

Selectivity of *Metarhizium anisopliae* and *Beauveria bassiana* to adults of *Telenomus podisi* (Hymenoptera: Scelionidae)

Seletividade de *Metarhizium anisopliae* e *Beauveria bassiana* para adultos de *Telenomus podisi* (Hymenoptera: Scelionidae)

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Highlights

Entomopathogenic fungi can decrease the longevity of female *Telenomus podisi*.

Telenomus podisi parasitism is not affected by exposure to entomopathogenic fungi.

The emergence of *T. podisi* offspring may reduce when the female is exposed to fungi.

Abstract

The selectivity of entomopathogenic fungi to non-target organisms needs to be considered in Integrated Pest Management (IPM), because even though they are biological control agents, if used incorrectly, they can negatively alter the functioning of agroecosystems. Therefore, studies that assess the selectivity of these fungi to beneficial organisms are extremely important. The objective of this work was to evaluate the selectivity of *Metarhizium anisopliae* (Metarril®) and *Beauveria bassiana* (Boveril®) to adults of *Telenomus podisi* Ashmead (Hymenoptera: Scelionidae), under laboratory conditions. The products were evaluated on adult females of *T. podisi*, at the concentrations recommended by the manufacturer. To this end, 0.2 mL of suspensions of each product and control (treatments) were applied to the inner surface of glass tubes, and then a female *T. podisi* was placed in it (\leq 48 h of emergence). After 24 h of contact, cards with 25 eggs of *Euschistus heros* Fabricius (Hemiptera: Pentatomidae) were offered for 24 h (COF24). After this period, the COF24 were withdrawn. After 72 h of contact of the female with the tube surface, new *E. heros* egg cards

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were made available (COF72) for 24 h for *T. podisi* ovipositioning. The mortality of *T. podisi* females was evaluated daily to determine longevity, percentage of parasitism and emergence, sex ratio, and egg-adult period of the *T. podisi* offspring. Metarril® and Boveril®, considered selective for adult females of *T. podisi*, did not negatively affect most of the parameters evaluated.

Key words: Biological control. Pest insects. Egg parasitoid. Entomopathogenic fungi.

Resumo

A seletividade de fungos entomopatogênicos a organismos não-alvo deve ser considerada no Manejo Integrado de Pragas (MIP), pois mesmo sendo agentes de controle biológico, se utilizados de forma incorreta, podem alterar negativamente o funcionamento dos agroecossistemas. Portanto, estudos que avaliam a seletividade desses fungos a organismos benéficos são extremamente importantes. O objetivo deste trabalho foi avaliar a seletividade de *Metarhizium anisopliae* (Metarril®) e *Beauveria bassiana* (Boveril®) a adultos de *Telenomus podisi* Ashmead (Hymenoptera: Scelionidae), em condições de laboratório. Os produtos foram avaliados em fêmeas adultas de *T. podisi*, nas concentrações recomendadas pelo fabricante. Para tanto, 0,2 mL das suspensões de cada produto e controle (tratamentos) foram aplicados na superfície interna dos tubos de vidro, sendo então colocada uma fêmea de *T. podisi* (≤ 48 h de emergência). Após 24 h de contato, cartões com 25 ovos de *Euschistus heros* Fabricius (Hemiptera: Pentatomidae) foram oferecidos por 24 h (COF24). Após esse período, o COF24 foi retirado. Após 72 h de contato da fêmea com a superfície do tubo, foram disponibilizadas novas cartelas de ovos de *E. heros* (COF72) por 24 h para oviposição de *T. podisi*. A mortalidade de fêmeas de *T. podisi* foi avaliada diariamente para determinar longevidade, porcentagem de parasitismo e emergência, proporção sexual e período de ovo-adulto da prole de *T. podisi*. Metarril® e Boveril®, considerados seletivos para fêmeas adultas de *T. podisi*, não afetaram negativamente a maioria dos parâmetros avaliados.

Palavras-chave: Controle biológico. Insetos-praga. Parasitoide de ovo. Fungos entomopatogênicos.

Introduction

The excessive use of synthetic phytosanitary products has increased the negative impacts on the environment, and with that, organic production has been gaining ground as a more sustainable alternative for cultivation in the global market (Nodari & Guerra, 2015). Soybeans in particular have stood out in terms of productivity and cultivated area, compared to other oilseeds in global organic production (FIBL & IFOAM, 2019). In organic crops, natural biological control is generally greater due to the non-

use of synthetic chemical insecticides, which are generally less selective. However, when intervention for pest management is necessary, the lack of efficient organic alternatives is still a challenge. Thus, augmentative biological control (ABC) has been developed in recent decades, using entomopathogens, parasitoids, and predators, which can be applied massively on crops as an effective pest control method to replace synthetic phytosanitary products (Bueno, Bueno, Parra, Vieira, & Oliveira, 2010; Parra, 2014, 2019; van Lenteren, Bolckmans, Köhl, Ravensberg, & Urbaneja, 2018).

In ABC, egg parasitoids are considered as the main natural enemies of stink bugs of the Pentatomidae family, especially the species *Telenomus podisi* Ashmead (Hymenoptera: Scelionidae). Responsible for more than 80% of the parasitism observed in eggs of the brown stink bug *Euschistus heros* Fabricius (Hemiptera: Pentatomidae) (Pacheco & Corrêa-Ferreira, 2000; Tognon, Sant'Ana, & Jahnke, 2013) and capable of parasitizing 104 to 211 eggs of this insect-pest (Pacheco & Corrêa-Ferreira, 2000; Silva, Bueno, Neves, & Favetti, 2018), this biological control agent has been a commercial product in Brazil since 2019. In addition to the parasitoids, products based on entomopathogenic fungi are also being used in the country for the biological control of the soybean brown stink bug, with so far seven registered products formulated with isolates of *B. bassiana* and *M. anisopliae* (Sistema de Agrotóxicos Fitossanitários [AGROFIT], 2021). Studies have been carried out to select and characterize new fungal isolates that have the potential to infect *E. heros*, since the natural infection by entomopathogenic fungi is generally insufficient for population control of this insect; thus, requiring flooding application (Silva-Santana, Alves, Ferreira, & Bonini, 2021; Nora et al., 2021). With this growth in the use of entomopathogens comes the need to assess their lethal and sublethal effects on egg parasitoids, which in many occasions are applied in an associated way in the field (Polanczyk, Pratissoli, Dalvi, Grecco, & Franco, 2010; Potrich et al., 2015).

The associated use of *T. podisi* and entomopathogenic fungi, such as *B. bassiana* and *M. anisopliae*, can be a necessary and efficient strategy in Integrated Pest Management (IPM), especially when target pests of these fungi are occurring together

with bed bugs, targets of the parasitoid (Amaro, Bueno, Pomari-Fernandes, & Neves, 2015). Therefore, evaluating the effect of these fungi on *T. podisi* is extremely important as these entomopathogens can infect egg parasitoids, causing lethal or sublethal effects (Magalhães, 1998). From this perspective, it is important to emphasize that entomopathogenic fungi can affect the non-target organisms in different ways, varying according to the form of application, the species of organism, and dosage, among other factors. Therefore, the objective of this work was to evaluate the selectivity of *M. anisopliae* and *B. bassiana* to adults of *T. podisi*.

Material and Methods

Commercial natural products, obtaining *T. podisi* and *E. heros* eggs

Two commercial natural products based on entomopathogenic fungi *M. anisopliae* (Metarriil®) and *B. bassiana* (Boveril®) were tested at the dose recommended by the manufacturer for the control of *E. heros* (Table 1). Eggs of *E. heros* not parasitized and parasitized by *T. podisi* were acquired from a company specialized in breeding and marketing (Bug Agentes Biológicos). Non-parasitized eggs were stored in a refrigerator at a temperature of 4 °C for a maximum of two days until the experiments were carried out. Eggs parasitized by *T. podisi* were placed in plastic bottles (1000 mL volume), kept in a air-conditioned chamber (26 ± 1 °C, 12h photophase, 75 ± 10% U.R.) for two days for the emergence of adults. To feed the adult parasitoids, a fillet of organic honey was placed on the wall of the plastic container.

Table 1

Composition, trade name, recommended concentration and cultures used of the products tested on adults of *Telenomus podisi*

Composition	Commercial name	Recommended dose*	Concentration of active ingredients	Crops
<i>Metarhizium anisopliae</i>	Metarril®	0.5 kg/ha	1.39×10 ⁸ conidia /g	Pastures/Soybeans
<i>Beauveria bassiana</i>	Boveril®	0.5 kg/ha	1×10 ⁸ conidia /g	Soybeans

* Volume of syrup considered 200 L/ha.

Effects of *M. anisopliae* and *B. bassiana* on *T. podisi* adults

For each treatment, 25 non-parasitized *E. heros* eggs (up to 96 h of age) were fixed on 1.5 × 1.5 cm bond paper sheets with non-toxic glue made with wheat flour and distilled water. Suspensions of entomopathogenic fungi were prepared in Becker-type flasks containing 100 mL of distilled water + Tween 80® (0.01%), according to the concentration recommended by the manufacturer (Table 1). With the aid of a micropipettor, 0.2 mL of solution was applied inside a sterilized glass tube (8 cm height and 2.5 cm diameter), ensuring that the product is evenly distributed throughout the internal region of the glass tube. Subsequently, the tubes were left in a Veco® laminar flow chamber for approximately three hours until complete drying. In each treatment, 20 glass tubes (replications) were used, placing a copulated female *T. podisi* (≤48h of emergence), with no experience of parasitism, per tube, together with a fillet of organic honey as food. In the control, the tubes were applied with 0.2 mL sterilized distilled water + 0.01% Tween 80® solution.

After 24 hours of contact of the females with the surface of the tube contaminated with the treatments, a card with 25 eggs of non-parasitized *E. heros* (COF24) was inserted into each of them. The tubes were closed with polyvinyl chloride (PVC) film, and placed in an air-conditioned chamber (26 ± 1 °C, 12h photophase, 75 ± 10% U.R.). After 24 hours, the COF24 were removed and placed in other tubes, which were sealed, identified and kept in an air-conditioned chamber under the same conditions described above. The adult females were kept in the same sprayed tubes, and after 72 hours of contact of the *T. podisi* females with the treatments, new cards with 25 *E. heros* eggs (COF72) were made available, following the same procedures described above. Females of *T. podisi* were kept until they died to assess their longevity and confirm death by the fungus. The parameters evaluated were: longevity of adult females that carried out the parasitism, parasitism (%) of eggs offered at 24 and 72 h of contact of females with the treatments, emergence (%), sex ratio, and egg-adult cycle of the F1 generation.

Parasitism was calculated considering the number of eggs per card (25) as 100%. The emergence percentage (Pe) and the sex ratio (R) were calculated by the following equations according to Battisti et al. (2020):

$$Pp = \frac{n \cdot 100}{25} \text{ and } Pe = \left(\frac{Te}{To} \right) \cdot 100 \text{ e } R = \frac{Tm}{Tm + Tf}$$

where Pp is the percentage of parasitism, n is the number of parasitized eggs, Te is the total number of emerged adults, To is the total number of parasitized eggs, and Tm and Tf are the total number of emerged males and females, respectively.

The longevity (L) and the egg-adult period (Po) were calculated using a weighted average as per Battisti et al. (2020):

$$L = \left[(npm \cdot d1) + (npm \cdot d2) + (npm \cdot d3) \dots + \frac{npm \cdot dn}{TMO} \right] \text{ and } Po = \left[(np \cdot d1) + (np \cdot d2) + (np \cdot d3) \dots + \frac{np \cdot dn}{To} \right]$$

where npm is the number of parasitoids dead that day, d is the day the parasitoids died, dn is the total number of days that there were deaths, and TMO is the total number of parasitoids dead in all days. On the other hand, np is the number of parasitoids that emerged that day, d is the day the parasitoids emerged, dn is the total number of days in which there was an emergence, and

To is the total of number of parasitoids that emerged every day.

Statistical analysis

The data obtained were subjected to the assumptions of homogeneity of variance (Bartlett test) and normality (Lilliefors test), and those that did not show normality or homogeneity were transformed by the formula $ARC = (ASEN(RAIZ((n^\circ \text{ parâmetro})/100)))$, using Microsoft Office Excel®, and then submitted to homogeneity and normality tests. The results were analyzed using the nonparametric Kruskal-Wallis test, and the different contact strategies of the parasitoid with the fungus (COF24 and COF72) were compared by the nonparametric Mann Whitney test, using the Bioestat 5.3 statistical program (Ayres, Ayres, & Santos, 2007).

Results and Discussion

The longevity of adult *T. podisi* females exposed to *M. anisopliae* (14.1 days) and *B. bassiana* (13.4 days) was significantly reduced by 4.49 and 5.14 days, respectively, when compared to the control group (18.5 days) (Table 2).

Table 2
Longevity (\pm SE), in days, of adult females of *Telenomus podisi* exposed to treated surfaces. Temperature $26 \pm 2^\circ\text{C}$, 12 hours of photophase and U,R, $75 \pm 10\%$

Treatment	Longevity (days)
Control	18.5 \pm 0.35a
<i>Metarhizium anisopliae</i>	14.1 \pm 0.35b
<i>Beauveria bassiana</i>	13.4 \pm 0.35b
p	<0.05

Means (\pm SE) followed by the same letter, lowercase in the column, do not differ according to the Kruskal Wallis test ($p < 0.05$).

Although the longevity of adult *T. podisi* females was reduced, there was no reduction of parasitism in both contact strategies of the parasitoid with fungi (COF24

and COF72), compared to the control. There was also no significant difference in parasitism, on comparing the contact strategies used (Table 3).

Table 3
Percentage of parasitism in adult females of *Telenomus podisi* (in COF24 and COF72) exposed to treated surfaces and percentage of emergence of parasitized eggs. Temperature 26 ± 2°C, 12 hours of photophase and U.R. of 75 ± 10%.

	Treatment	COF24 ³	COF72 ⁴	p
Parasitism (%) ¹	Control	61.6 ± 6.11aA	49.0 ± 6.22aA	> 0.05
	<i>Metarhizium anisopliae</i>	51.4 ± 7.18aA	34.2 ± 6.33aA	
	<i>Beauveria bassiana</i>	51.4 ± 6.60aA	43.6 ± 6.31aA	
	p	>0.05		
Emergency (%) ²	Control	56,1 ± 8,07aA	46,1 ± 6,49aA	>0,05
	<i>Metarhizium anisopliae</i>	45,3 ± 8,75aA	10,3 ± 2,15bB	<0,05
	<i>Beauveria bassiana</i>	53,5 ± 8,12aA	10,2 ± 1,81bB	
	p	>0,05	<0,05	

³COF24: Cards with 25 eggs of non-parasitized *E. heros* offered 24 hours after *T. podisi* female contact with sprayed surface.

⁴COF72: Cards with 25 eggs of non-parasitized *E. heros* offered 72 hours after female contact with sprayed surface. Means (± SE) followed by the same letter, lowercase in the column, do not differ according to the Kruskal Wallis test (p<0.05).

Means (± SE) followed by the same letter, capitalized on the line, do not differ according to the Mann Whitney test (p<0.05).

Brown stink bug is one of the main pest insects of the soybean crop in Brazil (Associação Brasileira das Indústrias e Óleos Vegetais [ABIOVE], 2018), which requires constant phytosanitary application for its control, including products based on entomopathogenic fungi (Silva-Santana et al., 2021; Nora et al., 2021). Even though fungal-based biopesticides are less toxic than conventional chemical insecticides, they can affect non-target organisms such as egg parasitoids. In this study, it was observed that the evaluated products, based on *M. anisopliae* and *B. bassiana*, reduced the longevity of adult female *T. podisi* parentals.

Such reduction may be related to changes in walking behavior, cleaning and/or reduced feeding, which represent a greater energy expenditure for these insects. Energy expenditure is reported in the literature as a factor influencing egg parasitoid longevity (Pacheco & Corrêa-Ferreira, 1998).

Regarding the emergence of F1 of *T. podisi* from the parasitized eggs, in the COF72 strategy, both fungi caused a significant reduction in the percentage of emergence, with 10.3% for *M. anisopliae* and 10.2% for *B. bassiana*, compared to the control (45.3%). When comparing the two contact strategies between the parasitoid and the fungi, a

significant reduction in the emergence of F1 *T. podisi* in COF72 was observed in relation to COF24 (Table 3). The emergence of *T. podisi* offspring tends to decrease naturally with the age of adult females, because at the time of copulation, the females store the sperm in the spermatheca. The condition of the male gametes (quality and quantity) is an important factor for reproduction and development of the parasitoids. In this context, older females will have sperms stored for longer and in smaller quantities (Cingolani, Greco, & Liljesthröm, 2014; Cunningham, Farias, Nakagawa, & Chambers, 1971). This fact, added to the possible changes in behavior mentioned, possibly contributed to the reduction in the percentage of emergence observed in this study.

In a similar study, *M. anisopliae* neither reduced the percentage of parasitism when applied to adult females of *T. podisi*, nor have a negative effect on offspring emergence (Agüero, Neves, & Manuel, 2014). This difference might be related to the *M. anisopliae* isolates used and/or their concentrations, which differ between the products used in both studies. In the same sense, the Metarril® and Boveril® products did not affect the emergence of *Trichogramma pretiosum* Riley (Hymenoptera: Trichogrammatidae) offspring (Amaro et al., 2015). It is important to note that the lethal and sublethal effects of entomopathogenic fungi vary according to the species of fungus tested, the isolate, the organism evaluated, the form of application, the concentration, and the time interval between application of entomopathogens and release of parasitoids, among other abiotic factors.

The adult-egg period, in days, of *T. podisi* offspring (male and female) emerged from *E. heros* eggs parasitized by *T. podisi*

females exposed to entomopathogenic fungi was not reduced in both contact strategies of the parasitoid with the fungi (COF24 and COF72), compared with the respective controls. On the other hand, only for females in COF24, *M. anisopliae* significantly increased the egg-adult period (12.8), with respect to the control (12.3). When comparing the egg-adult period of *T. podisi* offspring emerging from *E. heros* eggs parasitized by *T. podisi* females exposed to entomopathogenic fungi using the different strategies, a reduction is observed only in COF72. In case of females, *M. anisopliae* reduced the period (12 days), differing significantly from COF24 (12.8 days), while in case of males, *B. bassiana* reduced the period in COF72 (11.6 days), compared to COF24 (12.6 days) (Table 4).

The sex ratio of *T. podisi* offspring emerged from *E. heros* eggs parasitized by *T. podisi* females exposed to entomopathogenic fungi was not altered in both parasitoid-fungal contact strategies (COF24 and COF72), nor in comparison between such strategies (Table 4). The results observed in the duration of the egg-adult period, despite showing significant differences, have variations of a few hours, which probably would not change the maintenance of the population in the agroecosystems (Potrich et al., 2009, 2015). Sex determination in offspring egg parasitoids, such as *T. podisi*, can be influenced by several factors, such as the age of the parental female and the quality of the host egg (Cingolani et al., 2014; Vinson, 1997). In this study, no change in sex ratio was observed, inferring that the age of the parental female was not a determining factor. Furthermore, the host eggs used (up to 96 h of age) did not receive any treatment that could alter their texture or nutritional composition.

Table 4
Egg-adult period (days) and sex ratio (\pm SE) of *Telenomus podisi* emerged from *Euschistus heros* eggs (in COF24 and COF72) parasitized by females exposed to treated surfaces. Temperature $26 \pm 2^\circ\text{C}$, 12 hours of photophase and U.R. of $75 \pm 10\%$

	Treatment	Female			Male		
		COF24 ¹	COF72 ²	<i>p</i>	COF24 ¹	COF72 ²	<i>p</i>
Egg-Adult Period (days)	Control	12.3 \pm 0.08bA	12.0 \pm 0.14aA	>0.05	12.3 \pm 0.13aA	11.8 \pm 0.16aB	<0.05
	<i>Metarhizium anisopliae</i>	12.8 \pm 0.04aA	12.0 \pm 0.13aB	<0.05	12.7 \pm 0.08aA	11.9 \pm 0.09aA	>0.05
	<i>Beauveria bassiana</i>	12.6 \pm 0.10abA	12.3 \pm 0.20aA	>0.05	12.6 \pm 0.12aA	11.6 \pm 0.20aB	<0.05
	<i>p</i>	>0.05			>0.05		
Sex Ratio	Control	0.63 \pm 0.06 ^{ns}			0.69 \pm 0.08 ^{ns}		
	<i>Metarhizium anisopliae</i>	0.61 \pm 0.05			0.65 \pm 0.04		>0.05
	<i>Beauveria bassiana</i>	0.62 \pm 0.06			0.58 \pm 0.08		
	<i>p</i>	>0.05			>0.05		

¹COF24: Cards with 25 eggs of non-parasitized *E. heros* offered to females 24 hours after *T. podisi* female contact with the sprayed surface.

²COF72: Cards with 25 eggs of non-parasitized *E. heros* offered to females 72 hours after *T. podisi* female contact with the sprayed surface.

ns = not significant Kruskal Wallis (columns) and Mann Whitney (rows) tests.

Means (\pm SE) followed by the same letter, lowercase in the column, do not differ according to the Kruskal Wallis test ($p < 0.05$).

Means (\pm SE) followed by the same letter, capitalized on the line, do not differ according to the Mann Whitney test ($p < 0.05$).

Some negative effects of the products, based on entomopathogenic fungi tested here, on *T. podisi* were observed. However, such fungi can be considered selective for *T. podisi* adults. This is because in these experiments, the parasitoids were placed in extreme conditions of contact (24 and 72 consecutive hours) with the products, at the recommended concentrations for use in the field, without changing the parasitism. According to (Potrich et al., 2009) parasitism is considered the main factor for the efficiency of parasitoids in the field. Battisti et al. (2020) also observed that when the parental females of *T. podisi* are exposed to Metarril® and Boveril® products, applied on the host eggs, the

percentage of parasitism and the percentage of offspring emergence are not affected. It is important to point out that these extreme conditions of contact will hardly occur in the field, as the parasitoid can fly and seek other environments and/or its host's eggs. Thus, we suggest the possible use of both control strategies (the fungi tested here and the parasitoid), since both act at different stages of *E. heros* life and might be complementary for controlling this and/or acting on different targets. Although laboratory studies show the selectivity of the fungi evaluated on adults of *T. podisi*, field studies are important to define the best strategies for joint use.

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Conflict of Interest Declaration

The authors declare no conflict of interest.

Authors' Contributions

All authors contributed to the development of the work, from conception to writing the manuscript. All authors critically reviewed the manuscript and approved the final version.

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