Susceptibility of *Elasmopalpus lignosellus* (Lepidoptera: Pyralidae) larvae to entomopathogenic nematodes (Rhabditida)[1]

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10.1590/0034-737X2024710028

ABSTRACT

The cornstalk borer, *Elasmopalpus lignosellus* (Lepidoptera: Pyralidae), reduces the productive potential of maize crops and is a difficult pest to manage. A management program using different methods could improve the control of *E. lignosellus*. Considering the potential of entomopathogenic nematodes (EPN) in reducing insect pest populations in soil, the objective of this study was to evaluate the virulence of these EPN and adjust their concentration for controlling *E. lignosellus* larvae under laboratory and greenhouse conditions. In the laboratory, the virulence of five EPN populations was tested; then, *Heterorhabditis amazonensis* MC01 was tested at four concentrations. In the greenhouse, *H. amazonensis* MC01 was tested at four concentrations and was applied to vessels containing maize plants and six larvae. After five days, mortality was evaluated, and means were compared using Tukey's test (p-value < 0.05). *Heterorhabditis amazonensis* MC01 and *S. carpocapsae* All were equally virulent, reducing the larva population by more than 90%. The concentration of *H. amazonensis* MC01 that caused the highest mortality of larvae in the laboratory was 182 infective juveniles (IJ) larva^{-1}. In the greenhouse, the nematode was also considered virulent to *E. lignosellus* since all concentrations tested caused larval mortality greater than 70%.

Keywords: biological control; *Heterorhabditis*; lesser cornstalk borer; plant protection; *Steinernema*.

INTRODUCTION

The lesser cornstalk borer, *Elasmopalpus lignosellus* (Zeller) (Lepidoptera: Pyralidae), feeds on several plant species of high economic value. The larvae penetrate the base of the plant in the initial stages of development of the culture, just below the ground, creating a gallery in the stem that can cause the breakage and death of the plants, resulting in stand reduction. Chemical control is the main method that has been used with seed treatment, but it is not always effective to reduce pest populations (Vieira *et al.*, 2020).

The larvae typically occur inside the seedlings or in cocoons made of web and soil particles, and they remain close to the stem, which makes handling them difficult. In the 1980s, new management strategies using natural enemies for the lesser cornstalk borer were developed (Jham *et al.*, 2005; 2007). Nevertheless, chemical control is still the most used method, even though its incorrect use may cause problems such as the development of resistant populations, reduction of natural enemies, and outbreaks of secondary pests (Neri *et al.*, 2005).

Among the organisms studied for *E. lignosellus* population control, entomopathogenic nematodes (EPN) have potential, as they inhabit the soil and have the ability to maintain their viability and remain active, controlling larvae in different instars (Grewal *et al.*, 2001). Moreover, they have a symbiotic association with bacteria that are released

Submitted on January 22nd, 2022 and accepted on March 24th, 2024.
[1] This work is part of the dissertation defended by the first author, Maria Eduarda Berlatto Magnabosco, in the Graduate Program in Qualidade Ambiental at the Universidade Federal de Uberlândia.
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into the hemolymph of the insect, causing its death (Cagnolo *et al.*, 2004; Dolinski, 2006). Georgis (1992) and Jaramillo & Saavedra (2007) highlighted the potential of using EPN as biological control agents for *E. lignosellus*, verifying the pest control potential in peanut and asparagus crops.

Before being field-tested, the virulence of EPN against target insects must be evaluated, as nematode populations cause different mortality rates in different hosts. In addition, the optimal concentration of EPN that cause pest mortality also varies (Kaya & Hara, 1981; Fuxa *et al.*, 1988). Therefore, the objective of the present study was to select EPN with potential to control *E. lignosellus* larvae and, depending on the laboratory virulence data, adjust the concentration of the most virulent nematode to be applied under laboratory and greenhouse conditions.

MATERIALS AND METHODS

The experiments were performed at the Federal University of Uberlândia, Umuarama Campus (18°53'40''S, 48°15'35''W). Nematodes were multiplied in *Tenebrio molitor* L. (Coleoptera: Tenebrionidae) larvae raised following Potrich *et al.* (2007). Dead *T. molitor* larvae were washed with water and placed in a dry chamber (petri dish with filter paper) for five days. After drying, they were removed and placed in White traps (1927) to collect infective juveniles (IJ), following Molina & López (2001). Infected larvae were maintained in a biochemical oxygen demand (B.O.D.) incubator at 26 ± 2 °C. IJ were used up to three days after emergence and stored at 16 ± 2 °C for up to five days.

Fifteen adult *E. lignosellus* couples were placed in cylindrical PVC cages (15 cm × 20 cm) lined with filter paper, where the adults were fed with a 10% honey aqueous solution. Every two days, the papers containing the postures were removed and stored in capped plastic Gerbox® plates. Daily, as the eggs hatched, the first instar larva were transferred to 100-mL plastic pots containing modified Chalfant (1975) artificial diet, without tetracycline and Vanderzant's mixture, and added with 0.2 g of benzoic acid and 2 mL of corn oil. An approximately 2-cm layer of autoclaved vermiculite (120 °C, 1 atm, 20 min) was added over the larvae to make the breeding environment similar to the natural conditions used for the construction of larval shelters. After 15 days, the larvae were removed for use in the tests.

Virulence of entomopathogenic nematodes against larvae

Initially, the virulence of *Heterorhabditis amazonensis* MC01, *H. amazonensis* JPM3, *H. amazonensis* GL, *Steinernema carpocapsae* All, and *Heterorhabditis amazonensis* Nepet 11 against *E. lignosellus* larvae was analyzed under laboratory conditions. Ten second and third instar larvae were arranged in glass Petri dishes (9-cm diameter) lined with two sheets of filter paper containing an approximately 8-cm^3 block of artificial diet. For each case, 1 mL of nematode suspension was applied at a concentration of 100 IJ $pupa^{-1}$ per plate. A control treatment received only distilled water.

Five replications per treatment were performed in a completely randomized design. The plates were kept in a B.O.D. incubator at 25 ± 2 °C, 70% relative humidity (RH), and 24 h in the dark. Mortality assessments were performed after 24, 48 and 72 h.

Data with normal distribution and homoscedasticity were submitted to analysis of variance (ANOVA) with Tukey's test comparison between the means obtained for each nematode ($p < 0.05$). Data without normal distribution were fitted to the Generalized Linear Model with binomial distribution (ANODEV), and Tukey's test was used to compare the means ($p < 0.05$).

Concentration of Heterorhabditis amazonensis MC01 against larvae under laboratory conditions

To adjust the concentration of application of IJ, *H. amazonensis* MC01 was applied to *E. lignosellus* larvae at concentrations of 50, 100, 150, and 200 IJ $larva^{-1}$ on Petri dishes containing 10 third and fourth instar larvae. Control plates received only distilled water. The volume of suspension/water per plate was 1 mL at the respective concentrations with five replications in a completely randomized design, totaling 25 plates.

The experiments were kept in a B.O.D. incubator at 25 ± 2 °C, 70% RH, and 24 h in the dark. Dead larvae were counted after 24, 48, 72 and 96 h. Data were subjected to ANOVA and subsequent regression analysis using the software Sigma Plot v.12.0, after meeting the assumptions of normality of residuals and homoscedasticity.

Concentration of Heterorhabditis amazonensis MC01 against larvae under greenhouse conditions

Untreated corn seeds of the BM 3061 hybrid were sown

in 2-L plastic pots containing approximately 1.5 kg of sieved soil, classified as Dystrophic Dark Red Latosol (Embrapa, 2006). Four seeds were sown in each pot, thinning to one plant per pot after emergence. The pots were fertilized with approximately 1.8 g of the 4-14-8 formulated per pot (i.e., 750 kg ha^{-1}).

When the plant reached approximately 20 cm in height, six fourth instar larvae were released per pot, and then the suspensions containing four concentrations of *H. amazonensis* MC01 190, 210, 230, and 250 IJ $larva^{-1}$ were applied on the soil surface using an automatic pipette. A treatment was maintained without the application of nematodes as a control. Each treatment was performed with five replications, totaling 25 pots distributed in a completely randomized design. In order to prevent the larvae from escaping, the pots were protected by a metal structure covered with an anti-aphid screen.

Assessment was performed after five days, verifying the percentage of larva mortality caused by the nematode. Means were compared using Tukey's test ($p < 0.05$). In all tests, the dead larvae were kept in B.O.D. at 25 ± 2 °C in a dry chamber for four days for subsequent dissection, and then observed under a stereoscopic microscope to confirm nematode mortality.

RESULTS AND DISCUSSION

Virulence of entomopathogenic nematode isolates to larvae

After 24 h of application of the EPN isolates, none of the observed larvae were dead. At 48 and 72 h after application, statistical differences were found by ANOVA and ANODEV, respectively. After 48 h, all the tested nematodes caused mortality of the *E. lignosellus* larvae, differing from the control, in which low mortality from natural events was observed. In addition, no difference in virulence was observed between the isolates, all of which caused more than 60% mortality. After 72 h, the isolates that showed the highest virulence, with mortality > 90%, were *S. carpocapsae* All and *H. amazonensis* MC01, with no differences between them (Table 1).

Based on the obtained results, only *H. amazonensis* MC01 was selected for the subsequent tests, as it was isolated from the same region of the experiments and was thus more adapted to the local conditions than the other isolates. This isolate searches for the insect in the soil, with horizontal displacement of the soil (Campbell & Gaugler, 1997). Moreover, it has appendages on the cephalic region (Griffin *et al.*, 2005) that contribute to the penetration into the insect's body (Geden *et al.*, 1985; Andaló *et al.*, 2012).

Several studies have verified the action potential of steinermatids on lepidopteran larvae (Van Damme *et al.*, 2015; Kamali *et al.*, 2017). However, Steyn *et al.* (2019) highlighted the importance of performing studies that include *Heterorhabditis* in the isolation selection tests, as they observed *Heterorhabditis* spp. controlling *Holocyst capensis* Van Nieukerken & Geertsema (Lepidoptera: Heliozelidae). Kepenekci *et al.* (2013) also observed *H. bacteriophora* causing high mortality (80%) in larvae of *Phthorimaea operculella* (Zeller) (Lepidoptera: Gelechiidae).

Concentration of Heterorhabditis amazonensis MC01 against larvae under laboratory conditions

Significant differences were found by ANOVA in all hours evaluated after application of *H. amazonensis*. The mortality of *E. lignosellus* after 24 and 48 h of application of the nematode was low, but after 72 h, a mortality of 48% was observed for 50 and 100 IJ $larva^{-1}$, 60% for 150 IJ $larva^{-1}$, and 70% for 200 IJ $larva^{-1}$.

Table 1: *Elasmopalpus lignosellus* larvae' mean mortality after 48 and 72 h of application of entomopathogenic nematode isolates

Treatment	Mortality 48 h (%)*	Mortality 72 h (%)*
Heterorhabditis amazonensis MC01	72.0 ± 3.74 a	92.0 ± 3.83 a
Steinernema carpocapsae All	72.0 ± 3.74 a	90.0 ± 4.24 a
Heterorhabditis amazonensis Nepet 11	76.0 ± 5.09 a	80.0 ± 5.65 b
Heterorhabditis amazonensis GL	68.0 ± 3.74 a	72.0 ± 6.35 b
Heterorhabditis amazonensis JPM3	60.0 ± 3.16 a	68.0 ± 6.59 b
Control	2.0 ± 2.00 b	2.0 ± 1.98 c
CV (%)	14.17	13.96

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

For 72 and 96 h, the relationship between larvae mortality and isolate concentrations was adjusted by a quadratic regression model with coefficient of determination (R^2) of 91.32% and 90.31%, respectively. From the derivative of the parabola equation, maximum mortality was calculated at 190 IJ larva^{-1} for 72 h (66%) and at 182 IJ larva^{-1} for 96 h (78.7%) (Figure 1).

The pathogenicity and virulence of *H. amazonensis* GL were assessed for *E. lignosellus* pupae in the laboratory, with CL_{50} = 6.49 IJ/cm^2 after 48 h of nematode contact with pupae, and CL_{90} = 39.7 IJ/ cm^2 after 48 h in the laboratory (Magnabosco *et al.*, 2020). This shows that both *H. amazonensis* populations are virulent to *E. lignosellus* with potential for pest control.

Few studies have been performed on EPN with *E. lignosellus*. Nevertheless, concentration tests were performed for other lepidopteran larvae. *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) inoculated with 100 and 500 IJ of *H. bacteriophora* presented mortality between 12% and 30% after 72 h of exposure, reaching up to 50% after 96 h (Kary *et al.*, 2012). However, *S. frugiperda* exposed to different *Heterorhabditis* isolates at a concentration of 100 IJ larvae^{-1} had mortality rates between 40% and 85%, indicating the variability between the infection processes of different isolates at different dosages and specific hosts (Andaló *et al.*, 2010). Observing the results for pupae of *E. lignosellus*, *H. armigera*, and *S. frugiperda* and the variations between the concentrations, we reinforce the importance of specific tests between entomopathogen and host.

A reduction in maximum mortality values was also observed in other studies and can be explained by a possible competition between nematodes interfering with infection rates (Selvan *et al.*, 1993; Giometti *et al.*, 2011; Santos *et al.*, 2011; Rohde *et al.*, 2012). Thus, we infer that the increase in the concentration of nematodes does not always cause greater host mortality, as the nematode may tend to be more attracted to insects previously infected by the same organism. The scarce knowledge on the interaction between IJ and host makes it difficult to interpret the dynamics of infection caused by EPN (Lewis, 2002).

Concentration of Heterorhabditis amazonensis MC01 against larvae under greenhouse conditions

Under the greenhouse conditions, no significant difference was observed among the concentrations tested (Tukey's test at 5% probability). This shows that the suspension with the lowest concentration (190 IJ larva^{-1}) offered the maximum control these pathogens could achieve, and that a higher concentration did not significantly increase control. Therefore, *H. amazonensis* MC01 was considered virulent to larvae of *E. lignosellus* under the conditions tested, and at all concentrations, the average larval mortality was greater than 70% (Figure 2).

Similar results were found by Leite *et al.* (2007) for *H. indica* (IBCB-n05), which applied at 5.7 and 22.6 IJ cm^{-2} showed no statistical difference, reaching the efficiency of 75% and 85%, respectively, in the control of larvae of *Bradysia mabiusi* (Lane) (Diptera: Sciaridae).

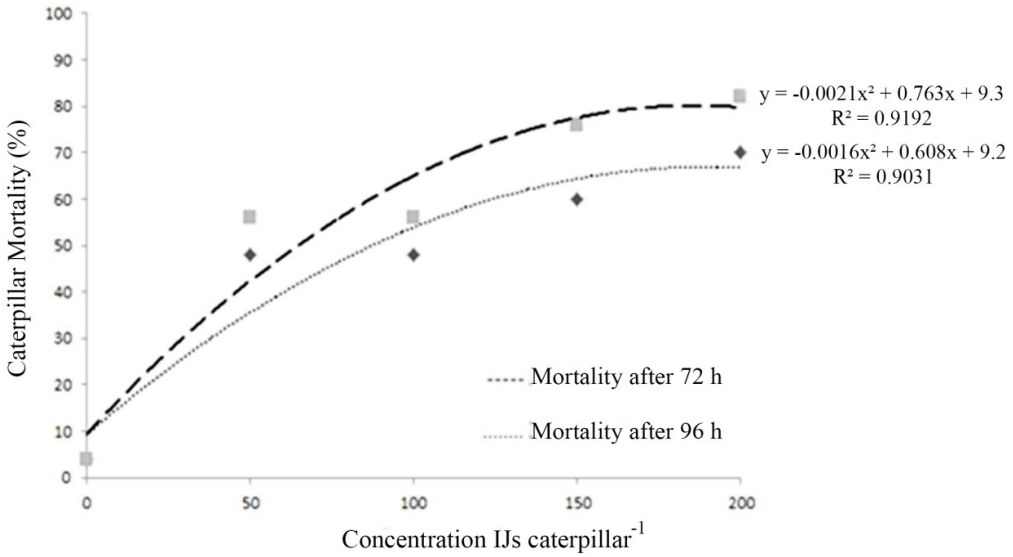

Figure 1: Mortality of *Elasmopalpus lignosellus* larvae at different *Heterorhabditis amazonensis* MC01 concentrations after 72 and 96 h of exposure.

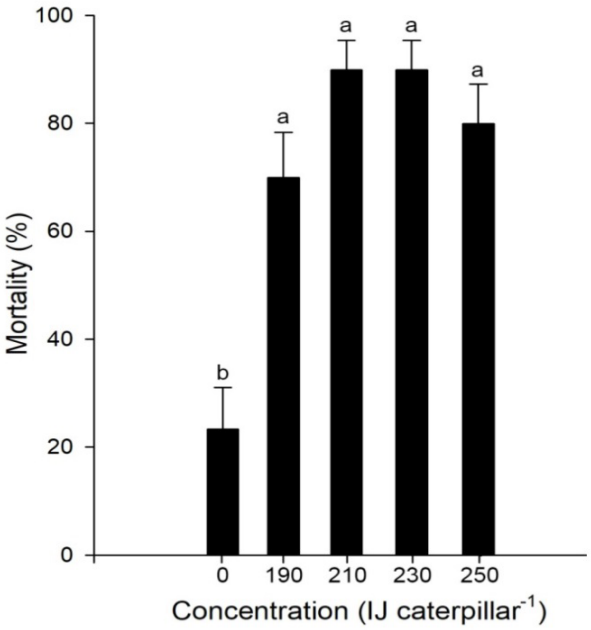

Figure 2: Mean mortality of *Elasmopalpus lignosellus* larvae inoculated at different concentrations of *H. amazonensis* MC01 in a greenhouse.

Different concentrations of *Heterorhabditis* sp. RSC01 also did not significantly affect the mortality of larvae of *Diabrotica speciosa* (Germar) (Coleoptera: Chrysomelidae) in corn (Santos *et al.,* 2011). Likewise, different concentrations of *H. amazonensis* RSC1 did not influence the efficiency against nymphs of *Mahanarva spectabilis* (Hemiptera: Cercopidae) in a greenhouse, and all concentrations tested caused a mortality of 57.14% (Batista *et al.*, 2014).

Overall, the results show that the optimal concentration under conditions of maximum exposure of the larvae to nematodes is similar to that under greenhouse conditions. However, this does not exclude the need for field tests, in which other variables must be considered.

CONCLUSIONS

From the isolates tested on the *E. lignosellus* larvae, *H. amazonensis* MC01 and *S. carpocapse* All reducing the larva population by more than 90%.

The concentration of *H. amazonensis* MC01 that caused the highest mortality of larvae in the laboratory was 182 IJ larva^{-1}. In the greenhouse, the nematode was also considered virulent to *E. lignosellus* since all concentrations tested caused larval mortality greater than 70%. In general, we found that the longer the exposure time, the greater the mortality of the larvae.

ACKNOWLEDGEMENTS, FINANCIAL SUPPORT, AND FULL DISCLOSURE

To the Federal University of Uberlândia and the Graduate Program in Agronomy for the financial support. The authors report that there is no conflict of interest.

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