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Virulence of Entomopathogenic Nematodes against Neotropical Brown Stink Bug (*Euschistus heros* [Fabricius], Hemiptera, Pentatomidae) and Compatibility with Phytosanitary Products under Laboratory Conditions

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ABSTRACT

The Neotropical brown stink bug, *Euschistus heros* (Fabricius), is considered a pest in soybean that is difficult to control and leads to deterioration of grains and reduced production. Entomopathogenic nematodes can be used to control insect pests and can also be a complementary tool in the management of the Neotropical brown stink bug. They also exhibit significant compatibility with chemical phytosanitary products. Thus, this study aimed to determine the virulence, production, and concentration of entomopathogenic nematodes in the Neotropical brown stink bug, as well as their compatibility with chemical phytosanitary products. Six nematode isolates, administered in the concentration of 100 Infective Juveniles (IJs) adult^{-1}, were evaluated. Subsequently, *Heterorhabditis amazonensis* MC01 was evaluated at concentrations of 50, 100, 150, 200, and 250 IJs adult^{-1}. The evaluations were carried out by determining Neotropical brown stink bug mortality and the production of IJs. The compatibility tests consisted of evaluating the viability and infectivity of two nematode isolates incubated in contact with 11 phytosanitary products for 48 hours. The virulence test showed up to 48% Neotropical brown stink bug mortality after 7 days. A greater concentration of IJs was produced for *H. amazonensis* MC01, compared to *Steinernema feltiae*, reaching 101,000 and 97,800 IJs adult^{-1}, respectively. The application of 150 IJs adult^{-1} was associated with the highest mortality of *E. heros* and the highest production of IJs. Methomyl and profenofos were incompatible with both tested nematodes and chlorpyrifos was incompatible with *H. amazonensis* MC01. The compatibility of the chemical products with nematodes highlights the possibility of application in association with entomopathogenic nematodes to control *E. heros.* Products considered incompatible should be avoided, and further tests should be performed to confirm the results in field conditions.

Keywords: Biological control, Glycine max, Heterorhabditis, Steinernema, Soybean.

INTRODUCTION

The Neotropical brown stink bug, *Euschistus heros* (Fabricius) (Hemiptera: Pentatomidae), is one of the most abundant pest species found in soybeans, being present throughout Brazil (Sosa-Gómez *et al*., 2014; Pereira *et al*., 2021). From the vegetative period to the reproductive period, it sucks branches and stems, injecting toxins that cause leaf retention and make it difficult for the grains to mature, thereby making them deformed and hollow (Corrêa-Ferreira, 2005). These factors reduce production and harm the harvest, causing qualitative and quantitative losses of the marketed product (Corrêa-Ferreira *et al*., 2009; Papa *et al*., 2018).

The control of *E. heros* has been considered difficult, with chemical insecticides being the most used tool. However, the control is not considered

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effective (Silva *et al.*, 2014). Moreover, improper use of chemicals can lead to resistance of insect pests, including *E. heros*, and the elimination of natural enemies that contribute to the reduction of the stink bug population (Sosa-Gómez and Silva, 2010; Hoffmann-Campo *et al*., 2012; Ecco *et al*., 2020).

Studies have reported possible cases of Neotropical brown stink bug resistance to chemical insecticides, demonstrating losses in the efficiency of molecules (Sosa-Gomes and Silva, 2010). The risk of failure to control *E. heros* using beta-cyfluthrin and imidacloprid has already been reported in the state of Goiás, Brazil (Tuelher *et al*., 2017).

Implementation of integrated management helps to keep the pest population below the damage level (Hoffmann-Campo *et al*., 2000). The inclusion of practices aimed at controlling *E. heros*, such as the use of biological control, is fundamental to enable better management of the pest. Fungi such as *Beauveria bassiana* (Bals.-Criv.) Vuill. and *Metarhizium anisopliae* (Metchnikoff) Sorokin, the bacterium *Bacillus thuringiensis* var. *kurstaki* (Berliner), and the parasitoid *Telenomus podisi* Ashmead (Hymenoptera: Scelionidae) are registered for the control of *E. heros* (Agrofit, 2021).

Among the biological control agents, entomopathogenic nematodes have potential in pest control, considering their association with symbiotic bacteria of the genera *Photorhabdus* and *Xenorhabdus* that can cause death in the insect by septicemia in 48 to 72 hours (Stock, 2005). Infectious juveniles can live in the soil for prolonged periods, which enables their use in conservative biological control programs (Fuga, 2012).

Entomopathogenic Nematodes (EPNs) can be successfully used for controlling insect pests of different orders, including Hemiptera, as observed for the green-belly stink bug, *Dichelops melacanthus* (Dallas) (Hemiptera: Pentatomidae) (Guide *et al*., 2015), and *Halyomorpha halys* (Stal) (Rhynchota: Pentatomidae) (Burjanadze *et al*., 2020). These organisms are compatible with various chemical and biological phytosanitary products having synergistic action in mixtures, in addition to host's search behavior and persisting in the environment for a long time (Grewal *et al.*, 2001; Magnabosco *et al*., 2019). Results of incompability are also observed, as stated by Sabino *et al*. (2014) who found that the insecticides abamectin and chlorpyrifos maintained the infective juveniles of *H. amazonensis* JPM4 and *S. carpocasae* All viable, however, their infectivity capacity after exposure to these insecticides were reduced, indicating the incompatibility. The compatibility of EPNs with phytosanitary products can be influenced by nematode species, exposure time, and temperature (Bajc *et al*., 2017). Laznik and Trdan (2014) highlight that the compatibility is not only related to a species, but also to a specific characteristic of each strain of EPN.

Considering the potential of EPNs to control *E. heros* and the possibility of applying a mixture of EPNs and phytosanitary products, in this research, our objective was to select EPNs virulent to the Neotropical brown stink bug and determine their multiplication in the insect, the application concentration, and the compatibility with chemical products registered for soybean crops.

MATERIALS AND METHODS

The experiments were conducted at the Entomology Laboratory of the Federal University of Uberlândia (UFU), Monte Carmelo Campus.

The stink bugs used in the experiments were obtained using a beating cloth in an area of soybean cultivation established in the experimental area of the UFU (geographic coordinates 18° 43' 28.0" S, 47° 31' 25.6" W), when the crop was in the R6 stage of development. The cultivar used was Desafio RR, planted in an area of 400 m² (20×20 m). Cultural treatments followed the recommendations for soybean crops. The

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IOBC/WPRS Protocol (Vainio, 1992) was used in the compatibility test.

In vivo Multiplication of Entomopathogenic Nematodes

The EPNs used in the experiments were obtained from the entomopathogen bank of the Entomology Laboratory at UFU. The Infective Juveniles (IJs) were inoculated using a micropipette in Petri dishes (9 cm), containing sterilized filter paper and larvae of *Tenebrio molitor* L. (Coleoptera: Tenebrionidae), and were reared according to the methodology proposed by Potrich *et al*. (2007).

Petri dishes were incubated in a climate chamber at 25±1ºC. The larvae killed by the IJs were transferred to a dry chamber, which consisted of Petri dishes containing sterilized filter paper, and remained there for 3–6 days for the multiplication of the nematodes within the insect's body. Subsequently, the larvae were transferred to a White trap (White, 1927) to obtain the IJs used in the bioassays.

Selection, Concentration, and Production of Entomopathogenic Nematodes

Six newly emerged isolates of entomopathogenic nematodes were tested to select those with greater virulence at the same concentration in the Neotropical brown stink bug. The nematodes tested were *Heterorhabditis* sp. UENP 01, *Heterorhabditis* sp. UENP 03, *H. amazonensis* MC01, *Steinernema carpocapsae*, *S. feltiae*, and *S. brazilense*.

The experiment consisted of seven treatments, six nematode isolates, and the control. For each treatment, 10 replicates were used. Each nematode was applied, using a manual pipette, in 9-cm Petri dishes containing two sheets of filter paper, a pod of about 3 cm, and five insects in each dish. Next, 1.5 mL of suspension was applied per plate at the concentration of 100 IJs insect^{-1}. In the control, only distilled water was applied. The plates were sealed with Parafilm®. The experiment was maintained under controlled conditions in the climate chamber, with temperature of 25±1ºC, relative humidity of 70±10%, and 12 hours of photophase.

Evaluations were performed 5 and 7 days after nematode inoculation. Mortality was confirmed after the cadavers remained 3 days in a dry chamber, by identifying characteristic symptoms of death caused by nematodes. The mortality data obtained were corrected by the formula of Abbott (1925), subjected to analysis of variance, and transformed into y+1.0-√(y+1.0) for the comparison test between the Scott-Knott mean values at 5% probability level.

After remaining in the dry chamber, the dead stink bugs were transferred to White traps to evaluate the production of IJs by each nematode species. Following the beginning of the emergence of the IJs in the traps, they were collected for up to 7 days and quantified using a stereoscopic microscope. Production data were subjected to analysis of variance and comparison test between Scott-Knott mean values at 1% probability level.

To determine the application concentration of IJs, the nematode *H. amazonensis* MC01 was tested at concentrations of 50, 100, 150, 200, and 250 IJs insect^{-1} in adult Neotropical brown stink bugs.

The experiment followed the same methodology as the nematode selection assay. The treatments were composed of the five concentrations and the control. Ten replicates were used for each treatment. Only distilled water was applied to the control. Evaluations were performed 5 and 7 days after nematode inoculation. Mortality was confirmed after the corpses remained in a dry chamber for 3 days, by identifying characteristic symptoms of death caused by nematodes, such as the change in the color of the insect's body.

After remaining in the dry chamber, the dead stink bugs were transferred to White traps to evaluate the production of IJs at each application concentration. After the beginning of the emergence of IJs in the

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traps, they were collected for up to 7 days and quantified using a stereoscopic microscope. Mortality and production data were subjected to analysis of variance and regression analysis.

Compatibility with Phytosanitary Products

The nematodes used were *H. amazonensis* MC01 and *S. carpocapsae*. The phytosanitary products used (Table 1) were prepared with twice the highest concentration of the dose recommended by the manufacturer per mL of water (in order to consider the posterior dilution with the nematode suspension). An aliquot of 1 mL was taken from this solution and placed in five glass tubes (8 cm high×2.5 cm in diameter) per treatment, in which 2,000 IJs in 1 mL of distilled water were added. The tubes were incubated in a climate chamber at 25±1ºC and 70±10% RH and 24 hours of darkness.

The viability of nematodes was evaluated 48 hours after exposure to the products. For this purpose, an aliquot of 0.1 mL of the suspension was removed and 100 IJs were evaluated to determine mortality. Those that did not respond to stimuli with a stylet were considered dead.

Nematode infectivity was tested simultaneously with viability. The tubes were filled with distilled water (3 mL) and left to decant for half an hour in the refrigerator. The supernatant (about 3 mL) was discarded, and the washing was repeated three times. Subsequently, 0.8 mL was taken from the bottom of each tube and pipetted into five Petri dishes with two filter papers per treatment. Ten *T. molitor* larvae were placed in each plate, which were incubated in a climate chamber under the same conditions as the previous one for 5 days. After this period, the mortality of larvae by nematodes was determined by symptomatology and dissection.

The values of mortality of *T. molitor* larvae submitted to *S. carpocapsae* were corrected using Abbott's formula (1925).

The values of nematode viability and larval mortality were subjected to analysis of variance and Scott-Knott test ($P < 0.05$) for comparison between mean values. The statistical analysis was performed in Sisvar software (Ferreira, 2011).

A phytosanitary product was considered compatible when the values were greater than 50% and incompatible when they were less than 50% (Vainio, 1992).

Table 1. Phytosanitary products used in the study of compatibility with entomopathogenic nematodes (Agrofit, 2021).

Active ingredient	Formulation	Use[a]	Chemical group	Recommended dose[b]
Chlorantraniliprole	SC	I	anthranilamide	400 mL ha^{-1}
Profenofos	EC	I	organophosphate	800 mL ha^{-1}
Cypermethrin	EC	I	pyrethroid	250 mL ha^{-1}
Lambda-cyhalothrin	CS	I	pyrethroid	100 mL ha^{-1}
Methomyl	SL	I	methylcarbamate	150 mL 100 L water^{-1}
Gamma-cyhalothrin	SC	I	pyrethroid	100 mL ha^{-1}
Chlorpyrifos	EC	I	organophosphate	150 mL 100 L water^{-1}
Cuprous oxide	WP	F	Inorganic	150 g 100 L water^{-1}
Fenpropathrin	EC	A/I	pyrethroid	400 mL ha^{-1}
Bifenthrin	EC	A/I	pyrethroid	1200 mL ha^{-1}
Glyphosate	SL	H	substituted glycine	4.2 L ha^{-1}

[a] A= Acaricide; I= Insecticide; F= Fungicide; H= herbicide. [b] Average concentration recommended on the product label for application per hectare.

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RESULTS

Selection, Concentration, and Production of Entomopathogenic Nematodes

After 5 days, the nematodes *H. amazonensis* MC01, *S. feltiae*, and *S. brazilense* differed from the others, causing up to 44% mortality of adults of *E. heros*. After 7 days, the previously mentioned nematodes and *Heterorhabditis* sp. UENP03 did not differ and were associated with the highest mortality rates, reaching 48% (Table 2).

There was a greater number of IJs in *E. heros* corpses when they were recovered from *H. amazonensis* MC01 and *S. feltiae*, reaching 101,000 and 97,800 IJs per adult, respectively. *S. carpocapsae* was the host that produced the least IJ, with 4.1 times fewer IJs being recovered from it than from *H. amazonensis* MC01 (Table 3). Thus, this nematode was selected for the concentration assay.

The highest insect mortality rates, 40 and 50%, were obtained at 5 and 7 days, respectively, when 150 IJs adult^{-1} were applied. On both days, a decline in the percentage of dead stink bugs was observed from the concentration of 200 IJs adult^{-1}, which may have occurred due to the greater number of juveniles penetrating the insect's body, increasing the competition for food resources (Figure 1).

There was a lower production of IJs in *E. heros* corpses when 50 IJs adult^{-1} were applied, with 90,000 IJs being recovered in a White trap. At the concentration of 100 IJs adult^{-1}, there was an increase in reproduction, reaching approximately 94,000 IJs. The highest production occurred when 150 IJs were applied per adults and the population of IJs reached 100,000 IJ. A decreasing trend can be observed in the reproduction curve from the concentrations of 200 and 250 IJ adult^{-1}, with a decrease in the production of IJ (Figure 2).

Compatibility with Phytosanitary Products

The compatibility test between the phytosanitary products and *H. amazonensis* MC01 shows that the products that least affected the viability of the nematode were bifenthrin, chlorantraniliprole, and gamma-cyhalothrin, and that most products maintained the viable IJs, as they did not differ from the control. Only the profenofos insecticide negatively affected the nematode, thereby reducing viability (Table 4).

With respect to the mortality caused by IJs in *T. molitor,* it was found that the products in which the nematodes remained viable did not affect their infectivity, except for methomyl and chlorpyrifos, which did not reduce the viability of the IJ, but reduced the insect's ability to infect. Thus, as the infectivity rates were below 50%, the products profenofos, methomyl, and

Table 2. Corrected cumulative mortality (%) of *Euschistus heros* adults caused by different entomopathogenic nematode isolates under laboratory conditions. [a]

Treatment	5 days	7 days
Heterorhabditis amazonensis MC01	44.0 ± 8.9 a	48.0 ± 11.0 a
Steinernema feltiae	32.0 ± 11.0 a	44.0 ± 16.7 a
Steinernema brazilense	32.0 ± 11.0 a	32.0 ± 11.0 a
Heterorhabditis sp. UENP03	20.0 ± 11.0 b	36.0 ± 11.0 a
Heterorhabditis sp. UENP01	8.0 ± 8.4 b	12.0 ± 16.4 b
Steinernema carpocapsae	8.0 ± 14.1 b	12.0 ± 16.7 b
CV (%)	31.84	35.69

[a] (a-b) Mean values followed by the same letter in the column do not differ from each other, as per the Scott-Knott test at 5% probability level. Data transformed into y+1.0-√(y+1.0). Mortality corrected by Abbott's formula (1925).

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Table 3. Production of infective juveniles of entomopathogenic nematodes by adult *Euschistus heros* under laboratory conditions. [a]

Treatment	Production of infective juveniles per adult of *E. heros*
Heterorhabditis amazonensis MC01	101000 ± 11,130 a
Steinernema feltiae	97800 ± 8,205 a
Heterorhabditis sp. UENP03	71000 ± 10,983 b
Heterorhabditis sp. UENP01	61500 ± 2,179 c
Steinernema brazilense	54200 ± 6,130 c
Steinernema carpocapsae	24600 ± 2,945 d
CV (%)	11.38

[a] (a-d) Mean values followed by the same letter in the column do not differ from each other, as per the Scott-Knott test at 1% probability level.

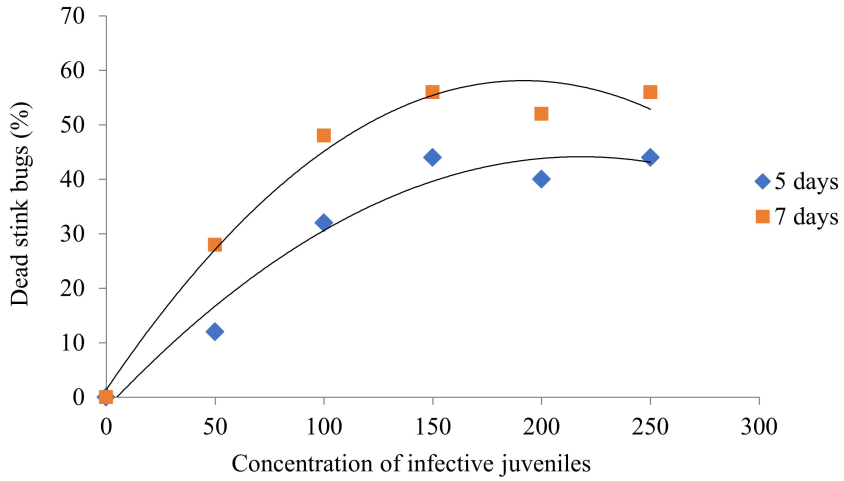

Figure 1. Corrected mortality (%) of *Euschistus heros* adults caused by the entomopathogenic nematode *Heterorhabditis amazonensis* MC01 after 5 and 7 days. Equations (**5 days**): $y= -0.001x^2+0.4234x–2$; R^2 = 96.34%, (**7 days**): $y= -0.0015x^2+0.5914x+1.4286$; R^2 = 97.68%.

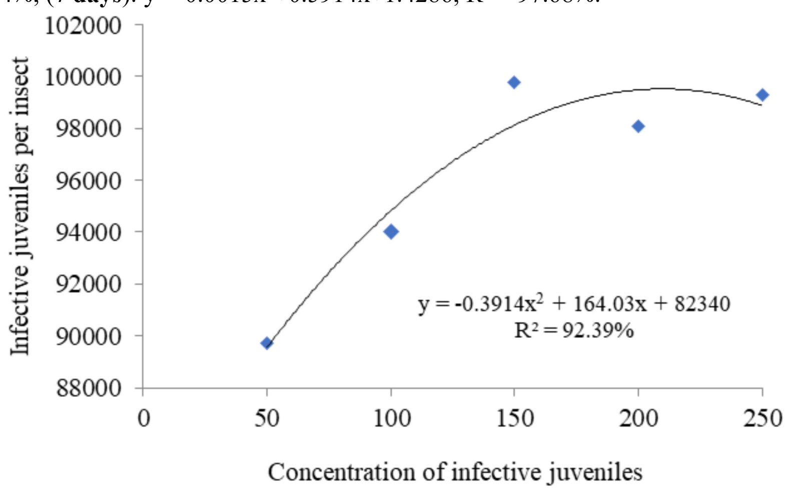

Figure 2. Production of infective juveniles of *Heterorhabditis amazonensis* MC01 applied at different concentrations in adults of *Euschistus heros*.

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chlorpyrifos were considered incompatible with *H. amazonensis* MC01. The profenofos insecticide reduced the viability of IJ by almost 20 times when compared to the control (Table 4).

Only the profenofos insecticide caused a reduction in viability of IJs of *S. carpocapsae* after contact with phytosanitary products (Table 5). Although profenofos and methomyl reduced the infectivity of IJs to *T. molitor* larvae, the latter did not reduce the viability of the nematodes. The other products did not affect the viability and infectivity of *S. carpocapsae*. Therefore, profenofos and methomyl can be considered incompatible with *S. carpocapsae*. A similar result was observed for the tests conducted with *H. amazonensis* MC01, demonstrating that profenofos and methomyl insecticides were incompatible with both nematodes (Tables 4 and 5).

DISCUSSION

Given that only a few studies have tested entomopathogenic effects of nematodes to *E. heros*, the present study can help evaluate the action potential of these entomopathogens in the mortality of the pest. The tested species were pathogenic to

Table 4. Viability (%) and infectivity (% mortality) of *Heterorhabditis amazonensis* MC01 in *Tenebrio molitor* larvae, after contact with phytosanitary products. [a]

Treatment	Viability of infective juveniles (%)	Mortality of *Tenebrio molitor* (%)	Compatibility
Bifenthrin	96.8 ± 1.3 a	84.0 ± 13.4 a	Compatible
Chlorantraniliprole	96.8 ± 1.3 a	88.0 ± 16.4 a	Compatible
Gamma-cyhalothrin	94.4 ± 3.3 a	88.0 ± 8.4 a	Compatible
Control	91.8 ± 1.5 b	70.0 ± 10.0 b	Compatible
Methomyl	91.8 ± 5.0 b	4.0 ± 5.5 d	Incompatible
Cypermethrin	90.8 ± 3.0 b	88.0 ± 8.4 a	Compatible
Cuprous oxide	90.0 ± 2.2 b	88.0 ± 4.5 a	Compatible
Lambda-cyhalothrin	98.6 ± 2.3 b	92.0 ± 8.4 a	Compatible
Fenpropathrin	88.8 ± 6.3 b	96.0 ± 5.5 to	Compatible
Chlorpyrifos	87.0 ± 3.2 b	38.0 ± 25.9 c	Incompatible
Glyphosate	86.8 ± 5.1 b	78.0 ± 8.4 b	Compatible
Profenofos	4.6 ± 1.5 c	8.0 ± 13.0 d	Incompatible

[a] (a-d)Mean values followed by the same letter in the column do not differ from each other, as per the Scott-Knott test ($P< 0.05$), M±SD(M).

Table 5. Viability (%) and infectivity (% mortality) of *Steinernema carpocapsae* in *Tenebrio molitor* larvae, after contact with phytosanitary products. [a]

Treatment	Viability of infective juveniles (%)	Mortality of *Tenebrio molitor* (%) [b]	Compatibility
Lambda-cyhalothrin	89.8 ± 3.7 a	88.9 ± 12.2 a	Compatible
Chlorantraniliprole	89.6 ± 8.1 a	84.4 ± 5.5 a	Compatible
Cuprous oxide	89.2 ± 4.4 a	53.3 ± 22.8 b	Compatible
Chlorpyrifos	89.0 ± 3.2 a	62.2 ± 15.2 b	Compatible
Gamma-cyhalothrin	88.8 ± 2.8 a	73.3 ± 15.2 a	Compatible
Fenpropathrin	88.0 ± 5.6 a	80.0 ± 8.4 a	Compatible
Methomyl	87.2 ± 4.3 a	0.0 ± 4.5 c	Incompatible
Bifenthrin	85.6 ± 3.6 a	53.3 ± 11.0 b	Compatible
Glyphosate	84.4 ± 8.1 a	75.6 ± 8.4 a	Compatible
Cypermethrin	81.0 ± 2.7 a	57.8 ± 19.2 b	Compatible
Profenofos	1.1 ± 0.5 b	13.3 ± 13.0 c	Incompatible

[a] (a-c) Mean values followed by the same letter in the column do not differ from each other, as per the Scott-Knott test ($p<0.05$). M ± SD(M). [b] Mortality corrected by Abbott's formula (1925).

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the insect, and selected species showed higher virulence, causing up to 48% mortality (Table 2). Although the mortality rates obtained are not considered high, we suggest the use of the nematode as an additional measure to control the Neotropical brown stink bug and conducting further tests on nymphs in the field.

Different isolates of *Heterorhabditis* spp. were tested on *E. heros*. While up to 100% adult mortality was obtained under laboratory conditions, only 18% adult mortality was obtained in the field (Cecconello *et al*., 2022). The authors also found that Neotropical brown stink bug populations from the field were less susceptible to nematode infections than those reared in a laboratory setting.

The pathogenicity and virulence of nematodes to another pentatomid, i.e. *D. melacanthus*, was evaluated by Guide *et al*. (2015). They found that *Heterorhabditis* sp. (IBCB-n 46) and *Heterorhabditis* sp. (GL) at the concentration of 100 IJs adult^{-1} were the most virulent isolates for the stink bug, causing mortality rates up to 76%. Subsequently, the authors evaluated other nematode species and found mortalities of 88% (*H. amazonensis* RSC05) and 82% (*Steinernema* spp. IBCB-n27) under laboratory conditions and up to 38% in a greenhouse (Guide *et al*., 2019).

Moreover, it should be highlighted that the production of IJs obtained per adult cadaver of *E. heros* (more than 100,000 IJ adult^{-1} in the case of *H. amazonensis* MC01, Table 3) is a result that supports the continuity of the presence of nematode population in the field, which will be able to infect new hosts for a significant period of time.

Nezara viridula L. (Hemiptera: Pentatomidae) is another stink bug species that was considered susceptible to the EPN (in this case, *S. mushtaqi*) and was indicated to be an alternative host with the potential for *in vivo* use in commercial production. A significant titer of 0.94 x 10^5 IJs were already obtained per stink bug corpse (Pervez and Ali, 2011), which is a result similar to that obtained for *E. heros* in the present study.

After 7 days, the stink bug mortality rates were higher than at 5 days for all concentrations of IJs per adult, which may be due to the longer time available to the juveniles to colonize and develop in the insect's body, causing mortality. The higher production of IJs obtained when applying the concentration of 150 IJs adult^{-1}, an intermediate concentration among those tested may be because a larger population of IJs penetrating the insect's body caused greater competition for space and food, negatively affecting the reproductive function (Půža and Mráček, 2010; San-Blas *et al*., 2012).

Many factors may influence the production of IJs, including the species and host size and also the inoculum dose applied on the insect. Rahoo *et al*. (2019), when testing the production of EPNs in *Galleria mellonella* L. (Lepidoptera: Pyralidae), observed that the large sized *G. mellonella* larvae produced a greater numbers of IJs compared to medium and small sized. Rahoo *et al*. (2018) studied the production of EPNs in *T. molitor* and a greater number of EPNs were recovered per cadaver with doses of 50 and 500 IJs than dose 10 IJs.

The significant production of progeny of the nematodes tested in *E. heros* is combined with the results of compatibility with the phytosanitary products used in soybean. We found that only three were considered incompatible with *H. amazonensis* MC01 and two with *S. carpocapsae*, despite this nematode being associated with low laboratory mortality rates. Thus, the subsequent generations of the nematode are maintained in the field, causing mortality in the pest for prolonged periods.

Glyphosate was the only herbicide tested at the present work and was considered compatible with the tested species, since there was no decrease in viability and infectivity. Negrisoli Junior *et al*. (2008b) also considerate glyphosate compatible with *S. carpocapsae* and *H. bacteriophora*, with

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no differences related to glyphosate concentration and the time of exposition. Andaló *et al*. (2004) reported compatibility between Roundup CS®, an herbicide with the active ingredient as glyphosate, and *S. carpocapsae*, which corroborates results from the present study.

However, according to Laznik and Trdan (2016), *S. carpocapsae* was considered sensitive to herbicides, including glyphosate, which affected its survival. The reduction of infectivity could be influenced by the reduction of neutral lipids after contact with the product, as stated by Andaló *et al.* (2009) who obtained that the herbicides clomazon + hexazinone and simazine + ametryn did not kill *H. amazonensis* IJs; however, they reduced IJs infectivity and presented a smaller amount of lipids when compared to IJs kept only in water.

All products considered as incompatible with the tested nematodes are neurotoxic insecticides, two of them are organophosphate, and one is a methylcarbamate, acting on the insect nerve synapse region. Zimmerman and Cranshaw (1990), when testing the organophosphate insecticide Diazinon®, found that while it harmed *Heterorhabditis* sp. after 48 h of exposure to the product, it did not affect *S. carpocapsae*. According to Gordon *et al*. (1996), carbamate insecticides can influence the action of *S. carpocapsae* and *S. feltiae*, and these products should be applied cautiously, in order to minimize negative impacts on nematodes that are present in the soil or have been introduced in pest control programs.

Most of the products tested were compatible with nematodes, which highlights the possibility of joint application and expansion of *E. heros* control programs in areas of integrated management along with the possible reduction in application costs. The application of products that are incompatible with the tested nematodes should be avoided. However, specific studies should be developed, since the species and lineage of the pathogens, the chemical nature of the products, the temperature, the time of exposure and the concentrations used can change the action of phytosanitary products on IJs (Negrisoli Junior *et al*., 2008a; Laznik and Trdan, 2016).

The application of EPNs in the field should be studied to verify the survival and infectivity of the nematode under different environmental conditions and considering the techniques of application technology.

CONCLUSIONS

H. amazonensis MC01, *S. feltiae*, and *S. brazilense* caused higher mortality of *E. heros* adults in a shorter time. *H. amazonensis* MC01 and *S. feltiae* produced the highest number of IJs. A concentration of *H. amazonensis* MC01 of 150 IJs adult^{-1} was associated with the highest mortality of *E. heros* and the highest production of IJs.

Profenofos and methomyl were incompatible with *H. amazonensis* MC01 and *S. carpocapsae*. Chlorpyrifos was incompatible with *H. amazonensis* MC01. The other phytosanitary products were compatible with the nematodes under the conditions tested.

Chemical products compatible with nematodes makes possible the associated application with entomopathogenic nematodes to control *E. heros.* Incompatible products should be avoided, and further tests performed to confirm the results in the field.

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شدت بیماری‌زایی نماتدهای Entomopathogenic برعلیه حشره بدبو قهوه ای نوتروپیک ، Hemiptera Pentatomidae ، (*Euschistus heros* [Fabricius]) و سازگاری با محصولات بهداشتی‌گیاهی در شرایط آزمایشگاهی

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چکیده

حشره بدبو قهوه ای نوتروپیکال (*Euschistus heros* (Fabricius به عنوان یک آفت در سویا محسوب می شود که کنترل آن دشوار است و منجر به صدمه به غلات و کاهش تولید می شود. نماتدهای Entomopathogenic را می توان برای کنترل آفات حشرات مورد استفاده قرار داد و همچنین می توان از این نماتدها به عنوان یک ابزار مکمل در مدیریت حشره بدبوی قهوه ای نئوتروپیکال استفاده کرد. آنها همچنین سازگاری قابل توجهی با محصولات بهداشتی‌گیاهی شیمیایی نشان می دهند. بنابراین، این مطالعه با هدف تعیین شدت بیماریزایی، تولید، و غلظت نماتدهای Entomopathogenic در حشره بدبوی قهوه‌ای نئوتروپیکال و همچنین سازگاری آنها با محصولات بهداشتی‌گیاهی شیمیایی انجام شد. شش جدایه نماتد، تجویز شده در غلظت ۱۰۰ بچه نوجوان عفونت‌زا (IJs) در هربالغ، مورد بررسی قرار گرفت. متعاقبا، *Heterorhabditis amazonensis* MC01 در غلظت های ۵۰، ۱۰۰، ۱۵۰، ۲۰۰ و ۲۵۰ IJ در هربزرگسال مورد بررسی قرار گرفت. ارزیابی ها با تعیین مرگ و میر حشرات بدبو قهوه ای نئوتروپیکال و تولید IJs انجام شد. آزمایش‌های سازگاری شامل ارزیابی زنده‌مانی و عفونت‌پذیری دو جدایه نماتد بود که در تماس با ۱۱ محصول بهداشتی‌گیاهی به مدت ۴۸ ساعت انکوبه شدند. آزمون شدت بیماری‌زایی پس از ۷ روز تا ۴۸% مرگ و میر حشرات بدبو قهوه ای نئوتروپیک را نشان داد.غلظت بیشتری از IJs برای *H. amazonensis* MC01 در مقایسه با *Steinernema feltiae* تولید شد که به ترتیب به ۱۰۱۰۰۰ و ۹۷۸۰۰ IJ در هربزرگسال رسید. استفاده از ۱۵۰ IJ درهر بزرگسال با بالاترین مرگ و میر *E. heros* و بالاترین تولید IJs همراه بود. متومیل و پروفنوفوس (Methomyl and profenofos) با هر دو نماتد آزمایش شده و کلرپیریفوس (chlorpyrifos) با *H. amazonensis* MC01 ناسازگار بود. سازگاری محصولات شیمیایی با نماتدها، امکان کاربرد آنها همراه با نماتدهای بیماریزا برای کنترل *E. heros* را برجسته می کند. از محصولاتی که ناسازگار در نظر گرفته می شود باید اجتناب کرد و آزمایش بیشتری برای تایید نتایج در شرایط مزرعه انجام داد.

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