), probably due to suboptimal sperm or unfertilized eggs. This observation suggests that NJBb2101 infection of males decreases the *N. lugens* population. Although the underlying mechanism is not clear yet, the unbalanced sex ratio and reduced reproduction observed suggest additional benefits beyond direct mortality following *B. bassiana* NJBb2101 application for the control of *N. lugens* population.

In addition to affecting *N. lugens* directly after inoculation, NJBb2101 also affected the offspring derived from the inoculated parents which suffered reduced fitness and fecundity (Table 4). Similar results have been reported in *Helicoverpa armigera* inoculated with *Metarhizium anisopliae s. l.* isolate M14 (Jarrahi and Safavi, 2016) and in *Bemisia tabaci* inoculated with *B. bassiana* isolate AE101M1 (Torrado-León et al., 2006). We speculate that this continuation of sublethal effects into the next generation is as a result of nutritionally deficient and less healthy eggs, although no direct evidence for this in the present study. Feng and Pu (2005) reported that sublethal doses of imidacloprid in formulated *B. bassiana* conidia enhanced fungal activity against *N. lugens*. The increased insecticide susceptibility of NJBb2101-inoculated *N. lugens* and their offspring (Table 5) that we observed confirm this and suggest that combinations of fungal insecticides and chemical pesticides could be a promising and feasible way to control *N. lugens*. However, the sublethal effects were diluted in the offspring generation as has been reported also by Torrado-León et al. (2006). The present results require more dedicated research on the mechanism of NJBb2101-induced sublethal effects and expanded trials on the feasibility and efficacy of combining NJB2101 and chemical insecticides.

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The authors declare no commercial or associative interest that represents a conflict of interest in connection with the work submitted.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.biocontrol.2018.02.007.

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