PERFORMANCE OF ENTOMOPATHOGENIC NEMATODES ON *NEOLEUCINODES ELEGANTALIS* (GUENÉE) (LEPIDOPTERA: CRAMBIDAE)

DESEMPENHO DE NEMATOIDES ENTOMOPATOGÊNICOS EM *NEOLEUCINODES ELEGANTALIS* (GUENÉE) (LEPIDOPTERA: CRAMBIDAE)

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Resumo

O uso de nematoides entomopatogênicos no manejo de pragas é uma alternativa para reduzir as perdas causadas por pragas em diversas culturas. O presente estudo teve como objetivo avaliar o desempenho de *Heterorhabditis indica* (Nemata: Rhabditida) e *Steinernema carpocapsae* (Nemata: Rhabditida), em pré-pupas de *Neoleucinodes elegantalis* (Guenée) (Lepidoptera: Crambidae) e determinar a viabilidade temporal da patogenicidade de *S. carpocapsae* aplicado no solo. As espécies de nematoides foram diluídas em água destilada para 50, 65, 83, 107, 138, 178, 229, 295, 380 e 500 juvenis infectantes por pré-pupa (JIs inseto^{-1}) de *N. elegantalis.* A mortalidade de pré-pupa de *N. elegantalis* foi maior à medida que aumentou-se as concentrações de ambas as espécies estudadas. O nematoide *S. carpocapsae* foi o mais efetivo, causando mortalidade de 82,93% na concentração de 65 JIs inseto^{-1} e uma CL50 de 24,32 JIs inseto^{-1}. No teste de patogenicidade, *S. carpocapsae* foi aplicado na concentração de 100 JI/cm² em vasos previamente plantados com mudas de tomateiro. Como controle positivo foram utilizadas mudas de tomateiro tratadas com água destilada. *S. carpocapse* apresentou viabilidade no solo por 24 dias. Assim, *S. carpocapsae* pode ser uma ferramenta importante no manejo integrado de pragas (MIP) de *N. elegantalis.*

Palavras-chave: Broca pequena do fruto; juvenis infectivos, plantas de tomate.

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Abstract

The use of entomopathogenic nematodes in pest management is an alternative to reduce the yield and/or damage losses caused by pests in several crops. The present study aimed to evaluate the performance of *Heterorhabditis indica* (Nemata: Rhabditida) and *Steinernema carpocapsae* (Nemata: Rhabditida), in pre-pupae of *Neoleucinodes elegantalis* (Guenée) (Lepidoptera: Crambidae) and determine the temporal viability of the pathogenicity of *S. carpocapsae* applied to soil. The nematode species were diluted in distilled water to 50, 65, 83, 107, 138, 178, 229, 295, 380 and 500 infective juveniles per pre-pupae (IJs insect^{-1}) of *N. elegantalis*. The mortality of pre-pupa of *N. elegantalis* was higher as the concentrations of both studied species increased. The nematode *S. carpocapsae* was the most effective, causing mortality of 82.93% in the concentration of 65 IJs insect^{-1} and an LC50 of 24.32 IJs insect^{-1}. In the pathogenicity test, *S. carpocapsae* was applied in the concentration of 100 JI/cm² in pots previously planted with tomato seedlings. As positive control was used tomato seedlings treated with distilled water. *S. carpocapse* presented soil viability of 24 days. Thus, *S. carpocapsae* can be an important tool in the integrated pest management (IPM) of *N. elegantalis.*

Keywords: Small fruit borer; infective juveniles, tomato plants.

1. INTRODUÇÃO

The small tomato borer *Neoleucinodes elegantalis* (Guenée) (Lepidoptera: Crambidae) is a pest that causes direct damages to tomato fruits, causing yield losses of up to 90% (LEITE et al., 2012; MAIA et al., 2016). The use of several control measures has been used to manage this pest however, they have not showed to be efficient. An of the major obstacles to the management of this pest is due to the habit of the 1st instar of the caterpillar to penetrate and colonizing the interior of the fruit, while pre-pupa phase occurs in the soil. Without exposition of insect phases the use of management measures aimed the caterpillar as target are not viable (OLIVEIRA et al., 2017).

Thus, management methods that can help control this pest should be investigated. Among these methods, entomopathogenic nematodes (EPNs), biological pest-control agents, are efficient and of great importance, especially because they are not toxic to mammals and also not affect crops (KARY et al., 2018; SANDA et al., 2018; GULCU et al., 2019).

These nematodes have two genera of great importance: *Steinernema*, with 25 described species, and *Heterorhabditis*, with eight described species (ADAMS, 1998). The species *Heterorhabditis indica* and *Steinernema carpocapsae* have been studied, and their use has been shown to be effective in the management of pests such as *Mahanarva sp.* (Hemiptera: Cercopidae), *Spodoptera frugiperda* (Smith) (Lepidoptera: Noctuidae),

Ceratitis capitata Wiedemann, *Anastrepha fraterculus* (Wiedemann) (Diptera: Tephritidae), *Agrotis* spp. (Lepidoptera: Noctuidae), *Sphenophorus levis* Vaurie (Coleoptera: Curculionidae), *Leucothyreus* sp. (Coleoptera: Scarabaeidae), *Diaprepes abbreviatus* (Linnaeus) (Coleoptera: Curculionidae), *Aethina tumida* Murray (Coleoptera: Nitidulidae) and *Bradysia mabiusi* Lane (Diptera: Sciaridae) (LINDEGREN et al., 1990; KAYA and GRIEVE, 1982; LEITE et al., 2012; RICHTER and FUXA, 1990; SHAPIRO-ILAN et al., 2010).

In this way, *Steinernema* and *Heterorhabditis* may be promising tool in the integrated pest management (IPM) of *N. elegantalis*, because this pest pupates in the soil in most cases and can thus be affected by the action of EPNs. Thus, the present study aimed to investigate the pathogenicity and virulence of *H. indica* and *S. carpocapsae* in *N. elegantalis* pre-pupae, in addition to evaluating the time (days) required to start the mortality of small pre-pupae tomato small borer.

2. MATERIAL E MÉTODOS

2.1 *Multiplication of Neoleucinodes elegantalis*

The pest was multiplied in a temperature-controlled room (25 ± 2ºC, 70 ± 10% RH and 12 h photoperiod). Adults were kept in acrylic cages and fed a 10% honey solution. For oviposition, tomato fruits of cultivar Alambra F1 were placed in the cages and then, the tomato fruits were removed every day, and the eggs were distributed in *Solanum aethiopicum* (Solanaceae) fruits (average of 5 eggs/fruit) that remained in plastic trays covered with non-woven fabric (NWF), serving as a place for the pupation of caterpillars. In the end of this phase, the pupae were removed and placed into chambers acclimated to the conditions described above until the emergence of adults.

2.2 *Multiplication of Galleria mellonella (L.) (Lepidoptera: Pyralidae)*

The insects were created according to the methodology proposed by Mohamed and Coppel (1983) in artificial diet based on soybean and beeswax at a temperature of 32ºC, ambient relative humidity and photoperiod of 12 hours.

2.3 *Collection and multiplication of entomopathogenic nematodes*

The nematode isolates were obtained from the rearing stock of the Nucleus of Scientific and Technological Development in Phytosanitary Management of pests – NUDEMAFI of the Federal University of Espírito Santo – UFES, Alegre, Espírito

Santo, Brazil. *H. indica* (strain - LPP30) was originally obtained from the Darcy Ribeiro State University of North Fluminense – UENF, Campos dos Goytacazes, Rio de Janeiro, Brazil *S. carpocapsae* was obtained originally from a commercial strain donated by the Koppert Biological System company.

For multiplication of the specimens, *G. mellonella* larvae were placed in Petri dishes (8.5 cm diameter x 1.5 cm height) (5 per plate) on filter paper, and 1.5 mL of an aqueous suspension containing the entomopathogenic nematode species was applied over the larvae. After this procedure, the plates were sealed and kept in a temperature-controlled chamber (25 ± 1°C and 12 h photoperiod) until the death of the *G. mellonella* larvae. Once mortality was confirmed, the larvae were transferred to White traps (WHITE, 1927), consisting of a Petri dish with a layer of water and a moist filter paper disc for the removal of infective juveniles (IJs), and 100,000 to 300,000 IJs per infected larva were obtained (POINAR, 1990). The solution containing the nematodes was then stored in a temperature-controlled chamber (16 ± 1°C, 70% RH and 12-h photoperiod) for a maximum period of 1 month.

2.4 ***Pathogenicity of*** *Steinernema carpocapsae* ***and*** *Heterorhabditis indica* ***on*** *Neoleucinodes elegantalis*

For the experiment, *N. elegantalis* pre-pupae were removed from the stocks, placed in Gerbox plastic boxes (6.3 cm diameter by 2.3 cm height), lined with filter paper and subsequently moistened with distilled water.

To prepare the concentrations, the nematodes were diluted in distilled water in a ratio of 500 infective juveniles (IJs) per 0.2 mL. After stabilisation of this concentration, the solution was then diluted to the lower concentrations (50, 65, 83, 107, 138, 178, 229, 295, 380 and 500 IJs per pre-pupae) by counting the number of IJs 1 ml of suspension in Peters's chamber under light microscope. Subsequently, to homogenise the solution, the solutions were stirred at a moderate speed for 3 minutes and were then applied, with the aid of an automatic pipette, on the pre-pupae at a volume of 0.2 mL per insect.

After the application, the Gerbox containers were closed, labelled and placed in a temperature-controlled chamber (27 ± 1°C, 70% RH and 12-h photoperiod). Mortality assessments were performed daily up to seven days after inoculation with the nematodes. The mortality due to nematodes was confirmed after the eighth day based on the dissection of the insects in 5 cm Petri dishes with water.

The experiment was conducted in a scheme of split-splitplot (11 x 2 x 7), with the plots having two levels (species) (*Heterorhabditis indica* and *Steinernema carpocapsae*). The splitplot concentration had 11 levels (0 (control), 50, 65, 83, 107, 138, 178, 229, 295, 380 and 500 IJs insect^{-1}), and the sub-subplots of days had seven levels, in a completely randomised design with 10 replications with three pre-pupae per replicate. The mortality data were adjusted using Abbott's formula (1925) and subjected to analysis of variance. The means were compared using Tukey's test ($p \leq 0.05$).

From previous results of mortality, the lethal concentrations (LC_{50}) were estimated for the 4th day, and the mortality data were analysed by Probit regression using the software POLO-PC (LEORA SOFTWARE, 1987).

2.5 *Persistence of the* *Steinernema carpocapsae* ***in soil in greenhouse***

The persistence experiment of *S. carpocapsae* in the soil was carried out under greenhouse conditions. The persistence of EPNs in soil and the effect on mortality of *N. elegantalis* was performed with *S. carpocapsae*, the species that presented better results in the previous bioassay.

The concentration used was 100 IJ/cm², applied with a potted pipette (5L) containing Alambra F1 tomato seedlings. Only distilled water was applied to the control. Each treatment consisted of 10 pots (replicates), filled with soil in the proportion 1:1:1 (soil: manure: sand), previously sterilized in an autoclave (120 ºC for 2 h). The vessels were moistened with water daily during the experiment (200 mL). After application of the concentrations, five pre-pupae of *N. elegantalis*, individually placed in *eppendorf* containing 10 holes to allow entry of the NEPs. To evaluate the persistence of NEPs in the soil, pre-pupae of *N. elegantalis* were inoculated every seven days and then removed after 48 hours of exposure to evaluate mortality.

The design was completely randomized in a splitplot, in which the plots were constituted by the treatments (Control and 1 species of EPNs) and the splitplots by time, with 10 replications. Mortality data were submitted to the Mauchly (1940) test to evaluate the sphericity, that is, to evaluate if the data obtained in the treatments were homogeneous. After sphericity was confirmed, the data were transformed by root (x + 0.5) and submitted to analysis of variance. Subsequently the means were compared by the Tukey test ($p < 0.05$).

3. RESULTADOS

3.1 *Pathogenicity of Steinernema carpocapsae and Heterorhabditis indica on Neoleucinodes elegantalis*

Regarding pre-pupae mortality caused by entomopathogenic nematodes, there was not significant three-way interaction (nematode species *x* concentrations *x* assessment days) (F = 0.7986, p = 0.8513). A significant interaction was observed between plot and subplot (nematode species *x* concentrations) and between plot and sub-subplot (concentrations *x* days) (F = 6.8506, p < 0.0001; F = 2.6889, p = 0.0135, respectively). However, the interaction between subplot and sub-subplot was not significant (F = 0.9056, p = 0.6682).

Based on the analysis between plot and subplot (nematode species *x* concentrations), both of the species were pathogenic to the *N. elegantalis* pre-pupae. In this case, both nematodes caused insect mortality (Table 1).

The mortality of the pre-pupae differed both between species and among concentrations. For the nematode species studied, mortality was higher for *S. carpocapsae* in all concentrations, except in 295, 380 and 500 IJs insect^{-1}, which exhibited similar mortality values as those for *H. indica* (Table 1).

For *H. indica*, the concentrations from 178 to 500 IJs insect^{-1} did not differ from each other, causing mortality of 81.23 to 94.45% of the pre-pupae. However, only mortality in the concentrations of 380 and 500 IJs insect^{-1} differed significantly from the mortality in the concentrations of 50 to 138 (Table 1).

In the pre-pupae treated with *S. carpocapsae*, concentrations ranging from 65 to 500 IJs insect^{-1} caused the highest mortality rates (82.93 to 95.15%) (Table 1). Only the mortality in concentrations between 138 and 500 IJs insect^{-1} differed significantly from the mortality in the concentration of 50 IJs insect^{-1} (Table 1).

Table 1. Adjusted mortality (%) of *Neoleucinodes elegantalis* prepupae treated with the nematodes *Heterorhabditis indica* and *Steinernema carpocapsae* at different concentrations.

Conc. (IJ Insect^{-1})	Species[1]	
	(%) Mortality by *Heterorhabditis indica*[2]	(%) Mortality by *Steinernema carpocapsae*[2]
50	34.13 ± 0.18 dB	73.09 ± 0.21 bA
65	68.27 ± 0.13 cB	82.93 ± 0.12 abA
83	68.31 ± 0.14 cB	84.84 ± 0.13 abA
107	78.00 ± 0.14 bcB	84.56 ± 0.13 abA
138	78.26 ± 0.13 bcB	91.46 ± 0.11 aA
178	81.23 ± 0.12 abB	93.32 ± 0.12 aA
229	81.82 ± 0.10 abB	94.47 ± 0.08 aA
295	90.28 ± 0.13 abA	93.85 ± 0.09 aA
380	92.65 ± 0.15 aA	94.50 ± 0.09 aA
500	94.45 ± 0.11 aA	95.15 ± 0.09 aA

[1]Percentage mortality adjusted based on the control, using Abbott's formula (1925);
[2]Means followed by the same lowercase letter in the column and uppercase letter in the row do not differ based on Tukey's test ($p \leq 0.05$).

In the analysis of the interaction between plot and sub-subplot (nematode species *x* days), there was an increase in mortality over time for both of the nematode species (Table 2). Regardless of the day assessed, the adjusted mortality for *S. carpocapsae* was higher. For *H. indica* and *S. carpocapsae*, there was not significant increase in mortality: 82.55 to 83.62% and 93.78 to 96.43% between the 4th and 7th days after inoculation, respectively.

Table 2. Adjusted mortality (%) of *Neoleucinodes elegantalis* prepupae treated with the nematode species *Heterorhabditis indica* and *Steinernema carpocapsae* as a function of time.

Time after inoculation (days)	Species[1]	
	(%) Mortality by *H. indica*[2]	(%) Mortality by *S. carpocapsae*[2]
1	55.39 ± 0.80 cB	71.54 ± 0.60 dA
2	73.55 ± 0.90 bB	80.65 ± 0.54 cA
3	78.54 ± 0.94 abB	89.11 ± 0.31 bA
4	82.55 ± 0.92 aB	93.78 ± 0.38 abA
5	83.61 ± 0.90 aB	95.11 ± 0.30 abA
6	83.61 ± 0.90 aB	95.11 ± 0.30 abA
7	83.62 ± 0.90 aB	96.43 ± 0.32 aA

[1]Percentage mortality adjusted based on the control, using Abbott's formula (1925);
[2]Means followed by the same lowercase letter in the column and uppercase letter in the row do not differ based on Tukey's test ($p \leq 0.05$).

For lethal concentration (LC_{50}) estimation, the data fit the Probit model, showing a non-significant χ^2 and low heterogeneity. The confidence intervals indicated significant differences between the nematodes species (Table 3).

Table 3. LC_{50} values calculated for *Neoleucinodes elegantalis* prepupae treated with two species of entomopathogenic nematodes

Species	N[1]	Slope ± SE[2]	LC_{50} (95% CI)[3/6]	$\chi^{2(4)}$	Df[5]
Heterorhabditis indica	500	1.811 ± 0.238	122.230 (62.346 - 173.650)	12.774	8
Steinernema carpocapsae	500	1.106 ± 0.232	24.329 (6.880 - 24.804)	5.7835	8

[1]N: number of observations;

[2]Slope ± SE: slope of the curve ± standard error;

[3]LC: Lethal concentration (%); CI: confidence interval;

[4]χ2: Chi-squared;

[5]Df: degrees of freedom;

[6]: Test performed at 5% significance

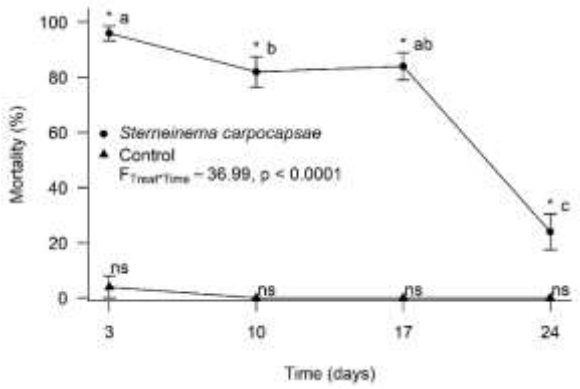
The LC_{50} differed between the two species (Table 3). The total of 24.32 IJs insect^{-1} of *S. carpocapsae* were required to cause 50% mortality of the population, while 122.23 IJs insect^{-1} of *H. indica* were required to cause the same mortality rate.

The slopes of the concentration-mortality curves differed, with the *H. indica* curve having the higher slope (1.811) and the *S. carpocapsae* curve having the lower slope (1.106) (Table 3). The viability data of *S. carpocapsae* in the times studied presented sphericity (W = 0.73762; p = 0.16625), that is, they presented similar variances in time.

3.2 *Persistence of Steinernema carpocapsae in soil in n a greenhouse*

S. carpocapsae presented pathogenicity on *N. elegantalis* pre-pupae in pots. Analyzing over time, it is verified that there was a statistical difference between the times studied, and that on the 24th day which the mortality decreased significantly (Figure 1).

Figure 1. Percentage of pre-pupal mortality of *Neoleucinodes elegantalis* infected by *Steinernema carpocapsae.*



4. DISCUSSÃO

At all concentrations or times studied, *S. carpocapsae* was superior to *H. indica* on the pathogenicity of *N. elegantalis*

The superiority of a species to a given goal may be due to several factors. Among these factors, it is possible that *S. carpocapsae* has better adaptability to *N. elegantalis* pre-pupae than *H. indica*, mainly due to the infection mode of this species. According to Almenara et al. (2012), *S. carpocapsae* infection occurs by ambush, in which, the juveniles positions itself to intercept an insect passing close to it, unlike the juveniles of *Heterorhabditis*, which have a search

behaviour, wandering through the water of the soil until finding an insect. Thus, the nematode species most suitable for the management of *N. elegantalis* is *S. carpocapsae*, since this species caused mortality of more than 70% from the 1st day after inoculation, reaching a mortality rate higher than 90% on the 3rd day.

The high mortality values found in the first evaluations may be associated with the ability of these nematodes to adapt to the soil. In addition, *S. carpocapsae* remained viable in the soil for 24 days, providing mortality of *N. elegantalis*. However, environmental conditions such as temperature and humidity may favor or decrease viability in EPNs in soil (SHAPIRO-ILAN et al., 2006).

The factors that influence the persistence of nematodes in the soil are temperature, humidity, soil texture, ultraviolet radiation and host presence (SHAPIRO-ILAN et al., 2006). The ideal temperature for nematode survival in the soil, which leads to better results, is between 15 ºC and 28 ºC (Akhurst & Smith, 2002). The soil was kept moist throughout the experiment, providing conditions for the nematodes to remain viable and to move for the evaluated period (24 days) (GRANT and VILLANI, 2003).

Thus, in spite of all the factors that directly influence the persistence of EPNs in the soil, the nematodes presented a considerably long time of viability in the soil, showing itself as an alternative in the management of *N. elegantalis*.

The present work is the first study to report the efficacy of entomopathogenic nematodes in *N. elegantalis*. These findings may lead to an improvement in the integrated pest management (IPM) because the present study is applicable for implementation under field conditions. In addition, the present study indicates a new opportunity to control *N. elegantalis*, which may help to reduce the use of pesticides in tomato plantations.

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