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SUSCEPTIBILITY OF *Elasmopalpus lignosellus* PUPAE TO ENTOMOPATHOGENIC NEMATODES IN MAIZE

Abstract – Virulence and concentration of entomopathogenic nematodes (NEPs) in *Elasmopalpus lignosellus* pupae were evaluated. In the laboratory, the virulence of the isolates *Heterorhabditis amazonensis* MC01, *H. amazonensis* JPM3, *H. amazonensis* GL, *Steinernema carpocapsae* All and *Heterorhabditis* sp. Nepet 11 was evaluated and, subsequently, *H. amazonensis* GL was applied at concentrations of 8; 16; 24 and 32 IJ cm⁻². The EPNs were applied to Petri dishes containing ten pupae with five replications. Mortality was assessed every 24 hours for three days. In a greenhouse, *H. amazonensis* GL was tested at concentrations 24, 25, 26 and 27 IJ cm⁻². The IJs were applied in pots containing a 20-cm high ‘BM 3061’ maize plant, besides six pupae with four replications. Knowing that the efficiency of EPNs is directly related to the ability to search and penetrate the host, it was found that *H. amazonensis* GL is highly virulent to *E. lignosellus*, presenting an LC₅₀ of 6.49 IJ cm⁻² after 48 hours, 5.61 IJ cm⁻² in 72 hours and LC₉₀ of 39.70 IJ cm⁻² in 48 and 27.73 IJ cm⁻² in 72 hours under laboratory conditions. In the soil, pupal mortality was lower and a concentration of 25 IJ cm⁻² was responsible for the death of 50% of the population, since environmental variability influences the dynamics of IJ infection and insect defense.

Keywords: Biological control, *Heterorhabditis*, integrated pest management, plant protection, *Steinernema*.

SUSCETIBILIDADE DE PUPAS DE *Elasmopalpus lignosellus* A NEMATÓIDES ENTOMOPATOGÊNICOS NO MILHO

Resumo – Avaliou-se a virulência e concentração de nematoides entomopatogênicos (NEPs) em pupas de *Elasmopalpus lignosellus*. Em laboratório a virulência dos isolados *Heterorhabditis amazonensis* MC01, *H. amazonensis* JPM3, *H. amazonensis* GL, *Steinernema carpocapsae* All e *Heterorhabditis* sp. Nepet 11 foi avaliada e, posteriormente, *H. amazonensis* GL foi aplicado nas concentrações de 8; 16; 24 e 32 IJ cm⁻². Os NEPs foram aplicados em placas de Petri contendo dez pupas com cinco repetições. A mortalidade foi avaliada a cada 24 h por três dias. Em casa de vegetação, *H. amazonensis* GL foi testado nas concentrações 24, 25, 26 e 27 IJ cm⁻². Os IJs foram aplicados em vasos contendo uma planta de milho cultivar BM 3061 com cerca de 20 cm de altura, e seis pupas com quatro repetições. Sabendo-se que a eficiência dos NEPs está diretamente relacionada com a capacidade de busca e penetração no hospedeiro, verificou-se que *H. amazonensis* GL é altamente virulento à *E. lignosellus*, apresentando CL₅₀ de 6,49 IJ cm⁻² após 48 horas e de 5,61 IJ cm⁻² em 72 horas e CL₉₀ de 39,70 IJ cm⁻² em 48 e 27,73 IJ cm⁻² em 72 horas nas condições de laboratório. No solo a mortalidade de pupas se mostrou inferior, sendo que uma concentração de 25 IJ cm⁻² foi responsável pela morte de 50% da população, já que as variabilidades ambientais influenciam na dinâmica de infecção dos IJs e de defesa dos insetos.

Palavras-chave: Controle biológico, *Heterorhabditis*, manejo integrado de pragas, proteção de plantas, *Steinernema*.

The lesser cornstalk borer *Elasmopalpus lignosellus* Zeller (Lepidoptera: Pyralidae) is an underground pest that occurs from the Southern United States to South America (Viana, 2011). In tropical and subtropical regions, it is considered one of the main pests of maize, attacking the plant at its initial development stage (Chittenden, 1980). Initially, even in the first instars, they feed on new leaves and then penetrate the collar region, making a gallery inside the stem, causing the plant to die (Dupree, 1965). Despite extensive knowledge about the species, management practices are still based on chemical control through seed treatment. However, in areas with low soil moisture, the effectiveness of these products is impaired as, like some herbicides, they require moisture to yield an effective control. These conditions are favorable to the appearance of the caterpillar (Viana, 2011). In addition, chemical control, when misused, can select resistant populations of the insect, causing deleterious effects to the environment, animal health and increased production costs (Dalvi et al., 2011; Zorzetti et al., 2017).

Research has shown interest in using biocontrol agents such as *Trichogramma pretiosum* Riley (Hymenoptera: Trichogrammatidae) and *Bacillus thuringiensis* Berliner (Xavier et al., 2011; Moar et al., 1995). Nevertheless, the impact on lesser cornstalk borer was considered low, since it is sheltered in the plant stem while feeding, or protected by the web shelter that it builds in the soil (Viana, 2011).

The potential use of entomopathogenic

nematodes (NEPs) in biological control has already been identified for different insect orders (Woltz et al., 2015; Giometti et al., 2011; Ma et al., 2013). However, it has been shown to occur more efficiently in insects that have at least one stage of their development in the soil, since it is in this environment that nematodes live (Tavares et al., 2007). Two nematode families are considered to have entomopathogenic potential: Steinernematidae and Heterorhabditidae (Nguyen & Hunt, 2007). Infective juveniles (JI) penetrate the host insect through its natural openings (mouth, spiracles and anal and genital pores) and release symbiotic bacteria that produce a wide range of hydrolytic toxins and exoenzymes, which kill the host insect by sepsis, leaving the nematode immersed in the material from which it feeds, enabling its growth and multiplication (Dolinski, 2006; Kaya & Gaugler, 1993).

The use of entomopathogenic organisms for biological control has vast potential, requiring a local search for organisms adapted to climatic conditions and the insect pest (Spiridonov, 2017). Therefore, the objective of this study was to evaluate species of entomopathogenic nematodes (EPNs) with potential to control *E. lignosellus* pupae and to adjust the application concentration of the most virulent nematode.

Material and Methods

The experiments were conducted at Universidade Federal de Uberlândia, Umuarama Campus, 18°53'40''S and 48°15'35''W.

The nematodes were obtained from the entomopathogen bank of the Entomology Laboratory of Universidade Federal de Uberlândia, Monte Carmelo Campus.

The IJs were multiplied in *Tenebrio molitor* L. (Coleoptera: Tenebrionidae) larvae, created according to Potrich et al. (2007). *T. molitor* larvae that showed symptoms of infection were washed with water and placed in a dry chamber for 5 days, after which they were removed and placed in White (1927) traps to collect the IJs, according to Molina and López (2001). IJs with up to three days of emergence were used and stored in a B.O.D. chamber at $16 \pm 2^\circ\text{C}$ for up to 5 days.

Elasmopalpus lignosellus pupae were created in the laboratory of Universidade Federal de Uberlândia. Fifteen adult couples were initially placed in PVC cages lined with filter paper, where they were fed 10% aqueous honey solution. The papers with layings were removed every two days and stored in plastic covered gerbox plates. Daily, as the larvae hatched, first-instar caterpillars were transferred to 100-mL plastic pots containing Chalfant's artificial diet (Chalfant, 1975), modified without using tetracycline, and Vanderzant's mixture, besides the addition of 0.2 g of benzoic acid and 2 mL of maize oil. On the caterpillars, a layer of approximately 2 cm of autoclaved vermiculite (120°C , 1 atm, 20 min) was added in order to make the breeding environment similar to the conditions that the insect finds in nature for the construction of larval shelters. After 35 days, the

pupae were removed for use in the tests.

Selection of entomopathogenic nematodes to *Elasmopalpus lignosellus* pupae

Five nematode isolates, four of the *Heterorhabditis* genus and one *Steinernema*, were evaluated for virulence on *E. lignosellus* pupae.

The assay had six treatments, with the following isolates: *Heterorhabditis amazonensis* MC01; *H. amazonensis* JPM3; *H. amazonensis* GL; *Steinernema carpocapsae* All; *Heterorhabditis* sp. Nepet 11 and the control. Each plot received ten *E. lignosellus* pupae arranged in glass Petri dishes (9 cm in diameter) lined with two sheets of filter paper. In each plate, 1 mL of suspension of the respective isolate was applied at a concentration of 16 IJ cm^{-2} , and distilled water was used for the control. For the preparation of the suspensions, the amount of IJ in each μL of the pre-existing suspensions arranged in ELISA plates was counted, with the aid of a stereomicroscope.

The plates were closed with plastic paraffin film and kept in a B.O.D. chamber at $25 \pm 2^\circ\text{C}$, 70% RH and 24 hours in the dark. Mortality was assessed after 24, 48 and 72 hours. The dead pupae were kept in B.O.D. at $25 \pm 2^\circ\text{C}$ for four days for subsequent dissection. They were then observed under a stereomicroscope to confirm mortality.

Five replications were performed, totaling 30 plots distributed in a completely randomized

design. Data were submitted to analysis of variance (ANOVA) ($p < 0.01$) with comparison between the means obtained by the Tukey test ($p < 0.05$). The assumptions of homoscedasticity, normality and independence of the residues were tested before the application of ANOVA.

Adequacy of *H. amazonensis* GL concentration for the control of *Elasmopalpus lignosellus* pupae

In the laboratory, an experiment was conducted under the same conditions as the previous test and had four concentrations: 8; 16; 24 and 32 IJ cm⁻² of *H. amazonensis* GL in *E. lignosellus* pupae and the control (distilled water).

In each Petri dish, 1 mL of aqueous suspension was applied at the respective concentrations and, in the control, only distilled water. Dead pupae were counted after 24, 48 and 72 hours. To confirm mortality by the nematode, the dishes were maintained in B.O.D. at 24 ± 2 °C for four days, and the pupae were subsequently dissected, observing the presence of nematodes with the aid of a stereomicroscope.

Five replications were performed, totaling 25 plates arranged in a completely randomized design. Data were adjusted to a Generalized Linear Model with binomial distribution and probit link function, with the effect of the concentrations verified by the Chi-Square test ($p < 0.01$). Subsequently, the data were adjusted to probit analysis to estimate the lethal concentrations of 50% (LC₅₀) and 90%

(LC₉₀) of the population and adjustment of the regression curves. The significance of the estimated coefficients was also assessed using the Chi-square test.

In a greenhouse, untreated 'BM 3061' maize seeds were sown in 2-L plastic pots containing approximately 1.5 kg of sieved dark-red latosol (LEd) (Santos et al., 2018). In each pot, four seeds were sown and, after emergence, thinning was carried out, leaving one plant per pot.

For fertilization calculations, a soil sample was taken for chemical analysis and the calculations were made according to the 5th approximation (Ribeiro et al., 1999). Approximately 1.8 g of the formulated 4-14-8, corresponding to 750 kg ha⁻¹, were used as a fertilizer. During the initial plant development, until reaching 20 cm, the pots were irrigated daily, keeping the soil moisture close to field capacity. After the application of the IJs, the pots did not receive irrigation and the plants did not show signs of water stress.

The trial had five treatments with four concentrations of *H. amazonensis* GL: 24, 25, 26 and 27 IJ cm⁻², and the control treatment. The tested concentrations were extrapolated to the pot area, starting from LC₅₀ and LC₉₀ found in the laboratory.

Fifteen days after sowing, when the plants reached about 20 cm in height, six pupae were buried in each pot at a depth of 3 cm. At this point, the treatments were sprayed onto the soil.

After five days, the percentage of pupal

mortality caused by the nematode was verified. Each treatment was performed with four replications, totaling 20 pots distributed in a completely randomized design. The data were adjusted to a Generalized Linear Model with binomial distribution and logit link function, with the effect of the concentrations verified by the deviance analysis ($p < 0.01$). With significant differences detected, the means were compared using the Tukey test ($p < 0.05$).

Results and Discussion

Selection of entomopathogenic nematode isolates to *Elasmopalpus lignosellus* pupae

No pupal mortality was identified 24 hours after the application of the isolates. After 48 and 72 hours, the mortality caused by the isolates differed statistically by the ANOVA ($F_c = 85.67$, GL-treatment = 5, GL-residue = 24 and P-value = 0.00). The data met the statistical assumptions for the application of the parametric test.

Pupal mortality differed statistically from the isolates in the control treatment, indicating that all nematodes were virulent to *E. lignosellus* pupae. However, under the conditions tested, *H. amazonensis* GL was more virulent when compared to the others, causing a mortality of 94% pupae in 48 hours and 96% after 72 hours (Table 1).

Under the same conditions, Stuart et al. (1997) also found a mortality of 90% of *Dysmicoccus vacinii* Miller (Hemiptera:

Pseudococcidae) using species of the *Heterorhabditis* genus. However, other experiments have shown that the mortality caused by *Heterorhabditis* nematodes varies according to the host insect. Machado et al. (2015) found a reduction of up to 25% in the pupal population of *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) when exposed to *H. amazonensis* GL for 72 hours. Salvadori et al. (2012) observed a mortality of up to 77% of *S. frugiperda* when exposed to *Heterorhabditis* for five days. This variation reinforces the need for selection tests of isolates, due to the characteristics and adaptations that each isolate has in relation to the environment and the host (Gaugler et al., 1997).

Adequacy of *H. amazonensis* GL concentration for the control of *Elasmopalpus lignosellus* pupae

No pupal mortality was identified 24 hours after treatment application. After 48 and 72 hours, differences were observed between mortality caused by the control and by the other concentrations tested. The data collected in the two periods showed a significant effect ($F_c = 112.33$, GL treatment = 4, GL-residue = 20 and P-value = 0.00) for the tested concentrations, when adjusted to the Generalized Linear Model.

A positive relationship was observed between the different concentrations of the *H. amazonensis* GL isolate and the mortality of *E. lignosellus* pupae (Figure 1). Probit analysis found that the LC_{50} was 6.49 IJ cm^{-2} in 48 hours and

Table 1 -Mortality of *Elasmopalpus lignosellus* pupae after 48 and 72 hours of application of EPN isolates.

| Treatment | Mortality 48 h (%) * | Mortality 72 h (%) * |
|-----------------------------------------|----------------------|----------------------|
| <i>Heterorhabditis amazonensis</i> GL | 94.0 ± 2.45 a | 96.0 ± 2.45 a |
| <i>Heterorhabditis</i> sp. Nepet 11 | 76.0 ± 5.09 b | 80.0 ± 5.47 a b |
| <i>Heterorhabditis amazonensis</i> JPM3 | 74.0 ± 2.45 b | 82.0 ± 3.74 a b |
| <i>Steinernema carpocapsae</i> All | 68.0 ± 3.74 b | 72.0 ± 3.74 c |
| <i>Heterorhabditis amazonensis</i> MC01 | 62.0 ± 3.74 b | 68.0 ± 3.74 c |
| Control | 2.0 ± 2.00 c | 2.0 ± 2.00 c |
| CV (%) | 12.19 | 12.40 |

*Means followed by the same letters do not differ by the Tukey test at 5% probability. Mean ± Standard Error.

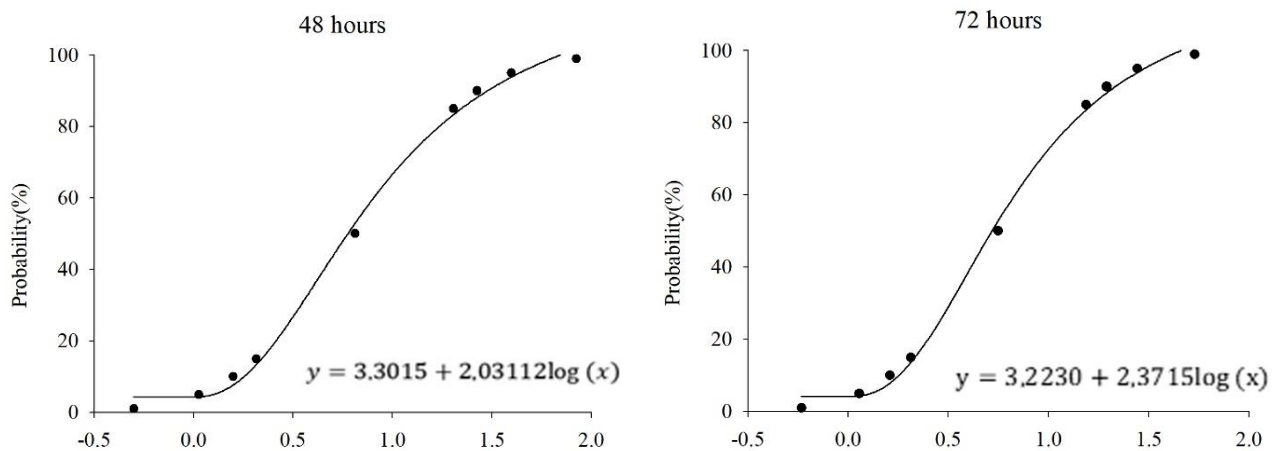


Figure 1 – Graphs of the concentration-mortality curve of *Elasmopalpus lignosellus* pupae as a function of *Heterorhabditis amazonensis* GL concentrations after 48 and 72 hours.

regressions were significant at 0.01 significance by the Chi-square test.

In a similar test, a reduction of more than 90% in the pupal population of *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) was identified, when exposed to *H. baujardi* LPP7 (Minas, 2008). Under the same conditions,

Santos et al. (2011) observed that the mortality of *Diabrotica speciosa* (Germar) (Coleoptera: Chrysomelidae) pupae increased as the concentration of *Heterorhabditis* sp. reached rates above 80%. In addition, they found a reduction in population mortality from the concentration of 31 IJ cm⁻².

5.61 IJ cm⁻² in 72 hours. For LC₉₀, concentrations of 39.70 IJ cm⁻² were observed in 48 hours and 27.73 IJ cm⁻² in 72 hours. Both adjusted Such behavior can be explained by a possible competition between nematodes, interfering in infection rates (Selvan et al., 1993).

Therefore, it can be inferred that the increased concentration of nematodes does not always cause higher host mortality, since there may be a tendency for IJs to be more attracted to insects previously infected by the same organism (Lewis et al., 2002).

In the greenhouse, mortality was statistically different (p-value < 0.001). The concentrations of 25 and 26 IJ cm⁻² did not differ between each other and showed a reduction between 33% and 50% in *E. lignosellus* pupal population (Table 2).

The maximum reduction in *E. lignosellus* pupal population caused by *H. amazonensis* GL in a greenhouse was lower than that identified in the laboratory, indicating that there is an

influence of the environment, both in the search process and infection of IJs in the hosts and defense strategies of insects to nematodes.

The efficiency of IJs on a given host is directly linked to their ability in search, penetration, pathogenicity and reproduction, bypassing the natural resistance of the insect immune system. Therefore, in view of this wide variability, each isolate should be tested on specific hosts, conditions and dosages (Lewis et al., 2006).

Andaló et al. (2010) identified a 97.6% mortality of *Spodoptera frugiperda* (Smith) (Lepidoptera: Noctuidae) in the laboratory when exposed to *Heterorhabditis* sp. RSC02; this value was slightly lower (87.5%) in a greenhouse using the same nematode at the same concentration. Therefore, due to the complexity of factors in agricultural systems with constant use of bioinsecticides, where pests are regulated by other pathogens, studies must be adjusted to confirm the potential of this control method

Table 2 – Mean mortality of *Elasmopalpus lignosellus* pupae inoculated with different *Heterorhabditis amazonensis* GL concentrations in a greenhouse.

| <i>Heterorhabditis amazonensis</i> GL (IJ cm ⁻²) | Mortality (%) |
|--------------------------------------------------------------|-----------------|
| 25 | 50.00 ± 10.20 a |
| 26 | 33.33 ± 9.62 ab |
| 24 | 12.50 ± 6.75 bc |
| 27 | 8.33 ± 5.64 bc |
| 0 | 0.00 ± 0.00 c |

*Means +/- standard error followed by the same letters do not differ by the Tukey test at 5% probability.

(Garcia et al., 2008).

The potential use of NEPs for the control of *E. lignosellus* was confirmed by this research. However, field tests must be carried out in order to understand the dynamics and behavior, both of the nematodes and of the insects on the various existing variables.

Conclusions

The isolate *H. amazonensis* GL showed high virulence to *E. lignosellus* pupae under the tested conditions. In addition, pupal mortality by entomopathogenic nematodes occurs 48 hours after inoculation.

The concentration of IJ in the laboratory was directly proportional to pupal mortality, with an LC_{50} estimate of 6.49 IJ cm^{-2} in 48 hours and 5.61 IJ cm^{-2} in 72 hours.

In the greenhouse, a lower pupal mortality was observed, since the variability existing in the environment can influence the dynamics of infection and defense of the nematodes and hosts, respectively. The concentration of 25 IJ cm^{-2} caused the highest percentage of mortality (50%).

References

- ANDALÓ, V.; SANTOS, V.; MOREIRA, G. F.; MOREIRA, C. C.; MOINO JUNIOR, A. Evaluation of entomopathogenic nematodes under laboratory and greenhouses conditions for the control of *Spodoptera frugiperda*. **Ciência Rural**, v. 40, n. 9, p. 1860-1866, 2010. DOI: [10.1590/S0103-84782010005000151](https://doi.org/10.1590/S0103-84782010005000151)
- CHALFANT, R. B. A. Simplified technique for rearing the lesser cornstalk borer (Lepidoptera: Phycitidae). **Journal of the Georgia Entomological Society**, v. 10, p. 33-37, 1975.
- CHITTENDEN, F. H. The smaller cornstalk borer (*Elasmopalpus lignosellus* Zell.). **Division Entomology Bulletin**, v. 23, p. 17-22, 1980.
- DALVI, L. P.; ANDRADE, G. S.; PRATISSOLI, D.; POLANCZYK, R. A.; MELO, R. L. DE. Compatibility of biological agents to control *Spodoptera frugiperda* (Lepidoptera: Noctuidae). **Agrarian**, v. 4, n. 12, p. 79-83, 2011.
- DOLINSKI, C. Nematoides como agentes do controle biológico de insetos. In: OLIVEIRA FILHO, E. C.; MONNERAT, R. G. Fundamentos para regulação de semioquímicos inimigos naturais e agentes microbiológicos de controle de pragas. Embrapa, Brasília, Brazil, p. 73-101, 2006.
- DUPREE, M. Observations on the life history of the Lesser cornstalk borer. **Journal of Economic Entomology**, v. 58, n. 6, p. 1156-1157, 1965. DOI: [10.1093/jee/58.6.1156](https://doi.org/10.1093/jee/58.6.1156)
- GARCIA, L. C.; RAETANO, C. G.; LEITE, L. G. Tecnologia de aplicação para os nematóides *Heterorhabditis* indica

- e *Steinernema* sp. (Rhabditida: Heterorhabditidae e Steinernematidae) para controle de *Spodoptera frugiperda* (Smith) (Lepidoptera: Noctuidae) na cultura do milho. **Neotropical Entomology**, v. 37, n. 3, p. 305-311, 2008. DOI: [10.1590/S1519-566X2008000300010](https://doi.org/10.1590/S1519-566X2008000300010)
- GAUGLER, R.; LEWIS, E.; STUART, R. J. Ecology in the service of biological control: the case of entomopathogenic nematodes. **Oecologia**, v. 4, n. 109, p. 483- 489, 1997. DOI: [10.1007/s004420050108](https://doi.org/10.1007/s004420050108).
- GIOMETTI, F. H. C. et al. Virulência de nematóides entomopatogênicos (Nematoda: Rhabditida) a *Sphenophorus levis* (Coleoptera). **Bragantia**, v. 70, n. 1, p. 81-86, 2011. DOI: [10.1590/S0006-87052011000100013](https://doi.org/10.1590/S0006-87052011000100013)
- KAYA, H. K.; GAUGLER, R. Entomopathogenic nematodes. **Annual Review of Entomology**, v. 38, p. 181-206, 1993. DOI: [10.1146/annurev.en.38.010193.001145](https://doi.org/10.1146/annurev.en.38.010193.001145).
- LEWIS, E. E.; BARBAROSA, B.; GAUGLER, R. 2002. Mating and sexual communication of *Steinernema carpocapsae* (Nemata: Steinernematidae). **Journal of Nematology**, v. 34, n. 4, p. 328-331, 2002.
- LEWIS, E. E.; CAMPBELL, J.; GRIFFIN, C.; KAYA, H.; PETERS, A. Behavioral ecology of entomopathogenic nematodes. **Biological Control**, v. 38, n. 1, p. 66-79, 2006. DOI: [10.1016/j.biocontrol.2005.11.007](https://doi.org/10.1016/j.biocontrol.2005.11.007)
- MA, J. et al. Efficacy of entomopathogenic nematodes (Rhabditida: Steinernematidae and Heterorhabditidae) against the chive gnat, *Bradysia odoriphaga*. **Journal of Pest Science**, v. 86, n. 3, p. 551-561, 19 abr. 2013. DOI: [10.1007/s10340-013-0497-7](https://doi.org/10.1007/s10340-013-0497-7).
- MACHADO, F. A. O.; GUZZARDI, M.; MIEKO, J.; FARIA, L. S. DE; MENDES, S. M.; ASSIS, G. A.; ARAÚJO, V.; ANDALÓ, V. Seleção de nematóides entomopatogênicos visando o controle de pupas de *Helicoverpa armigera*. 2015. Disponível em: <<https://ainfo.cnptia.embrapa.br/digital/bitstream/item/125884/1/Selecao-nematoides.pdf>>. Acesso em: 20 jul. 2018.
- MINAS, R. S. DE. Potencial dos nematóides entomopatogênicos como agentes de controle biológico da mosca-domediterrâneo *Ceratitis capitata* (Wied.) (Diptera: Tephritidae). 93p. (Tese de doutorado) - Universidade Estadual do Norte Fluminense Darcy Ribeiro, Campos dos Goytacazes, Brasil, 2008.
- MOAR, W. J.; PUSZTAI-CAREY, M.; MACK, T. P. Toxicity of purified proteins and the HD-1 strain from *Bacillus thuringiensis* against Lesser Cornstalk Borer (Lepidoptera: Pyralidae). **Journal of Economic Entomology**, v. 88, n. 3, p. 606-609, 1995. DOI: [10.1093/jee/88.3.606](https://doi.org/10.1093/jee/88.3.606).
- MOLINA, J. P.; LÓPEZ, N. J. C. Producción in vivo de tres entomonematodos con dos sistemas de infección en dos hospedantes. **Revista**

- Colombiana de Entomología**, v. 27, n. 1-2, p. 73-78, 2001.
- NGUYEN, K. B.; HUNT, D. J. Entomopathogenic nematodes: Systematics, Phylogeny and bacterial symbionts. Brill, Boston, EUA. 816 p., 2007.
- POTRICH, T. D.; LORINI, I.; VOSS, M.; STEFFENS, M. C. S.; PAVANI, D. P. Metodologia de criação de *Tenebrio molitor* em laboratório para obtenção de larvas. 2007. http://www.cnpt.embrapa.br/biblio/do/p_do82_12.htm.
- RIBEIRO, A. C.; GUIMARÃES, P. T. G.; ALVAREZ, V. A. H. Recomendação para uso de corretivos e fertilizantes em Mina Gerais – 5º aproximação. Viçosa, Brazil. 359 p. 1999.
- SALVADORI, J. D.; DEFFERRARI, M. S.; LIGABUE-BRAUN, R.; LAU, E. Y.; SALVADORI, J. R.; CARLINI, C. R. Characterization of entomopathogenic nematodes and symbiotic bacteria active against *Spodoptera frugiperda* (Lepidoptera: Noctuidae) and contribution of bacterial urease to the insecticidal effect. **Biological Control**, v. 63, n. 3, p. 253-263, 2012. DOI: [10.1016/j.biocontrol.2012.08.002](https://doi.org/10.1016/j.biocontrol.2012.08.002)
- SANTOS, H. G. DOS; JACOMINE, P. K. T.; ANJOS, L. H. C. DOS; OLIVEIRA, V. A. DE; LUMBRERAS, J. F.; COELHO, M. R.; ALMEIDA, J. A. DE; ARAÚJO FILHO, J. C. DE; OLIVEIRA, J. B. DE; CUNHA, T. J. F. **Sistema brasileiro de classificação de solos**. Embrapa, Rio de Janeiro, Brazil. 306 p. 2018.
- SANTOS, V.; MOINO JUNIOR, A.; ANDALÓ, V.; MOREIRA, C. C.; OLINDA, R. A. DE. Virulência de nematóides entomopatogênicos (Rhabditida: Steinernematidae e Heterorhabditidae) para o controle de *Diabrotica speciosa* germar (Coleoptera: Chrysomelidae). **Ciência e agrotecnologia**, v. 35, n. 6, p. 1149-1156, 2011. DOI: [10.1590/S1413-70542011000600015](https://doi.org/10.1590/S1413-70542011000600015)
- SELVAN, S.; CAMPBELL, J. F.; GAUGLER, R. 1993. Density-dependent effects on entomopathogenic nematodes (Heterorhabditidae and Steinernematidae) within an insect host. **Journal of Invertebrate Pathology**, v. 62, n. 3, p. 278-284, 1993. DOI: [10.1006/jjpa.1993.1113](https://doi.org/10.1006/jjpa.1993.1113)
- SPIRIDONOV, S. E. Entomopathogenic nematodes of the families Steinernematidae and Heterorhabditidae: morphology and taxonomy. In: Askary, T.H. (Ed.). **Biocontrol agents: entomopathogenic and slug parasitic nematodes**. CABI, Boston, EUA. p. 45-62, 2017.
- STUART, R. J.; POLAVARAPU, S.; LEWIS, E. E.; GAUGLER, R. Differential susceptibility of *Dysmicoccus vacinni* (Homoptera: Pseudococcidae) to entomopathogenic nematodes (Rhabdita: Heterorhabditidae and Steinernematidae). **Journal of Economic**

- Entomology**, v. 90, p. 925-932, 1997. DOI: [10.1093/jee/90.4.925](https://doi.org/10.1093/jee/90.4.925).
- TAVARES, F. M.; BATISTA FILHO, A.; LEITE, L.G.; ALMEIDA, L.C.; SILVA, A.C.; AMBRÓS, C.M.G. Efeito de *Heterorhabditis indica* e *Steinernema* sp. (Nemata: Rhabditida) sobre larvas do bicudo da cana-deaçúcar, *Sphenophorus levis* (Coleoptera: Curculionidae), em laboratório e casa-de-vegetação. **Nematologia Brasileira**, v. 31, n. 1, p. 12-19, 2007.
- VIANA, P. A. Principais pragas subterrâneas do milho no Brasil. Embrapa, Sete Lagoas, Brazil. 61 p., 2011. Disponível em: <<https://www.embrapa.br/busca-de-publicacoes/-/publicacao/914556/principais-pragas-subterraneas-do-milho-no-brasil>>. Acesso em: 20 out. 2018.
- WHITE, G. F. A method for obtaining infective nematode larvae from cultures. **Science**, v. 66, p.302-303, 1927. DOI: [10.1126/science.66.1709.302-a](https://doi.org/10.1126/science.66.1709.302-a).
- XAVIER, L.M.S., LAUMANN, R.A., BORGES, M., MAGALHÃES, D.M., VILELA, E.F., BLASSIOLI-MORAES, M.C. *Trichogramma pretiosum* attraction due to the *Elasmopalpus lignosellus* damage in maize. **Pesquisa Agropecuária Brasileira**, v.46, p.578-585, 2011. DOI: [10.1590/S0100-204X2011000600002](https://doi.org/10.1590/S0100-204X2011000600002)
- ZORZETTI, J.; RICCIETTO, A. P. S.; FAZION, F. A. P.; MENEGUIM, A. M.; NEVES, P. M. O. J.; VILAS-BÔAS, G. T. Isolation and characterization of *Bacillus thuringiensis* strains active against *Elasmopalpus lignosellus* (Zeller, 1848) (Lepidoptera, Pyralidae). **Acta Scientiarum Agronomy**, v. 39, p. 417-425, 2017. DOI: [10.4025/actasciagron.v39i4.32707](https://doi.org/10.4025/actasciagron.v39i4.32707).
- WOLTZ, J. M.; DONAHUE, K. M.; BRUCK, D. J.; LEE, J. C. Efficacy of commercially available predators, nematodes and fungal entomopathogens for augmentative control of *Drosophila suzukii*. **Journal Of Applied Entomology**, v. 139, n. 10, p. 759-770, 2015. DOI: [10.1111/jen.12200](https://doi.org/10.1111/jen.12200).