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Original Article

Infection and reinfection of *Stomoxys calcitrans* larvae (Diptera: Muscidae) by entomopathogenic nematodes in different times of exposure

Infecção e reinfecção de larvas de *Stomoxys calcitrans* (Diptera: Muscidae) por nematoides entomopatogênicos em diferentes tempos de exposição

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Abstract

Stomoxys calcitrans is a hematophagous dipteran. Several agents are used in biological control, including entomopathogenic nematodes (EPNs). Bioassay I involved an evaluation of the effect of *Heterorhabditis bacteriophora* HP88 and *Heterorhabditis baujardi* LPP7 on *S. calcitrans* larvae in different periods of exposure. Groups of 10 larvae were placed in Petri dishes and 200 EPNs/larva were added, which were divided into groups according to the exposure times of 2, 4, 6, 12, 24 and 48 hours. The purpose of Bioassay II was to evaluate the efficacy of the EPNs in infecting *S. calcitrans* larvae when they were isolated from stable fly larvae in Bioassay I. Groups of 10 larvae were placed in Petri dishes and 200 EPNs/larva were added. In bioassay I, *H. bacteriophora* caused mortality rates of 51.7, 83.3 and 91.7% in 12, 24 and 48 hours, respectively, while *H. baujardi* caused mortality rates of 9.3 (12h), 35 (24h) and 35% (48h). In Bioassay II, *H. bacteriophora* and *H. baujardi* resulted in mortality rates of 35% and 25%, respectively. It was concluded that the longest exposure times presented the highest larval mortality and that EPNs isolated from *S. calcitrans* are not efficient in controlling the larvae fly.

Keywords: Stable fly, *Heterorhabditis*, periods of exposure.

Resumo

Stomoxys calcitrans é um díptero hematófago. Vários agentes são usados no controle biológico, incluindo nematoides entomopatogênicos (NEPs). O bioensaio I objetivou avaliar o efeito de *Heterorhabditis bacteriophora* HP88 e *Heterorhabditis baujardi* LPP7 sobre larvas de *S. calcitrans* em diferentes períodos de exposição. Grupos de 10 larvas foram colocados em placas de Petri, adicionou-se 200 NEPs/larva, e foram divididos de acordo com tempos de exposição de 2, 4, 6, 12, 24 e 48 horas. O objetivo do Bioensaio II foi avaliar a eficácia dos NEPs na infecção de larvas de *S. calcitrans*, quando esses foram isolados de larvas da mosca oriundos do Bioensaio I. Os grupos de 10 larvas foram colocados em placas de Petri e 200 NEPs/larva foram adicionados. No bioensaio I, *H. bacteriophora* causou taxas de mortalidade de 51,7%, 83,3% e 91,7% em 12, 24 e 48 horas, respectivamente, enquanto *H. baujardi* causou taxas de mortalidade de 9,3% (12h), 35% (24h) e 35% (48h). No Bioensaio II, *H. bacteriophora* e *H. baujardi* resultaram em taxas de mortalidade de 35% e 25%, respectivamente.

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Concluiu-se que os maiores tempos de exposição apresentaram as maiores mortalidades larvais e que NEPs isolados de *S. calcitrans* não são eficientes no controle das larvas mosca.

Palavras-chave: Mosca dos estábulos, *Heterorhabditis*, períodos de exposição.

Introduction

Stomoxys calcitrans (Linnaeus, 1758) (Diptera: Muscidae) is a hematophagous dipteran commonly known as the stable fly, which can parasitize several animal species and may also affect humans. The duration of the life cycle of *S. calcitrans* depends directly on the environment and climate of the region (Bittencourt, 2012). Parasitism by this fly is detrimental to cattle in Brazil and around the world. The action of this fly causes annual economic losses estimated at 2.2 billion dollars in the United States (Taylor et al., 2012), and 335.5 million dollars in Brazil (Grisi et al., 2014). However, the latter figure does not take into account the impact of recent outbreaks reported in some regions of Brazil (Dominghetti et al., 2015).

Arthropod pests have become resistant to most chemical pesticides (Barros et al., 2019). In addition, society is becoming increasingly aware about the effects of these substances on nature and human health, so new means are needed to control these organisms. Biological control is an alternative method, which minimizes the accumulation of chemical residues in agricultural products and lowers pest resistance to these compounds. Thus, entomopathogenic nematodes (EPNs) are an alternative method for the control of economically important arthropods. The nematodes of genus *Heterorhabditis* are used in the control of arthropods, including dipterans, around the world (Dolinski et al., 2012; Aatif et al., 2019; Bream et al., 2018; Mahmoud et al., 2007), in Brazil there are studies that show the efficiency of these EPNs in the control of *S. calcitrans* (Leal et al., 2017; Monteiro et al., 2016).

The objectives of the present study was to evaluate the efficacy of *Heterorhabditis bacteriophora* (HP88) (Nematoda: Heterorhabditidae) and *H. baujardi* (LPP7) (Nematoda: Heterorhabditidae) infection on *S. calcitrans* larvae in different periods of exposure; and evaluate the efficacy of *H. bacteriophora* HP88 and *H. baujardi* LPP7, which were isolated from larvae of *S. calcitrans* on third instar larvae of the stable fly and also to verify if *S. calcitrans* larvae would be efficient for nematodes multiplication.

Material and Methods

The *S. calcitrans* colony was used in this study bred entirely on a bench in a laboratory environment (27 ± 1 °C and 70-80% relative humidity–RH), the larval development diet consisted of crushed sugarcane (330 g), wheat bran (125 g), meat meal (40 g), sodium bicarbonate (5 g) and distilled water (125 mL), using an adapted version of the method described by Moura (2015).The EPNs colony was raised using the method described by Lindegren et al. (1993), its maintenance and multiplication was done through *in vivo* multiplication in *Galleria mellonella* (Lepidoptera: Pyralidae). The infectious juveniles (IJs) were stored in an air-conditioned chamber of type B.O.D (Eletrolab®, model EL 202/4) at 16 ± 1 °C and 70-80% RH in a 40 mL cell culture flask for less than 7 days. The IJs were counted in twelve 10µL aliquots taken from an aqueous suspension of EPNs. After counting the IJs in the 12 aliquots, the largest and smallest number of EPNs/aliquot were discarded, and the average number of IJs in the remaining 10 aliquots was calculated. Based on this calculation, the concentration of suspensions was adjusted to IJs/mL (Taylor et al., 1998).

Bioassay I

Infection of *S. calcitrans* larvae by EPNs in different exposure times

Groups of ten 3rd third instar larvae of the fly were placed in Petri dishes (9 cm in diameter) containing filter paper. Using an adapted version of the method described by Taylor et al. (1998), 200 IJs/larva were added to each Petri dish containing the *S. calcitran*s larvae. The dishes were then sealed with plastic film to prevent the *S. calcitran*s larvae from escaping and to prevent moisture loss. Two species of EPNs were used: *H. bacteriophor*a HP88 and *H. baujardi* LPP7. After the exposure of *S. calcitrans* larvae to EPNs, the larvae were divided into groups according to the length of exposure of 2, 4, 6, 12, 24 and 48 hours. At the end of each exposure time, the larvae were removed from the Petri dishes and deposited in plastic containers (7.5×7.5×4 cm) containing a diet for larval development, without the presence of EPNs, where they were observed until they died or formed pupae and eventually emerged as adults. The volume of water used in the control group was 4 mL, which was the same as

that used in the treated groups, but without EPNs. The experiment was conducted with six replicates for each group and was observed for 14 days.

After the larvae died, they were placed in traps adapted from White (1927) in order to confirm infection by EPNs by observing the presence of adult nematodes (Figure 1) inside the fly larva. This provided proof that the cycle was continuous inside the dead larvae of *S. calcitrans*, showing that IJs developed from the larval phase to adulthood, and that the EPNs were the cause of their death.

The design used was completely randomized, with six replications. The treatments were arranged in a 2x6+1 factorial scheme, with two nematode species (*H. bacteriophora* HP88 and *H. baujardi* LPP7), six exposure times (2, 4, 6, 12, 24 and 48h) and a control, without the presence of nematodes. A statistical analysis was performed using the factorial method with an additional control. When significant ($p<0.05$), the interaction of 'nematodes vs. exposure time' was evaluated by breaking down the levels of each factor using the Tukey test. Dunnett's test at a probability of 5% was used to compare the control group to the other treatments, using SISVAR 5.1 software (Ferreira, 2011). The nematode *H. bacteriophora* HP88, for causing the highest mortality rates, was submitted to the test of Probit for the determination of the Lethal Time 50 (LT50) through the XLStat 7.5.2. Program, module XLstat-Dose 3.

Bioassay II

Reinfection of S. calcitrans larvae by EPNs

The nematodes used in this bioassay were recovered in White traps (White, 1927) containing *S. calcitrans* larvae killed due to the action of EPNs and from Bioassay I. After collection, the EPNs were stored in 40 mL cell culture flasks,

Figure 1. Infection by entomopathogenic nematodes, confirmed by the presence of adult nematodes inside stable fly larvae.

20 mL of the solution containing EPNs was collected from de White traps. A completely randomized design was used, with treatments (control, *H. bacteriophora* HP88 and *H. baujardi* LPP7) submitted to 6 replications.

Groups of 10 3rd instar larvae were placed on filter paper in Petri dishes, there was no larval development diet. Then, 200 IJs/larva were added to the dishes containing the *S. calcitrans* larvae. The dishes were sealed with plastic film and were monitored until the larvae died or formed pupae and eventually emerged as adults (seven days). The EPNs were in contact with the fly larvae throughout the bioassay. Each Petri dish contained 4 mL of water, but that of the control group contained no EPNs.

The normality of the data was determined by the Shapiro-Wilk test. The data were then subjected to an analysis of variance (ANOVA), which revealed a significant effect of the treatments, as well as to the Tukey test (P≤0.05). Both tests were performed using SISVAR 5.1 software (Ferreira, 2011).

Results and Discussion

Infection of *Stomoxys calcitrans* larvae by EPNs in different periods of exposure

An analysis of the data indicated that the effect of the EPNs varied significantly as a function of exposure times. A comparison of the two nematodes (*H. bacteriophora* HP88 and *H. baujardi* LPP7) in each period of exposure revealed that the effect of the nematodes did not differ statistically from each other in the first three exposure times (2, 4 and 6 h). However, after 12 h of exposure, *H. bacteriophora* HP88 caused higher mortality than *H. baujardi* LPP7, with larvae mortality rates of 51.7%, 83.3% and 91.7%, respectively (Table 1). An evaluation of the periods of exposure to each nematode showed little variation in larval mortality rates caused by *H. baujardi* LPP7, with differences observed only between exposure times of 4, 24 and 48 hours compared to 12 hours. As for the effect of *H. bacteriophora* HP88, the two longest exposure times (24 and 48 h) were significantly more effective than the others, although they did not differ statistically (Table 1). A comparison of the treatments and the control group, indicated that the following treatments were the most effective: *H. baujardi* LPP7 with exposure times of 24 and 48 hours, and *H. bacteriophora* HP88 with exposure times of 4, 12, 24 and 48 hours (Table 1). Therefore, the longer the exposure time of the stable fly larvae to the EPN *H. bacteriophora* HP88 the higher the larval mortality rate. Conversely, in the case of *H. baujardi* LPP7, the activity of this EPN against stable fly larvae remained practically constant, even after the longest period of exposure. *H. bacteriophora* HP88 presented LT50 of 8.32 hours. *H. baujardi* LPP7 had a low mortality rate, so the LT50 was not calculated for this nematode.

Studies involving exposure/infection times of arthropod pests by EPNs are still scanty in the literature. Mahmoud et al. (2007) used *Steinernema feltiae* N33 (Nematoda: Steinernematidae) to control third-instar larvae of *S. calcitrans*, which they exposed to EPN infecting juveniles for 24 and 48 hours. The authors reported a mortality rate of 16.6% after 24 hours and of 25% after 48 hours of exposure to a concentration of 200 EPNs/larva. These mortality rates are lower than those found in the present study, caused for both *H. bacteriophora* HP88 (83.3% after 24 hours and 91.7% after 48 hours) and *H. baujard*i LPP7 (35% after 24 and 48 hours of exposure). Apparently, the strains used in this study are more virulent than *S. feltiae* N33 in the control of stable fly larvae, because both caused higher mortality rates than those reported by Mahmoud et al. (2007). This is particularly true of the mortality rates caused by the EPN *H. bacteriophora* HP88, which were considerably higher when compared to the aforementioned

Table 1. Mean mortality rate of *Stomoxys calcitrans* larvae upon exposure to entomopathogenic nematodes *Heterorhabditis bacteriophora* HP88 and *Heterorhabditis baujardi* LPP7 for different periods of time.

Nematodes	Exposure time (hours)					
	2	4	6	12	24	48
HP88	23.3 Ac	35.0 Abc*	18.3 Ac	51.7 Ab*	83.3 Aa*	91.7 Aa*
LPP7	23.3 Aab	30.0 Aa	20.0 Aab	9.3 Bb	35.0 Ba*	35.0 Ba*
Control	18.86					
			DMS (Dunnet) = 14,594			

Means followed by the same letter (uppercase in columns and lowercase in rows) do not differ from each other according to Tukey's test. *Means differ from the control group according to Dunnett's test (p<0.05).

study. It should be noted that Mahmoud et al. (2007) recorded the mortality rates pointwise after 24 and 48 hours of exposure of the fly larvae to EPN. Conversely, in this study, after the various periods of exposure, the fly larvae were removed from the environment with EPNs and monitored for 14 days. This two-week period may have resulted in the better results obtained in this study, since it would correspond more accurately to what happens in the environment. Moreover, EPNs of the genus *Heterorhabditis* have a chitinous tooth (Kaya & Gaugler, 1993), which assists in the infection process, while nematodes of the genus *Steinernema* are devoid of this structure.

Leal et al. (2017) made a laboratory evaluation of the mortality rate caused by *H. bacteriophora* HP88 and *H. baujardi* LPP7 in third instar larvae of *S. calcitrans*. Their evaluation covered a period of 10 days, which is five times longer than the longest time used in this study (48 hours - two days). The aforementioned authors reported that a concentration of 200 infective juveniles of *H. bacteriophora* HP88 per *S. calcitrans* larva caused a mortality rate of 96.7% of the fly larvae, while in the present study, the mortality rate caused by *H. bacteriophora* HP88 at the same concentration was 91.7%. In addition, Leal et al. (2017) reported that *H. baujardi* LPP7 caused a larval mortality rate of 93.3%, unlike in this study, in which the highest mortality rate caused by *H. baujardi* LPP7 was 35%. Despite the difference in periods of exposure in the two studies, *H. bacteriophora* HP88 resulted a mortality rate of more than 90% in both studies. Therefore, it can be stated that this EPN is highly virulent against stable fly larvae, because even though the exposure time in the present study was considerably shorter than that used by Leal et al. (2017), *H. bacteriophora* HP88 was able to cause a larval mortality rate close to that reported by the aforementioned authors. However, *H. baujardi* LPP7 did not present same behavior, since Leal et al. (2017) reported a mortality rate of 93.3% in 10 days of exposure, while the mortality rate in the present study after two days of exposure to *H. baujardi* LPP7 was only 35%. This indicates that the EPN *H. baujardi* LPP7 needs more time than *H. bacteriophora* HP88 in contact with fly larvae to reach its peak mortality rate, since only two days of exposure of fly larvae to this EPN did not suffice to cause a mortality rate above 90%.

To control the fruit fly *Ceratitis capitate* (Diptera: Tephritidae), Minas (2008) exposed its larvae for 2 and 24 hours to the EPN *H. baujardi* LPP7, and reported mortality rates of 46.7 and 88.3%, respectively. These mortality rates are higher than those recorded in the present study after 2 (23.3%) and 24 (35.0%) hours of exposure of *S. calcitrans* larvae to *H. baujardi* LPP7. This indicates that *C. capitata* may be more susceptible to the action of EPN *H. baujardi* LPP7 than *S. calcitrans*, since just 2 hours of exposure of *C. capitata* resulted in a higher mortality rate than that observed after 24 hours of exposure of *S. calcitrans* in this study. Minas (2008) used a concentration of 70 IJs/larva, which is less than half the concentration used in present work (200 IJs/larva). Therefore, even at a lower concentration, *H. baujardi* LPP7 is more pathogenic to the fruit fly *C. capitata* than to the stable fly *S. calcitrans*, since the third instar larvae of the fruit fly and the stable fly are similar in size. Hence, this factor should not have been the cause of the higher mortality rate of *C. capitata*, which may, instead, be attributed to the immune system of *S. calcitrans*. In fact, Boulanger et al. (2002) identified peptides with antimicrobial properties inside the stable fly, and these peptides probably interfered in the action of bacteria released by the EPN on the host hemocele. This information matches the findings of Moraes et al. (2015), who also observed antimicrobial peptides inhibiting the action of *Beauveria bassiana* (Bals.) Vuill on immature stages of the stable fly. Aatif et al. (2019) used EPNs to control third instar larvae of *Bactrocera dorsalis* (Diptera: Tephritidae). The authors reported a mortality rate of 69.42% when the larvae were exposed to *H. bacteriophora* HP88 for 72 hours. In this study, when stable fly larvae were exposed to the EPN *H. bacteriophora* HP88 for 48 hours, the mortality rate was 91.7%, indicating that even in just half that exposure time, *S. calcitrans* appears to be more sensitive than *B. dorsalis* to the action of this EPN. According to Aatif et al. (2019), the mortality rate of *B. dorsalis* larvae after 72 hours of exposure to *H. indica* was similar to that found in the present study with stable fly larvae exposed for 48 hours to the EPN *H. baujardi* LPP7. In other words, the mortality rate of *B. dorsalis* was 37.15% and that of *S. calcitrans* was 35%, indicating that, like the stable fly, *B. dorsalis* appears to be more susceptible to the action of *H. bacteriophora* HP88 than to that of *H. indica* and *H. baujardi* LPP7. It should be noted that Aatif et al. (2019) used a concentration of 100 IJs/larva, half of that used in the present work (200 IJs/larva). This may have contributed to the differences in mortality rates of *B. dorsalis* and *S. calcitrans* attacked by *H. bacteriophora* HP88. However, comparing the mortality rates caused to the two dipteran species by *H. baujardi* LPP7 and *H. indica*, this factor did not have a significant impact, since their mortality rates were similar.

Carvalho et al. (2010), who evaluated different periods of exposure of ingurgitated females of *Rhipicephalus microplus* (Acari: Ixodidae) to the EPN *Steinernema glaseri* CCA strain (Nematoda: Steinernematidae), reported that treatments of 2, 6, 12, 24 and 48 h resulted mortality rates of 66%, 78% and 83.7%, 99.9%, and 99%, respectively. These rates are higher than those of this study with the stable fly, where the highest mortality rate was 91.7% (*H. bacteriophora* HP88- 48h). The differences between hosts and EPNs should be taken into account, because although

both hosts are arthropods, they belong to different classes, which indicates that ticks may be more susceptible than flies to the action of EPNs. However, this comparison should be made carefully, since the EPN (*S. glaseri* CCA) used in the aforementioned study belongs to another genus, different from the genus used in this study work, in which the two EPNs were of the genus *Heterorhabditis*. Another important fact is that Carvalho et al. (2010) used a concentration of 1000 IJs per ingurgitated female, while the concentration used in the present study was five times lower (200 IJs/fly larva). In fact, the superior results reported the aforementioned authors can be attributed to this higher concentration. Monteiro et al. (2012) evaluated different periods of exposure of *R. microplus* engorged females to the EPN *H. bacteriophora* HP88 (300 IJs/female), and reported that 6h and 12h of exposure resulted in mortality rates of 12.24% and 32.62%, respectively. Their results are lower than those of the present study with *H. bacteriophora* HP88, in which the larval mortality rate in 6h of exposure was 18.3% and in 12h it was 51.7%. However, Monteiro et al. (2012) reported that mortality rates were 82.54% after 24 hours of exposure, and 100% after 48 hours of exposure. Those mortality rates are higher than the rates achieved in the present study, where the highest mortality rate was 91.7% (48h), indicating that, apparently, after longer periods of exposure to EPNs, *R. microplus* engorged females are more affected than stable fly larvae.

The periods of exposure tested in this study were necessary to clarify how much time was required for the entomopathogenic nematodes to successfully infect and then kill *S. calcitrans* larvae. This knowledge is important because, if these EPNs are used in an environment where the target arthropod is present, the nematodes are subjected to abiotic factors such as ultraviolet radiation, temperature and humidity, bearing in mind that these organisms undergo loss of viability at relative humidity levels below 60% (Baur et al., 1995). Therefore, the faster the nematodes enter the host the lower their risk of exposure to these factors, since the infectivity of *H. bacteriophora* HP88 (which proved more virulent in this study) decreases at temperatures above 40.1ºC. EPNs do not have a well-adapted relationship to their hosts, so the stable fly and other arthropods have no short-term resistance to them (Kaya & Gaugler, 1993).

Greater attention should focus on studies of EPNs as a form of control of arthropods of veterinary importance, because although the mortality rates they cause are not higher than those required for registration with the Ministry of Agriculture, Livestock and Food Supply (MAPA), unlike chemical pesticides, they are quite virulent against arthropods. Moreover, they are mobile and can be produced at low cost, they survive storage for long periods, they are compatible with most chemical pesticides and they can be applied via irrigation or water spraying (Koppenhöfer & Grewal, 2005). All these factors make EPNs agents with a potential for use in the control of *S. calcitrans* and other arthropod pests that have a developmental stage in soil (Kaya & Gaugler, 1993). EPNs can be used separately or in combination with chemical pesticides (integrated control), since they will also act on populations of resistant insects, aiming to reduce losses and increase Brazil's agricultural productivity.

Reinfection of *Stomoxys calcitrans* larvae by EPNs

In terms of the variable of mortality, the EPNs used here differed statistically, showing mortality rates of 15% in the control group, 25% by *H. baujardi* LPP7 and 35% by *H. bacteriophora* HP88. As for the variable of emergence rate, the treated groups and the control group showed no significant difference, and the overall average emergence rate of the groups was 70.54%. To determine which treatment achieved the highest mortality rate, the Tukey test ($p \leq 0.05$) was performed (Table 2). This test indicated that the mortality rate caused by *H. bacteriophora* HP88 (35%) was statistically higher than that of the control group (15%), but did not differ statistically from that caused by *H. baujardi* LPP7 (25%), since a comparison of the mortality rate of *H. baujardi* LPP7 with that of the control group indicated that they were statistically the same. The fact that EPNs reproduce in *S. calcitrans* larvae could help maintain IJs in the environment, to control future generations of the stable fly.

Monteiro et al. (2016) used *H. bacteriophora* HP88 for the control of *S. calcitrans* larvae developing in filter cake. The EPNs used by these authors were bred in *G. mellonella* and they reported a mortality rate of 83.3% using 200 IJs/larva. Conversely, the larval mortality rate in the present study was only 35% when using the same concentration of nematodes. The mortality rate reached in the present study is close to that achieved by Monteiro et al. (2016) with a concentration of 25 IJs/larva (38.33%), indicating that when bred in *G. mellonella*, even the lowest EPN/larva concentrations used by those authors achieved higher than mortality rates than in this study with *H. bacteriophora* HP88 bred in *S. calcitrans*. The rate achieved by Monteiro et al. (2016) in the presence of filter cake is remarkable, given that filter cake is rich in organic matter, which decreases the oxygen levels for EPNs and might have had a negative effect on their action.

Table 2. Mean *Stomoxys calcitrans* larvae mortality rate and emergence rate in response to the action of EPNs *Heterorhabditis bacteriophora* HP88 and *H. baujardi* LPP7.

Treatments	Mortality Rate	Emergence Rate
Control	15.0 b	73.84 a
LPP7	25.0 ab	66.74 a
HP88	35.0 a	71.06 a

Means followed by the same letter in the columns do not differ according to Tukey's test (p≤0.05).

Mendes et al. (2016) reported emergence rates ranging from 74.5 to 84% using stable fly larvae bred on a diet of sugarcane, crude ash and vinasse. These values do not differ much from the emergence rate found in this work using EPNs to control *S. calcitrans*. Therefore, the EPNs may not have had a negative effect on the adult stable fly emergence rate, and its virulence may have been attenuated after being isolated from stable fly larvae. This decrease in entomopathogenic nematode virulence may be attributed to the action of antimicrobial peptides found in the intestine of stable fly larvae (Moraes et al., 2015), which may have affected the bacterial symbionts that EPNs harbor when they were still inside the *S. calcitrans* larvae from which they were isolated.

Conclusions

It was concluded that the EPNs tested proved effective in the control of third instar larvae of the stable fly and the longest exposure times presented the highest larval mortality. The EPN *H. bacteriophora* HP88 is the most recommended for control of the stable fly, presenting LT50 of 8.32 hours (Bioassay I). The EPNs isolated from *S. calcitrans* (Bioassay II) were not virulent for the fly larvae.

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