Metarhizium anisopliae and Beauveria bassiana (Hypocreales: Clavicipitaceae) are Compatible with Cotesia flavipes (Hymenoptera: Braconidae)

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Source: Florida Entomologist, 97(4) : 1794-1804

Published By: Florida Entomological Society

URL: https://doi.org/10.1653/024.097.0455
METARHIZIUM ANISOPLIAE AND BEAUVERIA BASSIANA (HYPOCREALES: CLAVICIPITACEAE) ARE COMPATIBLE WITH COTESIA FLAVIPES (HYMENOPTERA: BRACONIDAE)

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ABSTRACT

The aim of this study was to evaluate the effects of commercially available bioinsecticides based on Metarhizium anisopliae (Metschnikoff) Sorokin and Beauveria bassiana (Vuillemin, i.e., Biometha WP Plus® (M. anisopliae), Biovéria G® (B. bassiana), Boverril WP® (B. bassiana), Metarril WP® (M. anisopliae), and Metiê WP® (M. anisopliae)) on the pupae and adults of Cotesia flavipes (Cameron) (Hymenoptera: Braconidae) at concentrations of 1 × 10⁹, 5 × 10⁹, and 10 × 10⁹ conidia mL⁻¹. This braconid is released to control the sugarcane borer, Diatraea saccharalis. In the completely randomized first experiment with each commercial product, 10 C. flavipes female adults were held individually in disposable cups, which contained a 9-cm² sugarcane leaf that had been treated with the one of the entomopathogenic fungal products. The mortality of C. flavipes females was assessed at 24, 48, 72, 96, and 120 h after treatment. In the second experiment we assessed the emergence of adults from treated pupae, the capacity of these adults to parasitize Diatraea saccharalis caterpillars, numbers of progeny of these C. flavipes, longevity of C. flavipes males and females, total adults emerged, and the percent emergence and longevity of males and females of the F1 generation. The mortality levels of C. flavipes pupae and adults were not affected by the 2 Entomopathogenic fungi. Therefore the use of Beauveria bassiana and M. anisopliae to protect sugarcane is compatible with the use of C. flavipes to suppress D. saccharalis.

Key Words: biological control, entomopathogen, inter-specific interaction, larval parasitoid

RESUMO

O objetivo deste estudo foi avaliar o efeito de bioinseticidas comercialmente disponíveis com base em Metarhizium anisopliae (Metschnikoff) Sorokin e Beauveria bassiana (Balsamo) Vuillemin (Hypocreales: Clavicipitaceae) Biometha WP Plus® (M. anisopliae), Biovéria G® (B. bassiana), Boverril WP® (B. bassiana), Metarril WP® (M. anisopliae), e Metiê WP® (M. anisopliae) em pupas e adultos de Cotesia flavipes (Cameron) (Hymenoptera: Braconidae) nas concentrações de 1 × 10⁹, 5 × 10⁹ e 10 × 10⁹ con.mL⁻¹. No primeiro experimento, para cada produto comercial, dez fêmeas de C. flavipes foram individualizadas em copos descartáveis e sua superfície de contato dentro (9 cm² folha de cana de açúcar) foram tratados com o produto. O delineamento experimental foi inteiramente casualizado (DIC), com 16 tratamentos e cinco repetições, cada repetição, incluindo 10 fêmeas. A mortalidade das fêmeas de C. flavipes foi avaliada depois de 24, 48, 72, 96, e 120 horas após o tratamento com os produtos. No segundo experimento DIC, os bioinseticidas e as concentrações foram mantidas as mesmas que no primeiro experimento, com 16 tratamentos e 10 repetições, cada repetição usando um número de pupas com um potencial de emergência de 50 C. flavipes adultos. A emergência, o parasitismo, a progênie, a longevidade de machos e fêmeas, o total de adultos emergidos, e a longevidade de machos e fêmeas da geração filial (F1) deste parasitoide foram avaliados.
Brazil is the world’s largest sugarcane producer, and the major pests of this crop are *Diatraea saccharalis* (Fabricius) (Lepidoptera: Crambidae) and *Mahanarva fimbriolata* (Stål) (Hemiptera: Cercopidae) (Tiago et al. 2011; Tiago et al. 2012; Vacari et al. 2012; Simões et al. 2012). Caterpillars of *D. saccharalis* can cause direct losses in the cane stem by inducing biomass losses and the death of apical buds (White et al. 2008; Rossato et al. 2013). Chemical insecticides have low efficiency against this pest, because its third instar remains hidden inside the sugarcane stalk (Cruz et al. 2011; Rodrigues et al. 2013).

Microbial control agents such as entomopathogenic fungi can regulate insect populations through inundative and inoculative applications (Kurtti & Keyhani 2008; Mahdavi et al. 2013). These fungi can cause disease in up to 80% of the insects of a population, and they offer advantages of high genetic variability, infection at different development stages of the host, penetration through the integument, and high capacities of dispersal in the field (Destefano et al. 2004).

*Diatraea saccharalis* is susceptible to *Beauveria bassiana* (Balsamo) Vuillemin (Hypocreales: Clavicipitaceae) and *Metarhizium anisopliae* (Metchnikoff) Sorokin (Hypocreales: Clavicipitaceae), and contact with these entomopathogenic fungi can affect the biological characteristics of this insect (Williams et al. 2013). In Brazil, the entomopathogenic fungus *M. anisopliae* is produced on rice and then applied to the sugarcane crop to reduce populations of *M. fimbriolata* (Loureiro et al. 2005).

Entomopathogenic fungi and *Cotesia flavipes* (Cameron) (Hymenoptera: Braconidae) are used in sugarcane crops, and *M. anisopliae* may impact beneficial parasitoids and other non-target insects (Roy & Cottrell 2008) synergistically or antagonistically (Fuentes-Contreras & Niemeyer 2000; Stolz et al. 2002; Delpuech & Delahaye 2013). Thus host-parasitoid-entomopathogen interactions in agricultural systems can be harmful to populations of beneficial arthropods and ecological communities (Meyling et al. 2009; Meyling et al. 2011). Clearly, it is important to know the mortality patterns and interactions between fungi and natural enemies involved in integrated pest management programs (Santos Jr. et al. 2006).

In sugarcane fields in Brazil, *M. anisopliae* is applied to suppress *Mahanarva fimbriolata*, and *B. bassiana* is applied against termites, *M. anisopliae* and *B. bassiana* that are used for controlling pests in sugarcane fields.

**Materials and Methods**

The experiments were performed in the Laboratories of Entomology/Biological Control (LECOBIOLOG) of the Faculdade de Ciências Agrárias (FCA), Microbiology and Entomology of the Faculdade de Ciências Biológicas e Ambientais (FCBA) of Universidade Federal da Grande Dourados (UFGD), Dourados, Mato Grosso do Sul State, Brazil, as detailed below.

Obtaining Commercial Formulations Based on *Metarhizium anisopliae* and *Beauveria bassiana*

Commercial formulations used included Bio-metha WP Plus® (*M. anisopliae*), Biovéria G® (*B. bassiana*), Metarril WP® (*M. anisopliae*), Boverril WP® (*B. bassiana*), and Metiê WP® (*M. anisopliae*) provided by the companies Biotech Controle Biológico Ltda., Itaforte Bioprodutos, and Ballagro Agro Tecnologia, respectively. All commercial formulations showed over 95% viable spores.

Rearing of *Diatraea saccharalis*

Eggs of *D. saccharalis* were obtained by rearing this species in the LECOBIOL. Eggs were placed in glass jars (8.5-cm diam × 13-cm high) with artificial diet based on wheat germ, soybean, and the phagostimulant, sugarcane yeast (Saccharomyces cerevisiae Meyen ex E.C. Hansen; Saccharomyces: Saccaromycescaceae) to provide food for neonates and 2nd, and 3rd instars. Fourth instars were transferred to disposable Petri dishes (6.5-cm diam × 2.5-cm high) and fed the same diet until they reached the pupal stage. Pupae were selected depending on their morphological characteristics and each held individually in a plastic pot covered with a screen until they...
reached the adult stage. The adults were separated in groups of 20 males and 30 females per cage of polyvinyl chloride (PVC) tubes (10-cm diam × 22-cm high). These cages were closed with bond paper and elastic and internally lined with paper sheets to aid oviposition. Eggs of *D. saccharalis* were collected daily, washed with a solution of copper sulfate, and then stored in a climatic chamber at 25 ± 2 °C, 70 ± 10% RH, and 14:10 h L:D, i.e., methodology adapted from Parra (2007).

Rearing the Parasitoid *Cotesia flavipes*

Fourth instar *D. saccharalis* caterpillars were individually exposed to a mated 24-h-old *C. flavipes* female. After being parasitized, the fourth instars were placed in disposable Petri dishes (6.5-cm diam × 2.5-cm high) and provided with artificial diet. These disposable Petri dishes were placed at 25 ± 2 °C, 70 ± 10% RH, and 14:10 h L:D until *C. flavipes* pupae formed. Pupae were held individually in disposable cups with lids (100 mL) using a drop of honey to feed the adults at 25 ± 2 °C, 70 ± 10% RH, and 14:10 h L:D until emergence of parasitoids (Garcia et al. 2009).

Experiment I. Quantification of Mortalities of *C. flavipes* Female Adults Exposed to Various Doses of *M. anisopliae* and *B. bassiana*

The objective of Experiment I was to quantify the mortality of adult females of *C. flavipes* when exposed to various doses of *M. anisopliae* and *B. bassiana*. Newly emerged *C. flavipes* females were exposed to commercial formulations of *M. anisopliae* and *B. bassiana* at the concentrations of 1 × 10^5, 5 × 10^5, and 10 × 10^5 conidia mL^-1. These concentrations of *M. anisopliae* are recommended for the control of *M. ﬁmbriolata*. Doses of *B. bassiana* were identical. Ten *C. flavipes* females were enclosed per 100 mL disposable cup with a lid and with one droplet of honey inside. The surface of the sugarcane leaf was disinfected with a hypochlorite solution (0.02%). A disinfected sugarcane leaf (9 cm^2; 3-cm long × 3-cm wide) was introduced into each cup. The surface contacting the insect was treated with 1 mL of each standardized suspension of the biopesticide in a Neubauer® chamber (Alves & Leucona 1998). The contact surfaces were treated with the aid of a micropipette and placed on paper towels to dry (Cardoso et al. 2007). The experiment was developed in a completely randomized design (CRD) with 16 treatments and 10 replications, each replication including a mass of pupae with the potential for 50 *C. flavipes* adults to emerge. The data were subjected to analysis of variance (F test) and the means were compared by the Scott-Knott test at 5% probability.

Twenty females and 20 males of newly emerged *C. flavipes* were used per 1.5 mL Eppendorf® microtube, fed a droplet of honey, and capped with cotton wool to evaluate their longevity (days). The microtubes with insects were placed in a climatic chamber temperature at 25 ± 2 °C, 70 ± 10% RH, and 14:10 h L:D.

The percentage of parasitism (based on the number of parasitized larvae per treatment, discounting the natural mortality of the host) was evaluated with 5 *D. saccharalis* fourth-instar each exposed to five 24-h-old *C. flavipes* females. The experiment was performed with 10 replications, each replication included 5 parasitized caterpillars, totaling 50 caterpillars per treatment. Each caterpillar was parasitized by a female *C. flavipes* immediately when she found it. The caterpillars were placed in disposable Petri dishes (6-cm diam), fed with the above-mentioned artificial diet, and then transferred to an air conditioned room at 25 ± 2 °C, 70 ± 10% RH, and 14:10 h L:D.
The percentage of *C. flavipes* that emerged from parasitized larvae (filial generation - F1), the progeny (total individuals emerged), and the longevity (days) of this parasitoid were evaluated. The data were subjected to analysis of variance (F test) and the means were compared by Scott-Knott test at 5% probability.

**RESULTS**

Experiment I, Quantification of Mortalities of *C. flavipes* Female Adults Exposed to Various Doses of *M. anisopliae* and *B. bassiana*.

Cumulative mortality of *C. flavipes* exposed to *M. anisopliae* commercial products was similar to the control at 24 h of exposure (HAE) (Table 1). Mortality at 24, 48, and 72 HAE was zero with Metarril WP® at a concentration of 1 × 10⁻⁹ conidia mL⁻¹ (Table 1). Linear regression analysis showing mortality as a function of time was 80% at 96 HAE with Metarril WP® at 1 × 10⁻⁹ conidia mL⁻¹ (Fig. 1B). Metié WP® at 5 × 10⁻⁹ conidia mL⁻¹ and 10 × 10⁻⁹ conidia mL⁻¹ at 96 HAE caused cumulative mortalities of this parasitoid of 42% and 48%, respectively. Biovèria G® (10 × 10⁻⁹ conidia mL⁻¹) caused the least cumulative mortality (12%) after 24, 48, and 72 HAE (Fig. 1C). The cumulative mortalities of *C. flavipes* adults exposed to *M. anisopliae* products were similarly great among treatments after 96 and 120 h of the exposure to the fungi (Table 1).

Experiment II, Quantification of Mortalities and Other Effects When *C. flavipes* Pupae Were Exposed to Various Doses of *M. anisopliae* and *B. bassiana*.

Parental Generation. The number of *C. flavipes* progeny (males and females) that emerged was similar among treatments with both entomopathogens (Table 2). Longevities (days) of females and males that emerged from pupae treated with Biometha WP Plus® (10 × 10⁻⁹ conidia mL⁻¹), Metień WP® (5 × 10⁻⁹ conidia mL⁻¹) and Metarril WP® (1 × 10⁻⁹ conidia mL⁻¹) were not significantly different from the control. The levels of parasitism induced in *D. saccharalis* by *C. flavipes* females that had emerged from pupae treated with *B. bassiana*-based products did not differ significantly among treatments and the control with the exception of Boverril WP® at 1 × 10⁻⁹ conidia mL⁻¹ treatment (Table 2).

F1 Generation. The emergence of F1 adults was not significantly different from the control in the following *M. anisopliae* treatments: Biometha WP Plus® at 1 × 10⁻⁹ conidia mL⁻¹ and 5 × 10⁻⁹ conidia mL⁻¹, Metień WP® at 10 × 10⁻⁹ conidia mL⁻¹ and Metarril WP® at 1 × 10⁻⁹ conidia mL⁻¹. However in the remaining *M. anisopliae* treatments the emergence of F1 adults was significantly lower than in the control; and the lowest rate of emergence occurred with the Metarril WP® treatment at 5 × 10⁻⁹ conidia mL⁻¹ and 10 × 10⁻⁹ conidia mL⁻¹ (Fig. 2A).

The longevity of F1 *C. flavipes* female adults did not differ significantly from the control for the following *M. anisopliae* treatments: Biometha WP Plus® at 5 × 10⁻⁹ conidia mL⁻¹ and 10 × 10⁻⁹ conidia mL⁻¹ and Metier WP® at 1 × 10⁻⁹ conidia mL⁻¹, but female longevity was significantly reduced in all remaining *M. anisopliae* treatments (Table 3). The longevity of males did not differ significantly from that of the control except it was lower with Metień WP® 10 × 10⁻⁹ conidia mL⁻¹ (1.65 days) (Table 3).

The percent emergence of F1 adults from *D. saccharalis* larvae parasitized by *C. flavipes* females in treatments with *B. bassiana* was not significantly different from the control in the following treatments: Biovèria G® at 1 × 10⁻⁹ conidia mL⁻¹, Boverril WP® at 1 × 10⁻⁹ conidia mL⁻¹ and Boverril WP® at 10 × 10⁻⁹ conidia mL⁻¹. However the percent emergence of F1 adults was significantly lower than the control in the following treatments: Biovèria G® at 10 × 10⁻⁹ conidia mL⁻¹ and Boverril WP® at 10 × 10⁻⁹ conidia mL⁻¹ (Fig. 2B).

The longevity of F1 *C. flavipes* females was not significantly different from the control (3.20 days) for the treatments Biovèria G® at 1 × 10⁻⁹ conidia mL⁻¹ (3.05 days), Biovèria G® at 5 × 10⁻⁹ conidia mL⁻¹ (3.00 days) and Boverril WP® at 1 × 10⁻⁹ conidia mL⁻¹ (2.70 days). However the longevity of F1 females emerged was significantly shorter than the control for the following treatments: Biovèria G® at 10 × 10⁻⁹ conidia mL⁻¹ (2.00 days), Boverril WP® at 5 × 10⁻⁹ conidia mL⁻¹ (2.15 days) and Boverril WP® at 10 × 10⁻⁹ conidia mL⁻¹ (2.40 days). The longevity of F1 males was significantly different from the control only for the following 2 treatments: Biovèria G® at 10 × 10⁻⁹ conidia mL⁻¹ (1.90 days) and Boverril WP® at 5 × 10⁻⁹ conidia mL⁻¹ (1.80 days).

**DISCUSSION**

The very low of mortality of *C. flavipes* females at 24 and 48 HAE with the products *M. anisopliae* and *B. bassiana* is important because the life period of the adult of this parasitoid is approximate-
TABLE 1. PERCENT CUMULATIVE MORTALITY OF *COTESIA FLAVIPES* (HYMENOPTERA: BRACONIDAE) ADULTS AFTER CONTINUOUS EXPOSURE TO VARIOUS COMMERCIAL PRODUCTS BASED ON *METARHIZIUM ANISOPLIAE* (A) AND *BEAUVERIA BASSIANA* (B) IN THE LABORATORY ON SUGARCANE LEAVES FOR 24 TO 120 HOURS (HAE) AT 25 ± 2°C, 70 ± 10% RH AND 14:10 H.L.D.

| Treatments                                                                 | 24 HAE       | 48 HAE       | 72 HAE       | 96 HAE       | 120 HAE      | (n)  |
|----------------------------------------------------------------------------|--------------|--------------|--------------|--------------|--------------|------|
| *Metarhizium anisopliae* (A)                                               |              |              |              |              |              |      |
| Control (untreated)                                                        | 2.00 ± 2.00 a| 8.00 ± 3.00 c| 38.00 ± 2.29 b| 94.00 ± 2.25 a| 100.00 ± 0.00 a| 50   |
| Biometha WP Plus® (1 x 10⁹ con.mL⁻¹)                                      | 0.00 ± 0.00 a| 42.00 ± 2.70 b| 96.00 ± 1.25 a| 100.00 ± 0.00 a| 100.00 ± 0.00 a| 50   |
| Biometha WP Plus® (5 x 10⁹ con.mL⁻¹)                                      | 0.00 ± 0.00 a| 94.00 ± 4.00 a| 100.00 ± 0.00 a| 100.00 ± 0.00 a| 100.00 ± 0.00 a| 50   |
| Biometha WP Plus® (10 x 10⁹ con.mL⁻¹)                                     | 2.00 ± 2.00 a| 50.00 ± 2.76 b| 100.00 ± 0.00 a| 100.00 ± 0.00 a| 100.00 ± 0.00 a| 50   |
| *Beauveria bassiana* (B)                                                  |              |              |              |              |              |      |
| Control (untreated)                                                        | 2.00 ± 2.00 b| 8.00 ± 3.00 c| 38.00 ± 2.29 b| 94.00 ± 2.25 a| 100.00 ± 0.00 a| 50   |
| Biovéria G® (1 x 10⁹ con.mL⁻¹)                                            | 0.00 ± 0.00 b| 4.00 ± 1.44 c| 96.00 ± 1.50 a| 98.00 ± 2.00 a| 100.00 ± 0.00 a| 50   |
| Biovéria G® (5 x 10⁹ con.mL⁻¹)                                            | 0.00 ± 0.00 b| 2.00 ± 2.00 c| 60.00 ± 1.95 b| 96.00 ± 1.88 a| 100.00 ± 0.00 a| 50   |
| Biovéria G® (10 x 10⁹ con.mL⁻¹)                                           | 2.00 ± 2.00 a| 2.00 ± 2.00 c| 12.00 ± 1.84 a| 100.00 ± 0.00 a| 100.00 ± 0.00 a| 50   |
| Boverril WP® (1 x 10⁹ con.mL⁻¹)                                           | 0.00 ± 0.00 b| 56.00 ± 1.69 a| 100.00 ± 0.00 a| 100.00 ± 0.00 a| 100.00 ± 0.00 a| 50   |
| Boverril WP® (5 x 10⁹ con.mL⁻¹)                                           | 0.00 ± 0.00 b| 28.00 ± 1.95 b| 98.00 ± 2.00 a| 100.00 ± 0.00 a| 100.00 ± 0.00 a| 50   |
| Boverril WP® (10 x 10⁹ con.mL⁻¹)                                          | 8.00 ± 2.74 a| 28.00 ± 2.72 b| 96.00 ± 1.78 a| 100.00 ± 0.00 a| 100.00 ± 0.00 a| 50   |
| CV                                                                         | 131.23       | 84.22        | 26.83         | —            | —            |      |

Means followed by the same letter in the column do not differ by the Scott-Knott test at 5% probability; CV—Coefficient of variation; HAE—Hours after exposition; (n) — Number of individuals per treatment.
ly 24 h in the laboratory at 24 ± 2 °C (Simões et al. 2012). The similar selectivity of B. bassiana and M. anisopliae to the parasitoid C. flavipes should not be generalized, because this natural enemy was susceptible to other isolates of M. anisopliae and B. bassiana (Folegatti et al. 1990).

Differences in the cumulative mortality of C. flavipes with the commercial products based on B. bassiana and M. anisopliae after 72 HAE and increased mortality in the control (38%) suggests the compatibility of this parasitoid with these fungi, as reported for the selectivity of B. bassiana to immature Habrobracon hebetor (Say) (Hymenoptera: Braconidae) (Mahdavi et al. 2013). The strain IPA 139E of M. anisopliae did not reduce egg parasitism of D. saccharalis by Trichogramma galloi Zucchi (Hymenoptera: Trichogrammatidae), which demonstrates the safety of this entomopathogenic fungus to this parasitoid (Broglio-Micheletti et al. 2006). The shorter longevity of C. flavipes females with the bioinsecticides Metarril WP® by Trichogramma galloi Zucchi (Hymenoptera: Trichogrammatidae), which demonstrates the safety of this entomopathogenic fungus to this parasitoid (Broglio-Micheletti et al. 2006).

A similar longevity of C. flavipes females with the products based on M. anisopliae and B. bassiana corroborate the results with Oomyzus sokolowskii Kurdjumov (Hymenoptera: Eulophidae) and B. bassiana Esalq 447 and M. anisopliae E9 at the concentration of 10^7 conidia mL^-1. These entomopathogenic fungi did not reduce the longevity, but B. bassiana caused higher mortality of this parasitoid (21%) than did M. anisopliae (9%) (Santos et al. 2006). However, B. bassiana is rarely used in sugarcane crops, which can contribute for the efficiency of C. flavipes in controlling D. saccharalis larvae.

The high mortality of C. flavipes at 72 HAE in the control and the treatments with the entomopathogens may not reduce parasitism by C. flavipes, whose adults start to parasitize their hosts approximately 24 h after emergence (Simões et al. 2012). This suggests that the 2 methods of biological control may act synergistically in the management of D. saccharalis in sugarcane crops.

Experiment II

Parent Generation. The emergence of similar numbers of individuals and similar proportions of males and females per parasitized C. flavipes pupae treated either with M. anisopliae or B. bassiana showed that these bioinsecticides did not affect the development of immature parasitoid. This may be because they are in the pupal stage, which is resistant to penetration and infection by entomopathogens (Armitage & Siva-Jothy 2005; Lemaitre & Hoffmann 2007; Mahdavi et al. 2013).

The shorter longevity of C. flavipes females with the bioinsecticides Metarril WP® (M. aniso-
TABLE 2. PROGENY, NUMBER AND LONGEVITY OF FEMALES AND MALES IN THE PARENTAL GENERATION (G: P) OF *COTESIA FLAVIPES* THAT EMERGED FROM PUPAE TREATED WITH *METARHIZIUM ANISOPLIAE* (A) AND *BEAUVERIA BASSIANA* (B) AND THEIR CAPACITIES TO PARASITIZE *DIATRAEA SACCHARALIS* AT 25±2 °C, 70±10% RH AND 14:10 H:L.D.

| Treatments                  | Progeny | Females* | Males* | (n1) | L. Fem. (days) | L. Male (days) | (n2) | Parasit.% | (n3) |
|-----------------------------|---------|----------|--------|------|----------------|----------------|------|-----------|------|
| *Metarhizium anisopliae* (A) |         |          |        |      |                |                |      |           |      |
| Control (untreated)          | 82.00 ± 6.67 a | 46.40 ± 0.88 a | 28.70 ± 0.67 a | 10  | 2.25 ± 0.10 a  | 2.10 ± 0.10 a  | 40  | 94.00 ± 3.05 a | 50  |
| B. Plus® (1x10⁹ con.mL⁻¹)   | 63.70 ± 5.26 a | 28.20 ± 0.74 a | 35.50 ± 0.81 a | 10  | 1.65 ± 0.15 b  | 1.70 ± 0.14 b  | 40  | 80.00 ± 3.43 a | 50  |
| B. Plus® (5x10⁹ con.mL⁻¹)   | 52.80 ± 4.71 a | 29.40 ± 0.93 a | 23.40 ± 0.56 a | 10  | 1.90 ± 0.19 b  | 1.65 ± 0.18 b  | 40  | 88.00 ± 3.68 a | 50  |
| *Beauveria bassiana* (B)     |         |          |        |      |                |                |      |           |      |
| Control (untreated)          | 82.00 ± 6.67 a | 46.40 ± 0.88 a | 28.70 ± 0.67 a | 10  | 2.25 ± 0.10 a  | 2.10 ± 0.10 a  | 40  | 94.00 ± 3.05 a | 50  |
| B. Plus® (1x10⁹ con.mL⁻¹)   | 74.40 ± 3.05 a | 35.40 ± 0.95 a | 14.90 ± 0.64 a | 10  | 1.60 ± 0.10 b  | 1.75 ± 0.22 b  | 40  | 96.00 ± 3.01 a | 50  |
| B. Plus® (5x10⁹ con.mL⁻¹)   | 71.20 ± 3.19 a | 19.80 ± 0.83 a | 41.10 ± 0.67 a | 10  | 1.75 ± 0.16 b  | 1.85 ± 0.17 b  | 40  | 80.00 ± 5.88 a | 50  |
| B. Plus® (10x10⁹ con.mL⁻¹)  | 83.50 ± 3.86 a | 37.40 ± 0.98 a | 33.00 ± 0.78 a | 10  | 1.80 ± 0.18 b  | 1.45 ± 0.12 b  | 40  | 92.00 ± 4.42 a | 50  |
| *Metarril WP*® (1x10⁹ con.mL⁻¹) | 73.10 ± 5.38 a | 29.30 ± 1.04 a | 25.30 ± 0.85 a | 10  | 1.10 ± 0.05 c  | 1.15 ± 0.05 c  | 40  | 58.00 ± 5.57 b | 50  |
| *Metarril WP*® (5x10⁹ con.mL⁻¹) | 78.50 ± 4.26 a | 33.50 ± 0.83 a | 36.40 ± 0.69 a | 10  | 1.50 ± 0.10 b  | 1.60 ± 0.15 b  | 40  | 86.00 ± 5.33 a | 50  |
| *Metarril WP*® (10x10⁹ con.mL⁻¹) | 67.30 ± 3.27 a | 24.00 ± 1.02 a | 32.50 ± 0.58 a | 10  | 1.20 ± 0.14 c  | 1.05 ± 0.05 c  | 40  | 84.00 ± 5.68 a | 50  |

*Number that emerged from treated pupae.

*Biometha WP Plus®. CV- Coefficient of variation; Means followed by the same letter do not differ by the Scott-Knott test at 5% probability. n1- Mass number of pupae of *Cotesia flavipes* treated with bioinsecticides; n2- Number of adults used to assess the longevity of *Cotesia flavipes*. n3- Number of *Cotesia flavipes* adults and *Diatraea saccharalis* caterpillars used to evaluate parasitism; Control (untreated) (Test.); Biometha WP Plus® (B.Plus).
Fig. 2. Emergence (%) of adults in the F1 generation of Cotesia flavipes (Hymenoptera: Brachonidae) after exposure to the fungi Metarhizium anisopliae (A) and Beauveria bassiana (B) (Hypocreales: Clavicipitaceae) at 25 ± 2 °C, 70 ± 10% RH and 14:10 h L:D.
Control (untreated) & 3.20 ± 0.19 a & 3.00 ± 0.19 a & 40 \\
Biometha WP Plus® (1 x 10^6 con.mL^-1) & 2.55 ± 0.15 b & 2.40 ± 0.24 a & 40 \\
Biometha WP Plus® (5 x 10^6 con.mL^-1) & 3.40 ± 0.24 a & 2.30 ± 0.20 a & 40 \\
Biometha WP Plus® (10 x 10^6 con.mL^-1) & 2.75 ± 0.19 a & 2.25 ± 0.19 a & 40 \\
Metiê WP® (1 x 10^6 con.mL^-1) & 3.25 ± 0.21 a & 2.70 ± 0.21 a & 40 \\
Metiê WP® (5 x 10^6 con.mL^-1) & 2.20 ± 0.23 b & 2.45 ± 0.26 a & 40 \\
Metiê WP® (10 x 10^6 con.mL^-1) & 2.30 ± 0.16 b & 1.65 ± 0.15 b & 40 \\
Metarril WP® (1 x 10^6 con.mL^-1) & 2.45 ± 0.19 b & 2.25 ± 0.20 a & 40 \\
Metarril WP® (5 x 10^6 con.mL^-1) & 3.05 ± 0.25 a & 2.50 ± 0.17 a & 40 \\
Metarril WP® (10 x 10^6 con.mL^-1) & 2.25 ± 0.16 b & 2.10 ± 0.15 a & 40 \\
CV & 33.95 & 38.06 & -

Treatments & Beauveria bassiana (B) & (n) \\
Control (untreated) & 3.20 ± 0.19 a & 3.00 ± 0.19 a & 40 \\
Biovéria G® (1 x 10^6 con.mL^-1) & 3.05 ± 0.24 a & 2.30 ± 0.25 a & 40 \\
Biovéria G® (5 x 10^6 con.mL^-1) & 3.00 ± 0.25 a & 2.15 ± 0.16 a & 40 \\
Biovéria G® (10 x 10^6 con.mL^-1) & 2.00 ± 0.20 b & 1.90 ± 0.19 b & 40 \\
Boverril WP® (1 x 10^6 con.mL^-1) & 2.70 ± 0.14 a & 2.20 ± 0.26 a & 40 \\
Boverril WP® (5 x 10^6 con.mL^-1) & 2.15 ± 0.15 b & 1.80 ± 0.15 b & 40 \\
Boverril WP® (10 x 10^6 con.mL^-1) & 2.40 ± 0.15 b & 2.30 ± 0.14 a & 40 \\
CV & 33.95 & 38.06 & -

CV= coefficient of variation; Means followed by the same letter do not differ by the Scott-Knott test at 5% probability; n= number of adults to evaluate Cotesia flavesce longevity.

Cotesia flavesce longevity may not affect this parameter (Sagarra et al. 2000a, 2000b; Chichera et al. 2012). F1 generation. The lower emergence of C. flavesce adults in the F1 generation from D. saccharalis caterpillars after contact with M. anisopliae was also observed for Trichogramma gallo Zuchi with D. saccharalis eggs treated with isolated IPA 159E (M. anisopliae) (Broglio-Micheletti et al. 2006). However, the smallest percent emergence and longevity of C. flavesce males and females with B. bassiana differs from that reported for Trichogramma atopovirilia Oatman & Platner (Hymenoptera: Trichogrammatidae) (Polanczyk et al. 2010) exposed to M. anisopliae and B. bassiana. The infection by entomopathogenics fungi on more advanced stages of immaturity may not reduce parasitoid emergence (Mesquita & Lacey 2001; Rashki et al. 2009), as observed in the parental generation. However, the exposure to this fungus may compromise other biological
characteristics of C. flavipes females by producing offspring with lower parasitic capacity and development in D. saccharalis caterpillars.

Most formulations based on the entomopathogenic fungi B. bassiana and M. anisopliae reduced the longevity of C. flavipes males and females, but the percent emergence, number of progeny, and percent parasitism of D. saccharalis caterpillars were less affected. The contact with the entomopathogenic fungi with C. flavipes pupae did not affect parasitism by this parasitoid, and, thus, they are compatible.

CONCLUSION

The mortality of C. flavipes pupae and adults was not influenced by B. bassiana and M. anisopliae at the concentrations of 1 × 10⁹, 5 × 10⁹, and 10 × 10⁹ con.ML⁻¹. Thus B. bassiana and M. anisopliae, at the concentrations of 1 × 10⁹, 5 × 10⁹, and 10 × 10⁹ con.ML⁻¹, were compatible with the use of the parasitoid C. flavipes for biological control of sugarcane pests.

ACKNOWLEDGMENTS

To the Brazilian institutions “Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES)” for granting of the scholarship. To “Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq)” and “Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG).” Global Edico Services rewrote and edited this manuscript.

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