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Parasitism capacity of *Trichogramma pretiosum* Riley, 1879 and *Telenomus remus* Nixon, 1937 after ingestion of biological pesticides

Capacidade de parasitismo de *Trichogramma pretiosum* Riley, 1879 e *Telenomus remus* Nixon, 1937 após ingestão de pesticidas biológicos

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Highlights

Beauveria bassiana and *Trichoderma harzianum* reduced *Trichogramma pretiosum* longevity.
After ingestion, the tested entomopathogens are selective to *Trichogramma pretiosum*.
After ingestion, the tested entomopathogens are selective to *Telenomus remus.*

Abstract

Augmentative biological control (ABC) of insect pests is an environmentally sustainable alternative to synthetic insecticides. By performing BC, more than one control agent can be used for the same insect pest that is in different stages of its life cycle or for pests that simultaneously occur in the area. However, this relationship requires biosecurity for the control agents employed. The aim of this study was to evaluate the parasitism capacity of *Trichogramma pretiosum* Riley, 1879 and *Telenomus remus* Nixon, 1937 after ingestion of biological pesticides. The entomopathogens, Baculovirus *Anticarsia gemmatalis* nucleopolyhedrovirus (AgMNPV), *Bacillus thuringiensis* (Bt) var. *kurstaki*, Bt var. *aizawai*, *Beauveria bassiana*, and *Metarhizium anisopliae*; the microbiological fungicide, *Trichoderma harzianum*, at concentrations recommended by the manufacturer; and a negative control (pure honey) were employed in this study. Further, forced ingestion was adopted, with the treatments mixed in honey and offered as food at two dilutions (one-part product: one-part honey and one-part product: nine parts honey). Each treatment consisted of 20 individual females

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for each parasitoid (*T. pretiosum* or *T. remus*). The following parameters were evaluated: female longevity, number of parasitized eggs, egg viability, and number of females and males to determine the sex ratio. For *T. pretiosum, B. bassiana* (1×10^{13} viable conidia 100 L H_2O^{-1}), and *T. harzianum* (5×10^{12} viable conidia 100 L H_2O^{-1}), the longevity of their females was reduced by the 1:1 mixture; however, this mixture did not interfere with other parameters and other biological pesticides compared to the respective controls of both dilutions. The biological pesticides did not negatively influence the parameters evaluated for *T. remus.* The tested products had low toxicity to the egg parasitoids, *T. pretiosum* and *T. remus.* Overall, more work is still required with parasitoids in other stages of development and with other exposure methods to confirm the selectivity of products for egg parasitoids to recommend its combined use in the field.
Key words: Biological control. Egg parasitoids. Entomopathogens. Selectivity.

Resumo

O controle biológico aumentativo (CBA) de insetos-praga é uma alternativa ambientalmente sustentável em relação aos inseticidas sintéticos. Com a utilização do CBA, mais de um agente de controle pode ser utilizado para um mesmo inseto-praga que se encontra em diferentes estágios do seu ciclo de vida ou para pragas que ocorrem simultaneamente na área. No entanto, essa relação necessita de biossegurança para os agentes de controle envolvidos. O objetivo do trabalho foi avaliar a capacidade de parasitismo de *Trichogramma pretiosum* Riley, 1879 e *Telenomus remus* Nixon, 1937 após a ingestão de pesticidas biológicos. Os entomopatógenos Baculovírus *Anticarsia gemmatalis* nucleopoliedrovirus (AgMNPV), *Bacillus thuringiensis* var. *kurstaki, Bacillus thuringiensis* var. *aizawai, Beauveria bassiana, Metarhizium anisopliae,* o fungicida microbiológico *Trichoderma harzianum* nas concentrações recomendadas pelos fabricantes e um controle negativo (mel puro) foram utilizados nos bioensaios. A metodologia empregada foi de ingestão forçada, com os tratamentos misturados ao mel e oferecidos como alimento em duas diluições diferentes (1 parte do produto:1 parte de mel e 1 parte do produto:9 partes de mel). Cada tratamento constou de 20 fêmeas individualizadas de cada parasitoide (*T. pretiosum* ou *T. remus*). Os parâmetros avaliados foram: longevidade das fêmeas, número de ovos parasitados, viabilidade dos ovos e número de fêmeas e machos para determinação da razão sexual. Para *T. pretiosum, B. bassiana* (1×10^{13} conídios viáveis 100L H_2O^{-1}) e *T. harzianum* (5×10^{12} conídios viáveis 100L H_2O^{-1}) reduziram a longevidade das fêmeas na mistura 1:1, porém não interferiram nos demais parâmetros avaliados, bem como os demais produtos microbiológicos comerciais avaliados, quando comparados às respectivas testemunhas de ambas as diluições. Os pesticidas biológicos não influenciaram negativamente nos parâmetros avaliados de *T. remus.* Os produtos testados apresentam baixa toxicidade aos parasitoides de ovos *T. pretiosum* e *T. remus.* Ainda há necessidade de mais trabalhos com outros estágios de desenvolvimento dos parasitoides e outros métodos de exposição para confirmar a seletividade dos produtos aos parasitoides de ovos, para recomendar o uso conjunto destes no campo.
Palavras-chave: Controle biológico. Parasitoide de ovos. Entomopatógenos. Seletividade.

Introduction

Insect pest control is predominantly carried out with synthetic chemical insecticides (Goulson, 2013; Stacke et al., 2020), which are often used incorrectly or excessively, and may cause negative effects, such as contamination of the environment and animals, elimination of biological control agents and pollinators, selection of resistant populations, and resurgence of pest insects (Hladik et al., 2018; Koch et al., 2018; Khan & Ahmad, 2019; Boudh & Singh, 2019; DiBartolomeis et al., 2019; Lima et al., 2020; Abati et al., 2021). The adoption of integrated pest management (IPM) is a viable and sustainable alternative that proposes the joint use of several control strategies, including biological control (van Lenteren, 2012; van Lenteren et al., 2018; A. F. Bueno et al., 2021), to maintain the insect-pest population below the level of economic damage and cause the least possible damage to natural enemies and the environment (J. Parra, 2014; Bortolotto et al., 2015; R. C. de O. Bueno et al., 2017b).

Biological pest control agents are classified as entomopathogens and entomophages (Lacey et al., 2016; van Lenteren et al., 2018). Among the entomopathogens, fungi, viruses, bacteria, and nematodes, and among entomophages, parasitoids and predators (van Lenteren et al., 2018). The biological control agents, *Beauveria bassiana, Metarhizium anisopliae, Anticarsia baculovirus* (AgMNPV), and *Bacillus thuringiensis* (Bt); and the egg parasitoids, *Trichogramma* spp. (Hymenoptera: Trichogrammatidae), *Telenomus podisi* Ashmead, 1893, *Telenomus remus* Nixon, 1937, and *Trissolcus* spp. (Hymenoptera: Scelionidae), are well-known for their potential for control and/or use in the field (Aquino et al., 2018; Sosa-Gómez et al., 2020).

Although these control agents have proven efficiency when used alone, in augmentative biological control programs, the joint use of two or more natural enemies, such as entomopathogens and parasitoids, may be necessary to increase the control efficiency, either owing to the presence of different life stages of a pest insect or the simultaneous occurrence of more than one pest insect species in the field. In this context, the joint use of entomopathogens and parasitoids to control a particular pest or set of pests has important practical value for enhancing their management. However, the entomopathogens might negatively interfere in the parasitism capacity and efficiency of the egg parasitoids, decreasing their efficiency in the field (Goulson, 2013; A. de F. Bueno et al., 2017a).

Studies that verify the selectivity of entomopathogens to parasitoids mainly included contact bioassays performed on an inert surface or the spraying of products on the host's eggs in tests pre and post-parasitism (Potrich et al., 2015; Amaro et al., 2018); however, an evaluation of the ingestion of entomopathogens by parasitoids has not been extensively conducted. In the field, the application of entomopathogens can contaminate the nectar of plants, which adult parasitoids feed on; therefore, verifying the effect of ingesting these products in a laboratory setting is necessary. Thus, the objective of this study was to evaluate the parasitism capacity of *Trichogramma pretiosum* Riley, 1879 (Hymenoptera: Trichogrammatidae) and *T. remus* after ingestion of biological pesticides mixed with bee honey.

Material and Methods

Insects used in the bioassays

The parasitoids (*T. pretiosum* and *T. remus*) and their respective hosts were reared at Embrapa Soja, Londrina, Paraná, Brazil, under controlled conditions of temperature (25 ± 2 °C), relative humidity (70 ± 10%), and a 14/10 h photoperiod (light/dark). The rearing of *T. pretiosum* was carried out according to the methodology described by Stein and Parra (1987) using eggs of *Anagasta kuehniella* (Zeller, 1879) (Lepidoptera: Pyralidae), which is considered the most suitable alternative host for rearing under laboratory conditions (Gomes, 1997). This host was maintained on a diet based on whole wheat flour (97%) and brewer's yeast (3%), according to the methodology proposed by J. R. P. Parra et al. (1989).

The rearing of *T. remus* was performed as described by Pomari et al. (2012) using the postures of *Spodoptera frugiperda* Smith, 1797 (Lepidoptera: Noctuidae), aged up to 24 h. The eggs of *S. frugiperda* were obtained by rearing caterpillars fed an artificial diet developed by Greene et al. (1976), which comprised beans, brewer's yeast, casein, soy protein, and wheat in 200 mL plastic jars. The adults were fed a 10% honey solution placed in a plastic bottle with cotton.

The parasitoids (*T. pretiosum* and *T. remus*) were kept separately in cages (transparent plastic containers with a capacity of 2 L) with honey drops as feed for adults with their respective host eggs for parasitism. The eggs of *A. kuehniella* were sterilized by exposure to ultraviolet light for 30 min before being offered to *T. pretiosum*. For *T. remus,* the egg masses of *S. frugiperda* with a maximum of 24 h without sterilization were used. *Telenomus remus* is known to avoid parasitism in sterilized *Spodoptera* eggs (Queiroz et al., 2016). Furthermore, using sterilized eggs for *T. pretiosum* and non-sterilized eggs for *T. remus* would better mimic rearing and field scenarios, respectively, in which parasitism will occur. Eggs were glued onto white cardboard sheets using white nontoxic glue and later offered for parasitism for 24 h. The emerging parasitoids were used to perform bioassays or to maintain parasitoid colonies.

Bioassays

Four independent bioassays were carried out, two for each species of parasitoid, with two dilutions prepared using honey for subsequent feeding of the insects. One-part treatment was diluted with one-part honey (1:1) in the first bioassay for each parasitoid. Further, one-part treatment to nine-parts honey (1:9) was tested in a second bioassay for each parasitoid species. Pure honey was used as a control in the bioassays for both parasitoids. These experiments were conducted under controlled laboratory conditions (25 ± 2 °C, 70 ± 10% RH, photoperiod of 14/10 h L/D) with a total of eight treatments (Table 1).

Table 1
Commercial products, formulation, active ingredient, and concentration of products used to verify the parasitism of *Trichogramma pretiosum* and *Telenomus remus* after ingestion of entomopathogens

Commercial product	Formulation	Active ingredient (a.i.)	Concentration (a.i. 100L H_2O^{-1})
Pure honey	-	-	-
Baculovirus AEE®	0.6 WP	AgMNPV	$1.4x10^{11}$ PIB
Thuricide®	3.2 WP	*Bacillus thuringiensis* var. *kurstaki*	$9.6x10^{9}$ IU
Agree®	50 WP	*Bacillus thuringiensis3* var. *aizawai*	$5x10^{9}$ IU
Dipel®	3.2 WP	*Bacillus thuringiensis* var. *kurstaki*	$6.2x10^{9}$ IU
Boveril®	5 WP	*Beauveria bassiana*	$1x\times10^{13}$ viable conidia
Metarril®	5 WP	*Metarhizium anisopliae*	$1.6x10^{12}$ viable conidia
Trichodermil®	48 SC	*Trichoderma harzianum*	$5x10^{12}$ viable conidia

PIB - polyhedral inclusion bodies
IU - international units.

In each bioassay, the parasitism capacity of *T. pretiosum* and *T. remus* after ingestion of the biological pesticides was evaluated (Table 1). The bioassays were carried out via a completely randomized experimental design with 20 females (each repetition = one female) ≤ 24 h old and previously mated (*T. pretiosum*), and 20 females (each repetition = one female) ≤ 48 h old and previously mated (*T. remus*) via treatment in each bioassay, according to the methodology described below.

Parasitism capacity of T. pretiosum after ingestion of biological pesticides

For the study of parasitism capacity, females (≤ 24 h old) of *T. pretiosum* reared without previous experience of parasitism or feeding were individually placed in Duran-type tubes (1 cm in diameter × 6 cm in height) and cardboard cards (0.8 cm × 5 cm) containing approximately 100 eggs of *A. kuehniella.* As food, a droplet of the treatment under evaluation diluted with honey (1:1 and 1:9 in independent bioassays) was added to the tube wall, which was sealed with a plastic film. The eggs were available for parasitism for 24 h, and cards with new eggs were provided daily until the females died. The food containing the diluted suspension of each treatment was offered to females only in the first 24 h of the experiment; thereafter, only pure honey was provided, according to adapted methodology proposed by Xu et al. (2004) and Stanley & Preetha (2016), which best approximates a condition of contamination of nectaries in the field following spraying with the biological pesticides (containing the products) under evaluation.

Cardboard cards containing parasitized eggs were placed on each day of oviposition and stored in plastic bags (4 cm × 23 cm) in climatized rooms [25 ± 2 °C, RH 70 ± 10%, and photoperiod: 14/10 h (L/D)] until the emergence and death of adults. The evaluated

parameters included the longevity of the parental females that ingested the products, the number of total eggs parasitized by these females, the emergence (%) of adults, and the number of females and males to determine the sex ratio (F_1 generation).

Parasitism capacity of T. remus after ingestion of biological pesticides

For the study of parasitism capacity, females (≤ 48 h old) of *T. remus* reared without previous experience of parasitism or feeding were individually placed in Duran-type tubes (1 cm in diameter × 6 cm in height) and cardboard cards (0.8 cm × 5 cm) containing one *S. frugiperda* posture with approximately 100 eggs. The other procedures, treatments, repetitions, conditioning, and parameters evaluated were the same as those used in the bioassays with *T. pretiosum.*

Statistical analysis

The data were subjected to exploratory analysis to evaluate the assumptions of normality of residues (Shapiro & Wilk, 1965) and homogeneity of variance of treatments (Burr & Foster, 1972). For longevity and number of parasitized eggs, ANOVA was performed. Further, means were compared using the Tukey test (5% significance). To fit the ANOVA assumptions, a Box-Cox transformation was performed for the longevity parameter for both species of parasitoids in the two tested dilutions and for the parameter number of eggs parasitized only for *T. remus* at both dilutions. For the evaluated parameters of offspring (F_1 generation), adult emergence and sex ratio were evaluated using the non-parametric Kruskal-Wallis test. Thereafter, the means were compared using Dunn's post-hoc test (5% significance).

Results and Discussion

Based on a comparison with females from the control treatment, Beauveria bassiana (1×10^{13} viable conidia 100 L H_2O^{-1}) and *T. harzianum* (5×10^{12} viable conidia 100 L H_2O^{-1}) were the only biological commercial products that reduced the longevity of the parental females of *T. pretiosum* after ingestion of contaminated honey at the 1:1 dilution (treatment/honey) (Table 2). The ingestion of conidia of *B. bassiana,* which may have adhered to the mouthparts of *T. pretiosum,* can cause infection and consequently reduce female longevity. Furthermore, after penetration and infection of the insect's body, *B. bassiana* can produce toxins, which are harmful to insects in general (Lacey et al., 2015; Mannino et al., 2019; Sosa-Gómez & D'Alessandro, 2019). In *T. harzianum,* the fungi were verified to produce substances and enzymes (proteases and chitinases) similar to those produced by entomopathogens. The combination of enzymatic degradation caused by enzymes and mechanical pressure by haustorium production can also cause *T. harzianum* entomopathogen, which may have reduced the longevity of *T. pretiosum* (Shakeri & Foster, 2007).

Table 2
Effect of ingesting Baculovirus *Anticarsia gemmatalis* nucleopolyhedrovirus (AgMNPV), *Bacillus thuringiensis* (Bt), *Beauveria bassiana, Metarhizium anisopliae,* and *Trichoderma harzianum* at a dilution of 1:1 (1-part treatment to 1-part honey) on the biological parameters of *Trichogramma pretiosum*

Treatment (Active ingredient 100L^{-1} H_2O)		Adults		F1 Generation	
		Longevity (days)[1,2]	N° of parasitized eggs[1]	Emergence (%)[3]	Sex ratio[3]
Control: Pure honey		6.50 ± 1.34 a	75.05 ± 5.76^{ns}	88.42 ± 3.2^{ns}	0.75 ± 0.28 ab
AgMNPV 1.4x10^{11} PIB		4.90 ± 1.80 a	78.7 ± 6.16	91.51 ± 2.64	0.62 ± 0.26 b
Bt var. *kurstaki* 9.6x10^{9} IU		5.55 ± 2.34 a	72.2 ± 5.22	91.19 ± 1.96	0.64 ± 0.28 ab
Bt var. *aizawai* 6.2x10^{9} IU		5.95 ± 2.36 a	63.4 ± 5.07	92.78 ± 2.06	0.62 ± 0.31 ab
Bt var. *kurstaki* 6.2x10^{9} IU		4.45 ± 1.74 a	78.65 ± 5.45	92.39 ± 2.10	0.71 ± 0.31 ab
B. bassiana 1x10^{13} conidia		3.00 ± 1.20 b	55.35 ± 5.56	90.11 ± 2.22	0.73 ± 0.33 ab
M. anisopliae 1.6x10^{12} conidia		4.25 ± 2.03 a	85.15 ± 6.47	92.43 ± 2.11	0.69 ± 0.30 ab
T. harzianum 5x10^{12} conidia		3.00 ± 1.37 b	64.16 ± 5.76	91.45 ± 2.44	0.81 ± 0.39 a
Statistic[4]	F or H	F = 2.00	F = 1.55	H = 8.36	H = 19.6
	p	0.029	0.152	0.399	0.011
	DF	171	171	8	8

[1]Means ± SEM followed by the same letter do not differ by Tukey test (5% significance)
[2]Original means followed by statistical analysis performed using Box-Cox transformation
[3]Means ± SEM followed by the same letter do not differ by Dunn's test (5% significance)
[4]F value for ANOVA and H for the Kruskal-Wallis test
^{ns}ANOVA not significant for the number of parasitized eggs and Kruskal-Wallis not significant for emergence (p>0.05)
PIB: polyhedral inclusion bodies
IU: international unit
DF: degrees of freedom.

Despite the reduction in the longevity of the parasitoid after ingestion of *B. bassiana* (1 × 1013 viable conidia 100 L H_2O^{-1}) and *T. harzianum* (5 × 10^{12} viable conidia 100 L H_2O^{-1}) within 1.2 days relative to females from the control treatment, no significant reduction was observed in the number of eggs parasitized by these females throughout their adult life. Further, there was no reduction in the emergence of adults from these parasitized eggs (Table 2). This result can be explained by *T. pretiosum* concentrating its parasitism in the first days of adult life, with the highest peak of parasitism occurring in the first 24 h after its emergence (R. C. O. de F. Bueno et al., 2012). *Trichogramma pretiosum* can store a full complement of mature eggs in the ovaries and complete oogenesis before or soon after adult emergence (pro-ovigenic parasitoids) (Mills & Kuhlmann, 2000). As adult *T. pretiosum* emerge ready to oviposite, the reduction in the longevity of *T. pretiosum* females was not sufficient to affect the period of greatest parasitism in adults soon after emergence.

The ingestion of *T. harzianum* (5 × 10^{12} viable conidia 100 L H_2O^{-1}) at a 1:1 dilution (treatment/honey) resulted in a higher sex ratio for the emerged *T. pretiosum* compared to AgMNPV (1.4x10^{11} PIB 100 L H_2O^{-1}). However, none of the evaluated entomopathogens altered the sex ratio relative to the progenies from the control treatment, with values above

0.62 in all evaluated treatments (Table 2), which can be considered adequate for the greater efficiency of the natural enemy due to the greater presence of females (Hardy, 1994).

When the biological pesticides were diluted in nine parts honey, none of products negatively affected *T. pretiosum* according to direct effects on adults and indirect effects on offspring (F_1 generation) compared to females administered the control treatments. The number of eggs parasitized by females that ingested *T. harzianum* (5 × 10^{12} viable conidia 100 L H_2O^{-1}) differed significantly from the parasitism of females that ingested Bt var. *kurstaki* (6.2 × 10^9 IU) and *M. anisopliae* (1.6 × 10^{12} conidia). However, the longevity of females that ingested the biological pesticides, the number of eggs parasitized by them, and the emergence (%) of adults of the F1 generation and their sex ratio did not differ from the evaluated parameters for insects from the control treatment (Table 3).

Table 3
Effect of ingesting Baculovirus *Anticarsia gemmatalis* nucleopolyhedrovirus (AgMNPV), *Bacillus thuringiensis* (Bt), *Beauveria bassiana, Metarhizium anisopliae,* and *Trichoderma harzianum* at a dilution of 1:9 (1-part treatment to 9-parts honey) on the biological parameters of *Trichogramma pretiosum*

Treatment (Active ingredient 100L^{-1} H_20)		Adults		F1 Generation	
		Longevity (days)[1,2]	N° of parasitized eggs[1]	Emergence (%)[3]	Sex ratio[3]
Control: Pure honey		4.20 ± 1.34 b	75.05 ± 5.77 ab	88.42 ± 3.21 b	0.75 ± 0.38[ns]
AgMNPV 1.4x10^{11} PIB		9.35 ± 2.73 a	96.85 ± 6.44 ab	96.41 ± 4.52 a	0.66 ± 0.33
Bt var. *kurstaki* 9.6x10^9 IU		8.65 ± 2.55 a	95.40 ± 7.10 ab	89.36 ± 2.32 b	0.64 ± 0.47
Bt var. *aizawai* 6.2x10^9 IU		4.55 ± 1.79 b	95.95 ± 7.02 ab	94.58 ± 2.99 ab	0.70 ± 0.43
Bt var. *kurstaki* 6.2x10^9 IU		4.70 ± 1.72 b	72.45 ± 5.38 b	93.99 ± 2.08 ab	0.66 ± 0.43
B. bassiana 1x10^{13} conidia		5.70 ± 1.89 ab	79.10 ± 5.11 ab	91.15 ± 2.16 ab	0.68 ± 0.37
M. anisopliae 1.6x10^{12} conidia		5.95 ± 2.65 ab	69.9 ± 6.55 b	93.59 ± 2.11 ab	0.68 ± 0.42
T. harzianum 5x10^{12} conidia		8.10 ± 2.65 a	104.95 ± 6.25 a	90.92 ± 2.22 ab	0.61 ± 0.41
Statistic	F or H[4]	F = 4.46	F = 2.34	H = 21.04	H = 12.75
	p	<0.001	0.02	0.01	0.12
	DF	171	171	8	8

[1]Means ± SEM followed by the same letter do not differ by Tukey test (5% significance)
[2]Original means followed by statistical analysis performed using Box-Cox transformation
[3]Means ± SEM followed by the same letter do not differ by Dunn's test (5% significance)
[4]F value for ANOVA and H for the Kruskal-Wallis test
[ns]Kruskal-Wallis not significant (p>0.05)
PIB: polyhedral inclusion bodies
IU: international unit
DF: degrees of freedom.

For *T. remus,* none of the studied biological pesticides negatively affected the biological parameters evaluated after ingestion of contaminated honey, even at a 1:9 dilution (treatment: honey) (Tables 4 and 5). As reported in the literature and observed in this study, in general, the use of biological products, such as those formulated with

fungi, bacteria, and viruses, presents a lower risk for parasitoids than synthetic insecticides (Amaro et al., 2018; Torres & Bueno, 2018) and is therefore compatible for combined use with IPM strategies. Viruses and bacteria used as biological insecticides are quite specific and thus usually more selective toward natural enemies.

AgMNPV is specific for the control of *Anticarsia gemmatalis* Hübner, 1818 (Lepidoptera: Erebidae) and does not affect other insect species (Song et al., 2016). Similarly, commercial products based on *B. thuringiensis* contain the proteins, Cry1 and Cry2, which are specific for the control of insects of the order Lepidoptera (Pinto & Fiuza, 2003). Despite acting only by ingestion, entomopathogenic bacteria require adequate pH of the midgut of insects for the activation of toxins and specific receptors to enable binding of the activated toxins (Takada et al., 2001; Valicente & Tuelher, 2009). In the literature, there is no information about midgut pH or the receptors present in the midgut of parasitoids. Even in other insect orders, AgMNPV and *B. thuringiensis* controls, the impact of products, whether biological or chemical, must be verified to guarantee the security of natural enemies and enable the use of more than one efficient strategy in the field.

Table 4
Effect of ingesting Baculovirus *Anticarsia gemmatalis* nucleopolyhedrovirus (AgMNPV), *Bacillus thuringiensis* (Bt), *Beauveria bassiana, Metarhizium anisopliae,* and *Trichoderma harzianum* at a dilution of 1:1 (1-part treatment to 1-part honey) on the biological parameters of *Telenomus remus*

Treatment (Active ingredient $100L^{-1}$ H_2O)		Adults		F_1 Generation	
		Longevity (days)[1,2]	N° of parasitized eggs[1]	Emergence (%)[3]	Sex ratio[3]
Control: Pure honey		10.55 ± 2.09[ns]	57.55 ± 5.17[ns]	94.16 ± 1.87[ns]	0.69 ± 0.43[ns]
AgMNPV $1.4x10^{11}$ PIB		10.10 ± 2.44	63.95 ± 5.69	94.33 ± 2.08	0.70 ± 0.34
Bt var. *kurstaki* $9.6x10^{9}$ IU		7.55 ± 2.19	43.40 ± 4.91	91.64 ± 1.92	0.72 ± 0.30
Bt var. *aizawai* $6.2x10^{9}$ IU		8.80 ± 2.09	61.05 ± 4.72	92.83 ± 2.16	0.71 ± 0.33
Bt var. *kurstaki* $6.2x10^{9}$ IU		9.85 ± 2.01	55.85 ± 4.69	93.68 ± 2.02	0.75 ± 0.32
B. bassiana $1x10^{13}$ conidia		7.15 ± 1.78	51.05 ± 5.01	93.40 ± 1.97	0.70 ± 0.38
M. anisopliae $1.6x10^{12}$ conidia		10.10 ± 2.39	41.45 ± 4.75	95.42 ± 1.53	0.76 ± 0.26
T. harzianum $5x10^{12}$ conidia		8.25 ± 1.92	54.25 ± 4.38	94.72 ± 1.87	0.74 ± 0.25
Statistic	F or H[4]	F = 4.46	F = 2.34	H = 21.04	H = 12.75
	p	<0.001	0.02	0.01	0.12
	DF	171	171	8	8

[1]Means ± SEM followed by the same letter do not differ by Tukey test (5% significance)
[2]Original means followed by statistical analysis performed using Box-Cox transformation
[3]Means ± SEM followed by the same letter do not differ by Dunn's test (5% significance)
[4] F value for ANOVA and H for the Kruskal-Wallis test
[ns]ANOVA not significant for longevity and number of parasitized eggs, and Kruskal-Wallis not significant for emergence and sex ratio ($p>0.05$)
PIB: polyhedral inclusion bodies
IU: international unit
DF: degrees of freedom.

Table 5
Effect of ingesting Baculovirus *Anticarsia gemmatalis* nucleopolyhedrovirus (AgMNPV), *Bacillus thuringiensis* (Bt), *Beauveria bassiana, Metarhizium anisopliae,* and *Trichoderma harzianum* at a dilution of 1:9 (1-part treatment to 9-parts honey) on the biological parameters of *Telenomus remus*

Treatment (Active ingredient 100L^{-1} H_2O)		Adults		F_1 Generation	
		Longevity (days)[1,2]	N° of parasitized eggs[1]	Emergence (%)[3]	Sex ratio[3]
Control: Pure honey		10.55 ± 2.08^{ns}	57.55 ± 5.16 ab	96.81 ± 3.73^{ns}	0.73 ± 0.28^{ns}
AgMNPV 1.4x10^{11} PIB		7.5 ± 1.98	44.2 ± 4.47 ab	95.26 ± 1.66	0.75 ± 0.26
Bt var. *kurstaki* 9.6x10^{9} IU		8.55 ± 2.03	55.7 ± 5.32 ab	93.87 ± 1.97	0.72 ± 0.28
Bt var. *aizawai* 6.2x10^{9} IU		11.1 ± 2.27	62.75 ± 4.00 ab	94.58 ± 1.64	0.70 ± 0.31
Bt var. *kurstaki* 6.2x10^{9} IU		10.95 ± 2.18	53.4 ± 5.26 ab	94.47 ± 1.82	0.71 ± 0.31
B. bassiana 1x10^{13} conidia		8.65 ± 2.15	48.1 ± 4.24 b	95.26 ± 1.81	0.66 ± 0.33
M. anisopliae 1.6x10^{12} conidia		10.7 ± 2.46	75.75 ± 4.93 a	94.22 ± 1.69	0.71 ± 0.30
T. harzianum 5x10^{12} conidia		9.85 ± 2.30	52.6 ± 5.27 ab	91.47 ± 3.49	0.67 ± 0.39
Statistic	F or H[4]	F = 19.66	F = 12.38	H = 8.17	H = 9.50
	p	0.07	<0.001	0.52	0.39
	DF	190	190	9	9

[1]Means ± SEM followed by the same letter do not differ by Tukey test (5% significance)
[2]Original means followed by statistical analysis performed using Box-Cox transformation
[3]Means ± SEM followed by the same letter do not differ by Dunn's test (5% significance)
[4]F value for ANOVA and H for the Kruskal-Wallis test
^{ns}ANOVA not significant longevity and Kruskal-Wallis not significant for emergence and sex ratio
PIB: polyhedral inclusion bodies
IU: international unit
DF: degrees of freedom.

Similar results of the selectivity of AgMNPV and *B. thuringiensis* for *T. remus* in different exposure methodologies and the two life stages of the insect (pupa and adult) have been reported (Silva et al., 2016). The selectivity of *B. thuringiensis* for *T. pretiosum* has been reported in studies that evaluated different commercial products, methodologies, and stages of development of the insect. The entomopathogen was identified to be selective to the parasitoid, as it does not affect the number of parasitized eggs, percentage of emergence, longevity of parental females, sexual reason, and does not interfere with the location of the host's eggs (Carvalho et al., 2006; Vianna et al., 2009; Amaro et al., 2015; Silva & Bueno, 2015; Feltrin-Campos et al., 2018; Nascimento et al., 2018). To our knowledge, this is the first study to report the selectivity of these biological pesticides after evaluating their ingestion at different dilutions.

Importantly, among the entomopathogens evaluated, fungi tend to be less specific than viruses and bacteria. The main mode of action of these biological control agents is contact and they can penetrate the insect body through the

integument and intersegmental membranes of the abdomen, tarsi, and mouthparts (Alves, 1998; Qu & Wang, 2018; Mannino et al., 2019). Therefore, antagonistic interactions between formulations of fungal pathogens and natural enemies have been reported in the literature (Torres & Bueno, 2018), demonstrating the importance of testing these interactions.

The selectivity of *B. bassiana* strain Unioeste 1 and *M. anisopliae* strain Unioeste 22 was evaluated against the egg parasitoid, *T. pretiosum,* by spraying pre- and post-parasitism entomopathogens. *Metarhizium anisopliae* was found to reduce the emergence of *T. pretiosum* and cause insect mortality, while *B. bassiana* did not interfere with the biological parameters evaluated (Potrich et al., 2009). Likewise, Potrich et al. (2015) found that *B. bassiana* strain Unioeste 57 interfered in the emergence of *T. pretiosum,* duration of the egg-adult period, and longevity of females, but did not reduce the number of parasitized eggs and sex ratio when sprayed on the parasitoid eggs.

Different results were reported by Araujo et al. (2020), who evaluated a commercial product based on *M. anisopliae* strain IBCB 348 for *T. pretiosum* by spraying pre- and post-parasitism. These researchers verified that the entomopathogenic fungus does not interfere with the biological parameters of the parasitoid. The difference in the results can be explained by the exposure methods used, such as contact versus ingestion, and the use of different entomopathogenic fungal isolates, which may have differences in pathogenicity and virulence to insects (Mannino et al., 2019). Therefore, to assess the selectivity of a product to natural enemies, it is important to consider a variety of aspects using a well-established and complete methodology that assesses the differences related to possible contamination routes of the natural enemy with the product being evaluated (Hassan et al., 2000; A. de F. Bueno et al., 2017a).

The impact of an insecticide (chemical or biological) on non-target insects not only includes lethal effects, but also sublethal effects, such as possible reductions in the fecundity of natural enemies, which can impact their ability to control the target pest in the field (Stark & Banks, 2003; Desneux et al., 2007). The trans-ovarian activity of some insecticides has been reported in the scientific literature when adults of natural enemies ingest products used in applications (A. de F. Bueno et al., 2017a). This harmful action was not observed for the biological pesticides evaluated in this trial, confirming their low toxicity to *T. pretiosum* and *T. remus* after ingestion at different dilutions.

Conclusion

The biological pesticides used in the bioassays (AgMNPV 1.4 × 10^{11} PIB, *B. thuringiensis* var. *kurstaki* 9.6 × 10^{9} IU, *B. thuringiensis* var. *aizawai* 6.2 × 10^{9} IU, *B. thuringiensis* var. *kurstaki* 6.2 × ×10^{9} IU, *B. bassiana* 1 × ×10^{13} conidia, *M. anisopliae* 1.6 × 10^{12} conidia, and *T. harzianum* 5 × ×10^{12} conidia por 100 L de H_20) were found to have low toxicity to the egg parasitoids, *T. pretiosum* and *T. remus*, when ingested by adult parasitoids.

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