

Original Research Article

Potential of entomopathogenic fungi as biological control agent of whitefly (*Bemisia tabaci* biotype B) (Genn.) (Hemiptera: Aleyrodidae)

ABSTRACT

The efficiency of isolates from entomopathogenic fungus *Beauveria bassiana*, *Lecanicillium muscarium* and *Metarhizium rileyi* in the control of eggs and third-instar nymphs of whitefly, *Bemisia tabaci* biotype B, was evaluated by pathogenicity and virulence tests, under laboratory conditions. For the pathogenicity tests, five leaflets of bean (*Phaseolus vulgaris*) plants containing 20 nymphs or 20 eggs of *B. tabaci* biotype B were used. The leaflets were immersed in suspension containing a concentration of 10⁸ conidia/ml of each fungus isolate. Nymph mortality and egg viability were evaluated after seven days. The isolates that caused nymph mortality and egg viability above 80% were submitted to virulence tests, in order to estimate the lethal concentration (LC₅₀) of each isolate. All isolates were pathogenic to eggs and third-instar nymphs of *B. tabaci* Biotype B. JAB07 of *B. bassiana* and LCMAP3790 of *L. muscarium* isolates presented the best results, with nymphs mortality of 96.68 ± 2.25% and 97.74 ± 1.56%, respectively. However, JAB07 was the most virulent isolate, both for eggs and third-instar nymphs, with LC₅₀ estimate of 0.012 and 0.006 × 10³ conidia/ml, respectively. However, future field trials are required in order to analyze the real efficiency of this isolate in the control of eggs and nymphs of *B. tabaci* biotype B in field conditions.

Keywords: Microbial control, pathogenicity, virulence, *Beauveria bassiana*, JAB07

1. INTRODUCTION

The whitefly, *Bemisia tabaci* (Genn.) (Hemiptera: Aleyrodidae), is one of the most important agricultural pests worldwide, its species has more than 600 host plant species, such as soybean, bean, cotton, tomato, among others [1, 2]. *B. tabaci* biotype B is the most important strain of *B. tabaci*, and distributed throughout Brazil causing economic losses estimated at 2 billion dollars in large-scale crops annually [3]. The immature and adult phases cause direct damage to the plant, by suctioning. Moreover, the indirect damage, by transmission of more than 100 virus species [4, 5, 6].

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The control of *B. tabaci* biotype B is performed primarily with chemical insecticides, which has increased the selection of resistant populations of this pest worldwide, as well as problems related to human health and environmental contamination [7]. Thus, integrated programs management using alternative control that do not cause risks to natural enemies, are harmless to humans and environmentally friendly are of great importance.

The biological control of whitefly using entomopathogenic fungi has **been** increased worldwide [8]. These fungi have the unique ability to infect their hosts directly through the integument and have important role in the pest population regulation in the field. The entomopathogenic fungi that have potential for whitefly control include **as well** *Isaria fumosorena*, Wize (1904), *Beauveria bassiana* (Bals.-Criv.) Vuill. (1912), *Metarhizium anisopliae* (Metchnikoff) Sorokin (1883), *Lecanicillium* spp. and *Aschersonia* spp. [13, 9]

However, several studies have reported low control efficiency of **these this pests** [10, 11, 12]. Thus, research for highly virulent fungi isolates is essential for the success of strategies for the whitefly control, as well as the evaluation of different isolates of entomopathogenic fungi that are used for the control of other pest species, such as *Metarhizium rileyi* (Farlow), an important biological control agent of lepidopteran pests. The objective of this work was to evaluate, in laboratory conditions, the efficiency of *B. bassiana*, *L. muscarium* and *M. rileyi* isolates for the control of eggs and nymphs of *B. tabaci* biotype B.

Comment [a2]: I guess you refer to the whitefly?

Comment [a3]: Why don't you try on adults as well?

2. MATERIAL AND METHODS

B. tabaci biotype B population

The population of *B. tabaci* biotype B was obtained in cabbage plants (*Brassica oleracea* L. var. Acephala) in experimental field of the Faculty of Agrarian and Veterinary Sciences (UNESP-FCAV), Jaboticabal, SP. Species identification was performed in the Department of Entomology of the Agronomic Institute of Campinas (IAC), Campinas-SP and was identified as biotype B. Insects were reared in acclimatized room (25 ± 2 ° C, 70 ± 10% RH and 12/12 h of photophase) in bean plants (*Phaseolus vulgaris* L. cv. Pearl) [13].

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Entomopathogenic fungi isolates

The isolates of *B. bassiana* (IBCB18, IBCB35, IBCB66 and JAB07), *L. muscarium* (LCMAP3790) and *M. rileyi* (NOM1950) were obtained from the entomopathogen bank of the Laboratory of Microbial Control of Arthropods Pests (LCMAP). Isolates from *L. muscarium* and *M. rileyi* were maintained in Petri dishes, containing culture medium Sabouraud Dextrose Agar with Yeast Extract (SDAY). The isolates of *B. bassiana* were maintained in Sabouraud Dextrose Agar (SDA) culture medium, incubated in a BOD at 28 ± 1 ° C, 70 ± 10% RH and 12/12h of photophase for eight days. Subsequently, a suspension (10 ml of distilled water + 0.05 of Tween® and entomopathogenic fungus) was mixed, and two serial dilutions were performed to quantify the number of conidia/ml in Neubauer chamber, and standardization at the concentration of 108 conidia/ml.

Pathogenicity bioassays with *B. tabaci* nymphs

Bean plants (*P. vulgaris* L. cv. Pearl) **with 25 days after emergence** were transferred to a rearing cage **25 days after emergence**. *B. tabaci* biotype B adult insects were infested near to the plants, for 24 h. After this period, the adult insects were removed from the plants and transferred to another rearing cage for 15 days until the nymphs reached the third instar. Third instar nymphs were used because of their immobility and ease of manipulation [14].

Comment [a5]: After emergence? Are you talking about the insect or the plant?

Comment [a6]: After emergence? Are you talking about the insect or the plant?

Comment [a7]: Inoculated ?

After that, the leaflets were detached from the infested plants and the nymphs present **in on** the leaves were selected by marking with a marker pen (1 mm) next to the nymph [15]. Each treatment consisted of five leaflets of bean, each one containing 20 nymphs of third instar of *B. tabaci* Biotipe B. The leaflets were immersed in suspension containing 10 ml of distilled water + Tween® 20 (0.05%) + entomopathogenic fungus, at the concentration of 108 conidia/ml, for 1 min.

After complete drying, the leaflets were transferred individually to glass containers (10 ml) and the petioles immersed in agar-water solution (1.5%) to maintain leaf turgidity [16]. A control treatment was performed by immersing the bean leaflets in solution containing 10 ml of autoclaved water + Tween® 20 (0.05%). The containers containing the leaflets and nymphs of *B. tabaci* Biotipe B were transferred to a glass cage (65 × 30 × 45 cm), with the upper opening covered with "voil". The experiment was maintained in acclimatized room (25 ± 2 ° C, 70 ± 10% RH and 12/12 h of photophase). The mortality of the nymphs was characterized by the flaccidity and whitish coloration of the insect tegument.

Pathogenicity bioassays with *B. tabaci* eggs

For the pathogenicity bioassays of the fungal isolates to eggs of *B. tabaci* biotype B, the same procedure used in the pathogenicity tests with nymphs was performed. After the oviposition period (24 h), the adults were removed from the infested plants and leaflets detached from the bean plants. The eggs were selected by lateral markings, as previously described. Each treatment consisted of five leaflets of bean, containing 20 eggs of *B. tabaci* biotype B (<48 h after

oviposition). After marking, the leaflets were immersed in suspension containing 10 ml of distilled water + Tween® 20 (0.05%) + entomopathogenic fungus, in the concentration of 108 conidia/ml, for 1 min.

After drying, the bean leaflets containing the whitefly eggs were transferred individually to glass containers (10 ml) and the petioles immersed in agar-water solution (1.5%), in order to maintain the turgidity of the leaf [16]. Control treatment was performed immersing the leaflets in solution of 10 ml of distilled water + Tween® 20 (0.05%). The containers were maintained in acclimatized room ($25 \pm 2^\circ\text{C}$, $70 \pm 10\%$ RH and 12/12h of photophase). Seven days after immersion of the leaves in the solution containing entomopathogenic fungus the eggs viability was evaluated. The unviability of eggs was verified by the observation of necrotic eggs and eggs that did not hatched [17]. Both pathogenicity tests (nymphs and eggs) were performed in triplicate.

Virulence of the entomopathogenic fungi isolates

For the virulence tests, we considered only the isolates that caused mortality above 80% of the nymphs or eggs of *B. tabaci* biotype B. Lethal concentration (LC50) bioassays were conducted according to methodology described previously in the pathogenicity tests for *B. tabaci* biotype B nymphs and eggs. Six concentrations of each entomopathogenic fungus isolate was tested (103, 104, 105, 106, 107 and 108 conidia/ml), in order to estimate the dose-response of each isolate [18].

Statistical analysis

The results of nymphs mortality and eggs viability of *B. tabaci* biotype B [19]. The results expressed in percentages were used the arcsine square root transformation [20]. All the experiment was conducted in a completely randomized design, the results were submitted to ANOVA and the means were compared by the Tukey test ($P < 0.05$). The LC50 was estimated by Probit analysis ($P < 0.05$). All analysis were performed in SAS package, version 9.1[21].

3. RESULTS AND DISCUSSION

All isolates evaluated were pathogenic to third instar nymphs and eggs of *B. tabaci* biotype B (Fig. 1 and Fig. 2). The isolates JAB07 of *B. bassiana* and LCMAP3790 of *L. muscarium* presented the greatest mortality rates, with mortality of $96.68 \pm 2.25\%$ and $97.74 \pm 1.56\%$ to third instar nymphs of *B. tabaci* Biotype B, respectively, differing from isolates IBCB66 of *B. bassiana*, with mortality of $67.51 \pm 8.11\%$ ($F = 5.07$; $P < 0.0001$) (Fig. 1). The most of the isolates tested caused more than 80% of eggs unviability, excepted *B. bassiana* isolate IBCB66, that caused only $27.54 \pm 2.67\%$ ($F = 20.96$; $P < 0.0001$) (Fig 2).

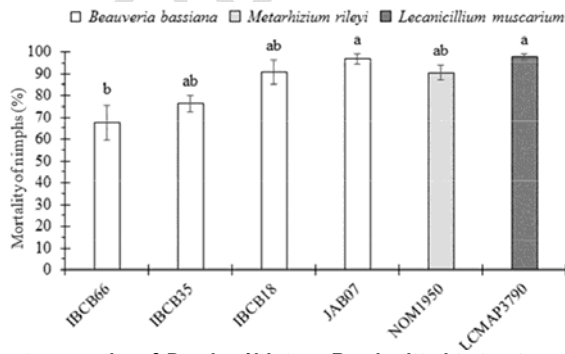


Figure 1. Mortality of third instar nymphs of *B. tabaci* biotype B submitted to treatments with entomopathogenic fungi isolates.

Columns followed by the same letter did not differ significantly from each other by the Tukey test ($P < 0.05$). The error bars represents the standard error of the mean (\pm SE).

Comment [a8]: How long does it take for an egg to hatch, when it is viable?

Comment [a9]: True, but not significantly different from 4 other treatments

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Comment [a10]: Correct «nymphs»: it takes a «y»

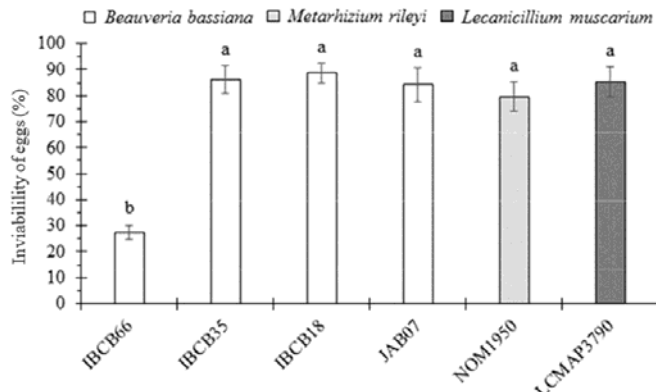


Figure 2. Unviability of eggs of *B. tabaci* biotype B submitted to treatments with entomopathogenic fungi isolates.

Columns followed by the same letter did not differ significantly from each other by the Tukey test ($P < 0.05$). The error bars represents the standard error of the mean (\pm SE).

For *B. tabaci* third instar nymphs, the LC₅₀ estimate varied between 0,006 and 0.217×10^3 conidia/ml, JAB07 isolate of *B. bassiana* was the most virulent among the isolate tested (Table 1). In relation to eggs viability results, JAB07 isolate also was the most virulent, with a LC₅₀ estimate of 0.012×10^3 conidia/ml (Table 2). The best results aiming the *B. tabaci* Biotype B control are related with *B. bassiana* isolates, with mortality rates above 70% [16, 2]. Several studies confirm the potential of *B. bassiana* for the control of *B. tabaci* Biotype B, that represented by the greater amount of products formulated worldwide for this pest [18, 22].

In relation to *L. muscarium* species, even though the results were expressive in the present study, many studies have demonstrated the low efficiency in whitefly control, with mortality varying between 20 and 60% [12, 2]. It is important to highlight that the genus *Lecanicillium* has been used worldwide as bioinsecticide for the control of *B. tabaci* Biotype B, although with unsatisfactory results [10, 11, 12].

Comment [a11]: Explain these numbers in the Material and Methods section. How do you read a LC₅₀ value?

Table 1. Lethal concentration (LC₅₀) of entomopathogenic fungi isolates applied in third instar nymphs of *B. tabaci* biotype B after seven days of evaluation.

Fungi	Isolate	n	LC ₅₀ ($\times 10^3$ con.ml ⁻¹)	CI 95% ^{a,d}	Slope \pm SE ^b	χ^2 ^c
<i>L. muscarium</i>	LCMAP3790	600	0.023	0.018 - 0.030	0.31 \pm 0.09	0.15
<i>B. bassiana</i>	IBCB18	600	0.084	0.062 - 0.115	0.23 \pm 0.05	49.81
<i>B. bassiana</i>	JAB 07	600	0.006	0.002 - 0.012	0.20 \pm 0.05	22.04
<i>M. rileyi</i>	NOM1950	600	0.217	0.098 - 0.428	0.14 \pm 0.02	3.41

^a Confidence interval with fiducial limit of 95% probability.

^b Angular coefficient \pm Standard error.

^c χ^2 = chi-square ($P < 0.05$).

^d 95% of probability confidence interval that does not overlap indicates statistically significates differences between LC₅₀ values.

Table 2. Lethal concentration (LC₅₀) of entomopathogenic fungi isolates applied in eggs of *B. tabaci* biotype B after seven days of evaluation.

Fungi	Isolate	n	LC ₅₀ ($\times 10^3$ con.ml ⁻¹)	CI 95% ^{a,d}	Slope \pm SE ^b	χ^2 ^c
<i>L. muscarium</i>	LCMAP3790	600	0.229	0.120 - 0.408	0.15 \pm 0.02	10.59
<i>B. bassiana</i>	IBCB18	600	0.244	0.110 - 0.484	0.14 \pm 0.02	1.78
<i>B. bassiana</i>	JAB 07	600	0.012	0.003 - 0.031	0.14 \pm 0.02	8.76

<i>M. riley</i>	NOM1950	600	0.223	0.099 - 0.446	0.14 ± 0.02	0.98
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^a Confidence interval with fiducial limit of 95% probability.

^b Angular coefficient ± Standard error.

^c χ^2 = Chi-square ($P < 0.05$).

^d 95% of probability confidence interval that does not overlap indicates statistically significant differences between LC_{50} values.

Most of the isolates of the entomopathogenic fungi tested caused high eggs unviability of *B. tabaci* Biotype B, especially *B. bassiana* JAB07 isolate, being the most virulent among the entomopathogenic fungi evaluated, with LC_{50} estimated of 0.012 and 0.006×10^3 conidia/ml for eggs and nymphs of *B. tabaci* Biotype B, respectively. However, only the IBCB66 isolate of *B. bassiana* did not present ovicidal activity [17, 23].

NOM1950 isolate of *M. rileyi* presented high mortality rate and activity ovicidal of *B. tabaci* biotype B. This entomopathogen is considered an important biological control agent against insect species of the Noctuidae family (Lepidoptera) [24, 25, 26, 27, 28]. However, this study evidenced high control efficiency of this entomopathogenic fungus against whitefly eggs and nymphs, demonstrating its potential as biological control agent of this important insect pest.

In conclusion, JAB07 of *B. bassiana* and LCMAP3790 isolates from *L. muscarium* presented the best results in control of *B. tabaci* Biotype B control. However, the isolate JAB07 was the most virulent against eggs and nymphs, therefore indicating its potential for further production of formulated products. Initial tests to select isolates of entomopathogenic fungi of high virulence are fundamental to increase the integrated programs management of this pest. However, future studies under field conditions are required, making it possible to analyze the real efficiency of these isolates, in addition to questions related to persistence and their compatibility with agrochemicals.

4. CONCLUSION

B. bassiana JAB07 was the most virulent for eggs and nymphs of *B. tabaci* biotype B, obtaining an LC_{50} estimated to 0.012 and 0.006×10^3 conidia/ml, respectively, and can be indicated for further field tests for the control of *B. tabaci* biotype B.

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