Original Research Article

Potential of entomopathogenic fungi as biological control agents 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 10^8 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 (LC_{50}) 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. Additionally, JAB07 was the most virulent isolate, both for eggs and third-instar nymphs, with LC_{50} estimated of 0.012 and 0.006 × 10^3 conidia/ml, respectively. We suggest, 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. The 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*, due to its high amount of host plants, high biotic potential and virus transmission capacity [3, 4]. *B. tabaci* biotype B is distributed throughout Brazil causing economic losses estimated at 2 billion dollars in large-scale crops annually [5]. The duration of the cycle life can varies according to the species, with temperature being one of the most determinant factors. *B. tabaci* life cycle lasts approximately 19 days at 32 °C, and can reach 73 days at 15 °C. Under favorable conditions, this pest can reach 11 up to 15 generations per year and each female being able to oviposit up to 300 eggs during its life cycle [6]. The immature and adult phases cause direct damage to the plant, by suctioning. Furthermore, this pest causes an indirect damage, due to the transmission of more than 100 species of plant viruses [3, 4, 7].

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 [8]. 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 increased worldwide [9]. 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 *Isaria fumosorea*, Wize (1904), *Beauveria bassiana* (Bals.-Criv.) Vuill. (1912), *Metarhizium anisopliae* (Metchnikoff) Sorokin (1883), *Lecanicillium* spp. and *Aschersonia* spp. [10, 11]

Indeed, isolates from *B. bassiana* and *Lecanicilium muscarium* has been used for control to whitefly with formulated sprays. Although, previously studies have reported low control efficiency of this pest [12, 13, 14]. In relation to *Metarhizium rileyi* (Farlow), this fungi species possessed high toxicity against lepidopterans larvae and their potential has been explored against this insect group [15]. However, for our knowledge, the activity of the *M. rileyi* against sucking pest, as whitefly is unknown. Searching 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. Thus, the objective of this work was to evaluate, in laboratory conditions, the efficiency of *B. bassiana*, *L. muscarium* and a new isolate from *M. rileyi* for the control of eggs and nymphs of *B. tabaci* biotype B.

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 São Paulo State University, School of Agricultural and Veterinarian Sciences (UNESP-FCAV), Jaboticabal, São Paulo, Brazil. Species identification was performed in the Department of Entomology of the Agronomic Institute of Campinas (IAC), Campinas, São Paulo, Brazil, and was identified as biotype B, based on the genetic characterization [16]. 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). Once these plants reached vegetative stage V3 (three leaves), they were infested with 60 adults of *B. tabaci* Biotype B in cages (50 cm x 50cm x 80 cm) for 24 hours to ensure oviposition and reduce the variability caused by the presence of different stages individuals. After 24 h, the adults were transferred and 7 days were waited to guarantee the egg stage and 15 days to guarantee the 3-instar stage. In this way, a biological cycle of the whitefly of 30 days is maintained and guarantees the next generation of the insect used during the study [17].

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), of the São Paulo State University, School of Agricultural and Veterinarian Sciences (UNESP-FCAV), Jaboticabal, Sao Paulo, Brazil. 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 distillated 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 10^8 conidia/ml.

Pathogenicity bioassays with *B. tabaci* nymphs

The bean plants (*P. vulgaris* L. cv. Pearl) in vegetative state V3, were placed in plastic cages (50 cm x 50 cm x 80 cm). Subsequently, the plants were infested with 60 adults of *B. tabaci* Biotype B for 24 h for oviposition. After this period, the adult insects were removed and the infested plants were placed in another plastic cage (50 cm x 50 cm x 80 cm) for 15 days, to guarantee whitefly third instar nymphs on the bean leaves. For the bioassays, third instar nymphs were used because their stylet remains immobile on the leaf and allows their manipulation [18].

After that, the leaflets were detached from the infested plants and the nymphs present on the leaves were selected by a marking close to the nymph [19]. Each treatment consisted five leaflets of bean, each one containing third instar 20 nymphs of *B. tabaci* Biotype B. The leaflets were immersed in suspension containing 10 ml of distillated water + Tween® 20 (0.05%) + entomopathogenic fungi, at the concentration of 10^8 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 a "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 distillated water + Tween® 20 (0.05%) + entomopathogenic fungus, in the concentration of 10^8 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 [20]. Control treatment was performed immersing the leaflets in solution of 10 m of distillated water + Tween® 20 (0.05%). The containers were maintained in acclimatized room (25 ± 2 °C, 70 ± 10% RH and 12/12h of photophase). Seven days after the immersion of the leaves in the solution with entomopathogenic fungi, egg mortality was evaluated. Mortality was confirmed by the necrotic eggs and fungus conidia observation, or by those eggs did not hatch 7 days after oviposition [21]. Both pathogenicity tests (nymphs and eggs) were performed in triplicate.

Virulence of the entomopathogenic fungi isolates

For virulence tests, the lethal concentration was estimated able to kill 50% of the population (LC_{50}) of the *B. tabaci* Biotype B of each isolate in order to evaluated the toxicity of the fungi isolates against *B. tabaci* biotype B and to estimate their dose-response. All the virulence tests were performed according to methodology described previously in the pathogenicity tests for *B. tabaci* biotype B nymphs and eggs. We considered only the isolates that caused mortality above 80% of the nymphs or eggs of *B. tabaci* biotype B. Six concentrations of each entomopathogenic fungus isolate was tested (10^3, 10^4, 10^5, 10^6, 10^7 and 10^8 conidia/ml) [22].

Statistical analysis

The results of the eggs and nymphs mortality of *B. tabaci* was previously corrected by the Abbott's formula [23]. The data were transformed used the arcsine square root transformation [24]. All the experiment were performed in a completely randomized design, the results were submitted to ANOVA test and the means were compared by the Tukey test ($P < 0.05$). The LC_{50} was estimated by Probit analysis ($P < 0.05$). All analysis were performed in SAS package, version 9.1[25].

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 ± 2.25% and 97.74 ± 1.56% to third instar nymphs of *B. tabaci* Biotype B, respectively, differentiating of the IBCB66 isolate of *B. bassiana* that cauded the lower mortality rate of 67.51 ± 8.11% ($F = 5.07$; $P < 0.0001$) (Fig. 1). Most of the isolates tested caused more than 80% of eggs unviability, except for *B. bassiana* isolate IBCB66, that caused only 27.54 ± 2.67% unviability ($F = 20.96$; $P < 0.0001$) (Fig 2).

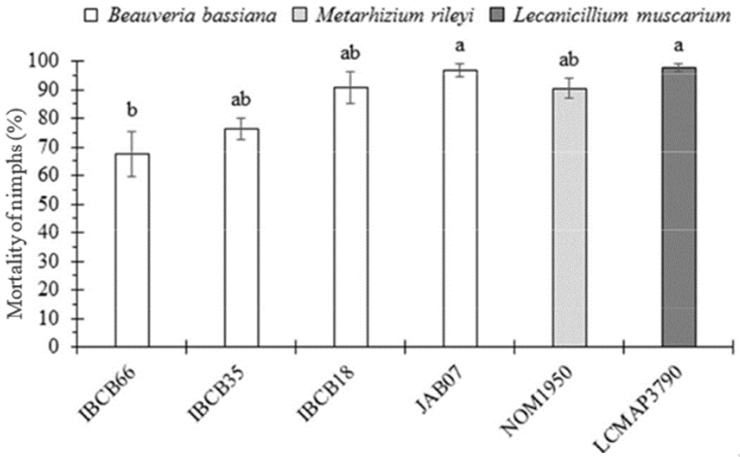

Figure 1. Mortality of third instar nymphs of *B. tabaci* biotype B submitted to treatments with entomopathogenic fungi isolates.

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 (± SE).

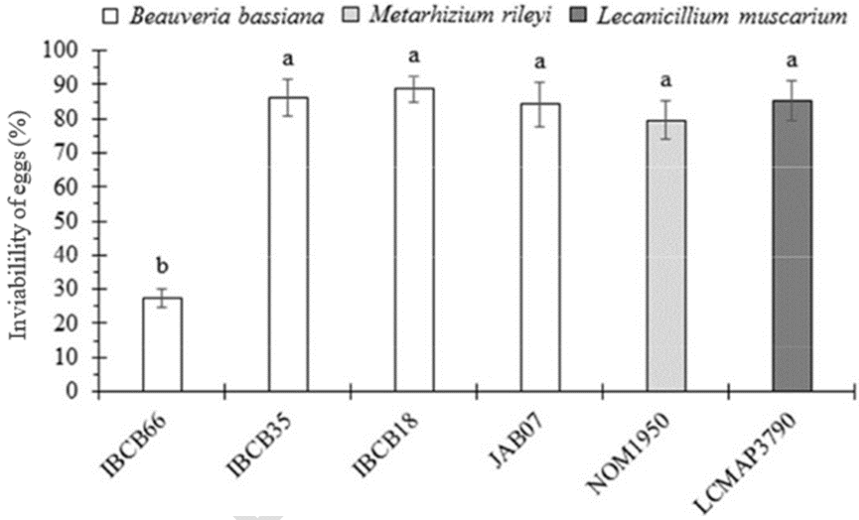

Figure 2. Unviability of eggs of *B. tabaci* biotype B submitted to treatments with entomopathogenic fungi isolates.

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 (± SE).

For *B. tabaci* third instar nymphs, the LC_{50} 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 LC_{50} 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 [22, 26].

In relation to *L. muscarium* species, even though the results were very promising, reaching mortalities above 80%, several studies have demonstrated the low efficiency in whitefly control, with mortality varying between 20 and 60% [14, 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 [12, 13, 14].

Table 1. Lethal concentration (LC_{50}) of entomopathogenic fungi isolates applied in third instar nymphs of *B. tabaci* biotype B after seven days of evaluation.

Fungi	Isolate	n	LC_{50} (x10^3 con.ml^{-1})	CI 95% [a d]	Slope ± SE [b]	χ^2 [c]
L. muscarium	LCMAP3790	600	0.023	0.018 - 0.030	0.31 ± 0.09	0.15
B. bassiana	IBCB18	600	0.084	0.062 - 0.115	0.23 ± 0.05	49.81

B. bassiana	JAB 07	600	0.006	0.002 - 0.012	0.20 ± 0.05	22.04
M. rileyi	NOM1950	600	0.217	0.098 - 0.428	0.14 ± 0.02	3.41

[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 significates differences between* LC_{50} *values.*

Table 2. Lethal concentration (LC_{50}) of entomopathogenic fungi isolates applied in eggs of *B. tabaci* biotype B after seven days of evaluation.

Fungi	Isolate	n	LC_{50} ($x10^3$ con.ml^{-1})	CI 95%[a d]	Slope ± SE [b]	χ^2 [c]
L. muscarium	LCMAP3790	600	0.229	0.120 - 0.408	0.15 ± 0.02	10.59
B. bassiana	IBCB18	600	0.244	0.110 - 0.484	0.14 ± 0.02	1.78
B. bassiana	JAB 07	600	0.012	0.003 - 0.031	0.14 ± 0.02	8.76
M. rileyi	NOM1950	600	0.223	0.099 - 0.446	0.14 ± 0.02	0.98

[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 significates 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 [21, 27].

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) [28, 29, 30, 31, 28]. Additionally, 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 for *B. tabaci* Biotype B control. Moreover, the isolate JAB07 was the most virulent against eggs and nymphs, 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 efficiency of the integrated program management of this pest. However, other biocontrol strategies should be considered for effective and more sustainable control of the whitefly. Furthermore, 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

The JAB07 and IBCB18 isolates of *B. bassiana* and LCMAP3790 isolate of *L. muscarium* were that caused higher mortality of nymphs and eggs of *B. tabaci* biotype B.

JAB07 isolate of *B. bassiana* was the most virulent for eggs and nymphs of *B. tabaci* biotype B, obtaining 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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