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Full Length Article

Reproduction of Entomopathogenic Nematodes on the Coffee Leaf-miner, *Leucoptera coffeella* (Lepidoptera: Lyonetiidae) and their Potential of Control

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Abstract

Coffee is one of the most important commodities in Brazil. *Leucoptera coffeella* (Guérin-Mèneville & Perrottet) (Lepidoptera: Lyonetiidae) is codamaged a relevant pest of coffee plants and has promoted severe damages to this crop. Chemical control has been considered difficult and there are already reports of resistance to insecticides. This study evaluated the control and reproductive potential of entomopathogenic nematodes to the coffee leaf-miner. Trials related to the virulence of entomopathogenic nematode were performed to select those that promoted higher mortality in larvae – as well as the concentration, lethal time, infection ability and reproductive potential assays. Regarding isolates selection *Heterorhabditis amazonensis* MC01 and *Steinernema feltiae* were selected for further assessments. LC_{80} values for *L. coffeella* by using *S. feltiae* were respectively 194 IJs larva^{-1} and 195 IJs pupa^{-1}; while 165 IJs larva^{-1} and 150 IJs pupa^{-1}, respectively, for *H. amazonensis*. Concerning the lethal time, from the fourth day there was stability in insect mortality. For infection ability it was demonstrated that at the lowest concentration the insect's body infection was decreased. For reproductive assay when the highest concentration was applied, 200 IJ insect^{-1}, an increased number of infective juveniles were recovered. Finally, it was evidenced that the nematode holds the ability to complete its life cycle in the insect, forming next generations. Herein, we demonstrated the potential of using *H. amazonensis* MC01 to control leaf-miner, highlighting the perspective for further tests under field conditions and the possible inclusion in management programs for *L. coffeella*, reducing the application of chemical insecticides. © 2023 Friends Science Publishers

Keywords: Biological control; *Coffea arabica*; Entomopathogen; *Heterorhabditis*; *Steinernema*

Introduction

Coffee is one of the most important commodities of Brazil, which has already produced 63 million bags in the 2020 season (Conab 2020). The Brazilian coffee production sector comprises about 2.18 million ha (Conab 2021). For this, selecting better sets of crop management is crucial to reach suitable results with reduced costs which requires special attention to pest control, for instance, leaf-miner, *Leucoptera coffeella* (Guérin-Mèneville and Perrottet) (Lepidoptera: Lyonetiidae) (Secex 2021).

The leaf-miner has promoted severe damage to coffee crops. The economic losses occur by the larvae feeding process, opening leaf mines, which reduces leaf area and triggers the defoliation event, thus culminating in the overall production impairment (Almeida *et al*. 2020). *Leucoptera coffeella* quickly develops resistance to the frequently chemical insecticides applied to control the pest, as organophosphate insecticide, one of the most applied insecticides to control the leaf-miner in Brazil (Ribeiro *et al*. 2003; Rocha *et al*. 2022).

Due to the high levels of infestations of *L. coffeella* besides the lower efficiency of chemical controls, alternative methods to insect pest management have been required to reduce the rates of populations in the field closely related to coffee production losses. High temperature and low precipitation increase the reproduction and reduces the life cycle of *L. coffeella*, which causes a greater number of chemical insecticides applications to control the insect (Almeida *et al.* 2020). Include other method to a management program, as biological control has been pointed out as a potential alternative, provides a more sustainable coffee production, and generates high-quality products demanded by internal and external markets (Rocha *et al*. 2022).

Entomopathogenic nematodes are biological control

agents able to effectively control pest insects in different environments, including those with cryptic habits (Lacey and Georgis 2012; Dolinski *et al.* 2017). The given protection to the insect facing the environment also provides a physical barrier against the action of chemical insecticides and other methods of controlling the pest. However, Gözel and Kasap (2015), verified that leaf-mines made by *Tuta absoluta* (Meyrick) (Lepidoptera Gelechiidae) in the tomato plant led to a suitable condition for nematodes intake into the insect, additionally to protection against abiotic factors such as desiccation and ultraviolet radiation.

Based on previous studies regarding the action of such organisms in controlling lepidopteran-related miners such as *T. absoluta* (Gözel and Kasap 2015) and *Phthorimaea operculella* (Zeller) (Lepidoptera: Gelechiidae) (Kary *et al*. 2018; Mhatre *et al*. 2020), besides the compatibility of action with chemical insecticides (Amizadeh *et al*. 2019), it was hypothesized the potential action of entomopathogenic nematodes on *L. coffeella*. Therefore, we investigate virulence, lethal concentration, lethal time and reproduction of entomopathogenic nematodes against the leaf-miner. Also, this is the first study to evaluate the reproduction of entomopathogenic nematodes applied on *L. coffeella* and the potential of controlling the insect-pest in laboratory tests.

Materials and Methods

Both larvae and pupae of *L. coffeella* were obtained from a growing field of *C. arabica* cv. Topázio MG-1190, located in the municipality of Monte Carmelo, MG, at geographic coordinates of 18°43'31.75"S, 47°31' 32.06"W, altitude 890 m.

The entomopathogenic nematodes were obtained from the Entomology laboratory bank from Federal University of Uberlândia, Brazil.

Selection of entomopathogenic nematode isolates – *Leucoptera coffeella* larvae

Each trial comprised eleven treatments, namely *H. amazonensis* MC01, *H. amazonensis* GL, *Heterorhabditis* sp. UENP01, *Heterorhabditis* sp. UENP02, *Heterorhabditis* sp. UENP03, *Heterorhabditis* sp. UENP04, *Heterorhabditis* sp. UENP07, *Steinernema feltiae* IBCB47, *S. carpocapsae* IBCB02, *S. brazilense* IBCB06 isolates and the control, which were tested on *L. coffeella* larvae under laboratory conditions by assessing the virulence ability to the insect. The early viability of infective juveniles (IJs) in suspensions from each isolate was confirmed by nematode mortality level before application.

Two leaves containing leaf-miners' mines were placed in a glass Petri dish (9 cm in diameter), which was covered by two layers of filter paper. Subsequently, each isolate was submitted to 1.2 mL suspension of nematode at a concentration of 80 IJs larva^{-1}, which was previously emerged for up to three days and stored for up to five days. The suspensions were prepared after quantification (counting) of IJs in each mL suspension by using a stereoscopic microscope (Leipzig® model GZ-500) in 96-well microtiter plates.

Each replicate was composed of a Petri dish, totaling twenty replicates per treatment, distributed in a model of completely randomized design. The dishes were sealed with Parafilm® and placed in a climatic chamber (Solab® model SL364) at 25 ± 2°C, 70% RH, and 12 h light/dark mode. Mortality assessments were performed after 96 h. Dead larvae were kept in climatic chamber (Solab® model SL364) at 25 ± 2°C in a dry condition for two days before dissection. Subsequently, the plates were observed under a stereoscopic microscope (Leipzig® model GZ-500) in which the nematode mortality was evaluated. Mortality values were corrected using the Abbott formula (1925).

The data were submitted to analysis of variance with the application of the F test, at 5% probability, after meeting the assumptions of normality of the residues by the Jarque-Bera test, homoscedasticity by the Levene test and additivity of blocks by the Tukey test, all at 5% probability. The means of the treatments were compared using the Scott-Knott test ($P < 0.05$) with the statistical software Sisvar (Ferreira 2019).

Lethal concentration (LC_{50}) of entomopathogenic nematodes

To evaluate the concentration of application, the entomopathogenic nematodes *S. feltiae* and *H. amazonensis* MC01 were selected based on the previous test. The experiments were conducted under the same conditions as described above.

Two experiments were carried out to assess the lethal concentration of nematode for both larvae and pupae of *L. coffeella*. Each experiment comprised ten treatments for each species of nematode, containing nine concentrations, namely 40, 60, 80, 100, 120, 140, 160, 180 and 200 IJ insect^{-1} and the control, which was treated with distilled water only. Afterward, 1.2 mL of aqueous suspension in their respective concentrations were applied on two sheets of filter paper placed in a Petri dish (9 cm in diameter), comprising five replicates per treatment (each repetition was represented by a Petri dish). Eight active leaf-miner mines were placed on each dish. It was selected and used emerged IJs of up to 3 d and stored for up to 5 d. The experiment was kept in a climatic chamber (Solab® model SL364) at 25 ± 2°C, 70% RH and 12 h light/dark mode.

The number of dead larvae was accomplished after 96 h. The nematode mortality was validated by keeping dishes in climatic chamber (Solab® model SL364) at 25 ± 2°C in a dry condition for two days, followed by the insect dissection, in which the presence of nematodes in it was observed *via* stereoscopic microscope (Leipzig® model GZ-500). Data were submitted to Probit analysis, where the estimated parameters of the model were tested by the Chi-

Square test ($P < 0.01$), to select the best lethal concentration of the nematode with regards to mortality promoted by leaf-miner larvae or pupae.

Lethal time (LT_{50}) of entomopathogenic nematodes

The lethal time (LT_{50}) in which nematodes of *S. feltiae* and *H. amazonensis* MC01 promote the mortality of larvae and pupae of *L. coffeella* was determined by following the same methods used to determine the lethal concentration, as well as the same previously selected concentration, thereby considering the highest mortality observed in larvae and pupae. Five replicates were used at each evaluated time. Eight leaves containing mines and leaf-miners' pupae were placed on each dish, respectively for the larva and pupae mortality experiments. The treatments consisted of seven evaluated times, namely 24, 48, 72, 96, 120, 144 and 168 h.

Mortality data were corrected by the equation of Abbott (1925). Then, a Generalized Linear Model was fitted *via* a binomial distribution and logit linkage function, in a 2 x 7 factorial, with the first factor as the nematode (*S. feltiae* and *H. amazonensis* MC01) and the second factor as the evaluated times. When significant differences were detected by Deviance Analysis (ANODEV) *via* Chi-Square test ($P < 0.01$), means within nematode factor were compared by Tukey's test ($P < 0.05$), while a regression adjustment was applied as a function of evaluated times.

Reproductive potential of the entomopathogenic nematode in *Leucoptera coffeella*

To evaluate the reproductive potential of entomopathogenic nematodes in larvae and pupae of *L. coffeella*, concentrations of 160, 180 and 200 IJs *H. amazonensis* MC01 larva^{-1} were used in the volume of 0.6 mL dish^{-1}. In the control, only water was used. The suspensions were added to a Petri dish (5 cm in diameter) with two sheets of filter paper, in which was placed either a *L. coffeella* mine or a leaf with pupa. To investigate penetration of IJs after the insect death, ten dead insects were randomly selected and transferred to a separate Petri dish (5 cm in diameter) containing dry filter paper and kept in the dark for 24 h.

After 24 h, dead insects were washed with distilled water, to remove the nematodes from the body's surface and then dissected in NaCl (1%). Subsequently, they were observed in a stereoscopic microscope (Leipzig® model GZ-500) to examine the number of IJs inside the body.

For the reproduction test, another ten dead insects were randomly selected, washed with distilled water, before being transferred to White's trap and incubated at 24 ± 2°C in the dark in climatic chamber (Solab® model SL364). The total number of IJs that emerged from each dead insect was quantified. Each body was considered a replicate.

A Generalized Linear Model (GLM) with Poisson distribution and log link function was fitted to assess the number of nematodes present in both larva and pupae, in which the significance of the treatment was accomplished by the Chi-Square test ($P < 0.01$) in ANODEV. If significant, the means were compared by the Tukey test ($P < 0.05$). To verify the correlation between infection ability of infective juveniles and reproduction, the Pearson correlation was then carried out.

Results

Selection of entomopathogenic nematodes

All evaluated species showed pathogenicity ability to *L. coffeella*. *Heterorhabditis amazonensis* GL, *H. amazonensis* MC01, *Heterorhabditis* sp. UENP1, *S. feltiae* and *Heterorhabditis* sp. and UENP2 provided higher mortality of *L. coffeella* larvae as compared to other evaluated nematodes, being up to 40% higher as compared to its counterparts, which did not differ among them (Table 1).

Lethal concentration (LC_{50}) of entomopathogenic nematodes

Larvae: The obtained average of LC_{50} to promote mortality in larvae in *L. coffeella* by *S. feltiae* was about 106 IJs larva^{-1}, while 194 IJs larva^{-1} for LC_{80}. It is observed that there was an increase in insect mortality of up to 80% in a concentration dependent-manner for larvae (Fig. 1). Thus, based on Fig. 1, it is reasonable to suggest that the mortality of *L. coffeella* larvae occurs as a function of increases in the applied concentration of *S. feltiae*.

Regarding *H. amazonensis* MC01, obtained averages of LC_{50} and LC_{80} were 96 IJs larva^{-1} and 165 IJs larva^{-1}, respectively. It was verified that there was an increase in the mortality of larvae as the applied concentration is increased. The mortality curve showed an exponential growth when CL was higher than 50 (Fig. 2).

Pupae: The obtained LC_{50} for pupae submitted to *S. feltiae* suspension was 95 IJs pupa^{-1}, while 195 IJs pupa^{-1} for the LC_{80}. Pupal mortality increased by up to 80% as a consequence of increases in the concentration of applied IJs (Fig. 3). This result was similar to that obtained in larvae of *L. coffeella*, in which increased mortality was observed from the LC_{50}.

Concerning *H. amazonensis* MC01, both LC_{50} and LC_{80} were 87 and 150 IJs pupa^{-1}, respectively. As previously observed in the concentration test, it was demonstrated that the higher the concentration, the higher is also the mortality of pupae (Fig. 4).

Lethal time (LT_{50}) of entomopathogenic nematodes

Larvae: There was no difference between treatments – *H. amazonensis* and *S. feltiae*. It is worth mentioning that the used levels were those obtained in the concentration test, which was based on the LC_{80} of each isolate. Such results are relevant for the selection of the nematode in further studies. The lethal time of stability to promote mortality of

Table 1: Corrected mortality (%)* of *Leucoptera coffeella* larvae submitted to different populations of entomopathogenic nematodes

Treatment	Mortality (%)**
Heterorhabditis amazonensis GL	79.42 ± 6.44 a
Heterorhabditis amazonensis MC01	76.48 ± 6.61 a
Heterorhabditis sp. UENP1	73.54 ± 6.71 a
Steinernema feltiae IBCB47	73.54 ± 6.71 a
Heterorhabditis sp. UENP2	70.60 ± 6.75 a
Heterorhabditis sp. UENP3	48.84 ± 6.50 b
Steinernema brazilense IBCB06	43.84 ± 6.35 b
Steinernema carpocapsae IBCB02	38.84 ± 5.98 b
Heterorhabditis sp. UENP7	35.02 ± 3.37 b
Heterorhabditis sp. UENP4	33.84 ± 5.35 b
CV (%)	29.99

Mean ± Standard error of the mean
*Corrected mortality by Abbott's formula
**Means followed by the same letter in the column do not differ by the Scott-Knott test at 1% probability. A √(y + 0.5) transformed value

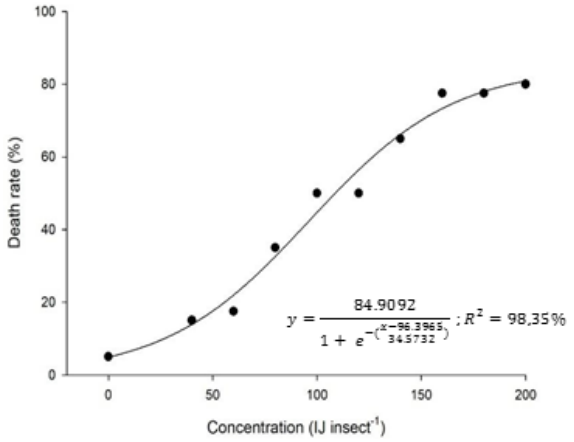

Fig. 1: Lethal concentrations of *Steinernema feltiae* (infective juveniles by larvae) to promote mortality in *Leucoptera coffeella* under laboratory conditions

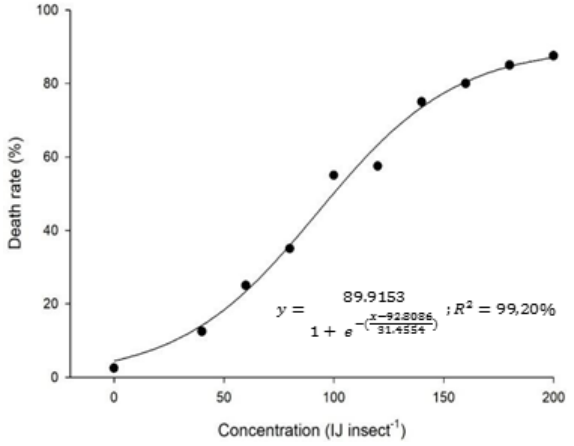

Fig. 2: Lethal concentrations of *Heterorhabditis amazonensis* MC01 (infective juveniles by larvae) to promote mortality in *Leucoptera coffeella* under laboratory conditions

larvae was 4 d. At the 3rd d, there was 45% mortality of larvae, thus not reaching LT_{50}. After the fourth day, the mortality remained stable, indicating that 4 d may be

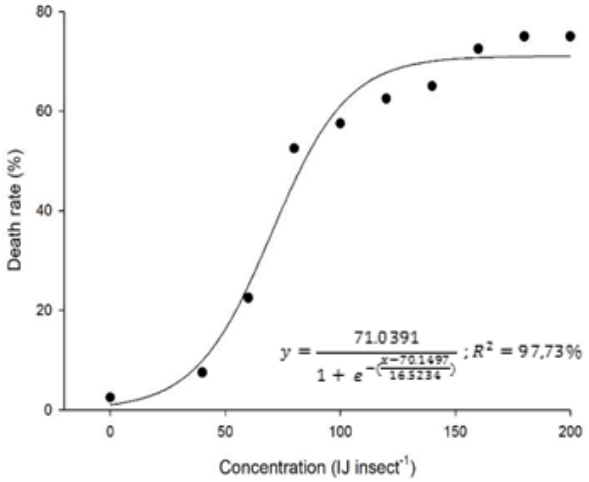

Fig. 3: Lethal concentrations of *Steinernema feltiae* (pupa-infective juveniles) to promote mortality of *Leucoptera coffeella* under laboratory conditions

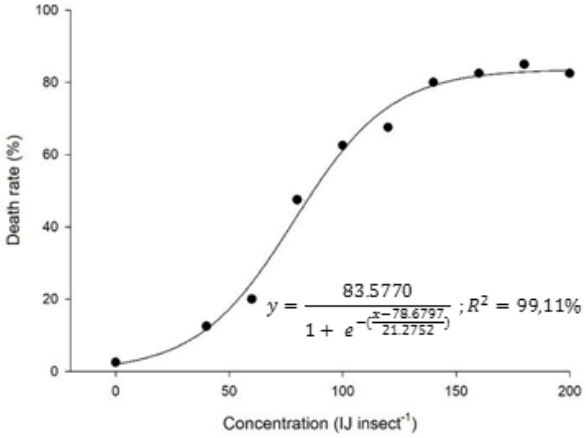

Fig. 4: Lethal concentrations of *Heterorhabditis amazonensis* MC01 (pupa-infective juveniles) to promote mortality in *Leucoptera coffeella* under laboratory conditions

indicated for an efficient incubation. Finally, five days after inoculation, the mortality was close to 80%, which remained stable throughout the evaluated period. Therefore, 4 d can be considered an ideal time for the death of larvae submitted to the evaluated nematodes in their appropriate concentrations, thereby reaching approximately 73% of larva mortality (Fig. 5).

Pupae: Regarding the mortality of *L. coffeella* pupae over time, no difference was observed between the treatments (*H. amazonensis* and *S. feltiae*) when the previously determined LC_{80} was applied for each nematode species.

The stability in pupae mortality was observed after four days, thus being the lethal time to promote approximately 71% of pupae mortality of *L. coffeella*. Similar values were obtained in the following evaluations, up to the seventh day. At 3rd d, there was a mortality of 28% larvae, not reaching the LT_{50} (Fig. 6).

Table 2: Penetration ability of infective juveniles of *Heterorhabditis amazonensis* MC01 at different concentrations in *Leucoptera coffeella* larvae and pupae. The evaluation was carried out after 4 d of application

Treatment (infective juveniles insect^{-1})	Number of nematodes larva^{-1}	Number of nematodes pupa^{-1}
160	2.3 ± 0.480 a	1.9 ± 0.436 a
180	3.9 ± 0.624 ab	3.7 ± 0.608 b
200	6.2 ± 0.787 b	5.0 ± 0.707 b

Table 3: Number of infective juveniles of *Heterorhabditis amazonensis* MC01 after reproduction of the nematode inside both larvae and pupae of *Leucoptera coffeella*. The evaluation performed after 12 days of dead insects

Treatment (infective juveniles insect^{-1})	Number of nematodes larva^{-1}	Number of nematodes pupa^{-1}
160	21.1 ± 1.45 a	18.1 ± 1.36 a
180	25.8 ± 1.61 ab	27.1 ± 1.65 b
200	31.4 ± 1.77 b	36.1 ± 1.90 c

Reproductive potential of the entomopathogenic nematode in *Leucoptera coffeella*

The result regarding the penetration ability of infective juveniles of *H. amazonensis* MC01 in larvae and pupae of *L. coffeella* was statistically significant by the Chi-square test at 0.01 significance.

It was observed that at lower concentrations, a smaller number of infective juveniles infected the insect, as evidenced in the concentration of 160 IJs insect^{-1}, in which a lower level of nematodes was observed for both larva and pupa (Table 2).

Regarding the infective juveniles of *H. amazonensis* MC01 obtained from reproduction in *L. coffeella*, it was demonstrated that the higher the used concentration, the higher the obtained progeny, thus, an increased number of infective juveniles were recovered at the highest applied concentration, for instance, 200 IJ insect^{-1} for both larvae and pupae (Table 3).

The correlation between penetration ability of infective juveniles and reproduction was significant by the *t*-test, being 0.49 for larvae and 0.68 for pupae. Therefore, it is worth mentioning that the nematodes that infected *L. coffeella* were able to complete their life cycle and generate progeny.

Discussion

The present study demonstrates the first data regarding the action of entomopathogenic nematodes *Steinernema* sp. and native *Heterorhabditis* sp. species on larvae and pupae of *L. coffeella* by assessing pathogenicity, virulence, concentration, and reproductive ability inside the insect.

Even though there are no previous studies concerning nematodes entomopathogenic to *L. coffeella*, several reports have demonstrated the potential way of controlling pests that live in cryptic environments, such as leaf-miners (Damme *et al*. 2015; Kary *et al*. 2018). The ability of entomopathogenic nematodes to target the host may be an important characteristic of entomopathogens that leads to higher pest mortality.

Batalla-Carrera *et al*. (2010) have demonstrated the ability of nematodes in going through mines and kill *T. absoluta* larvae, thereby reaching values between 77 and 91% of mortality. On the other hand, Garcia-del-

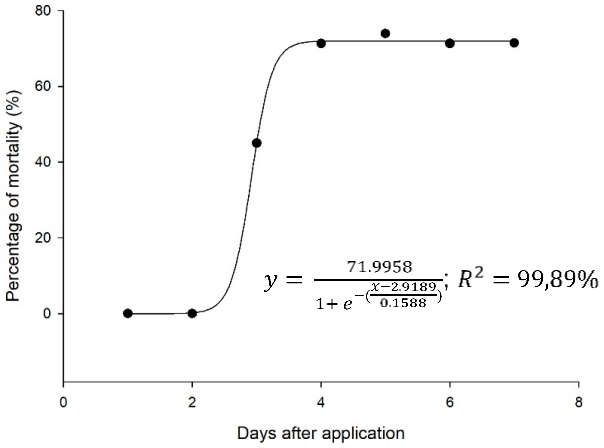

Fig. 5: Average of larva mortality (%) of *Leucoptera coffeella* promoted by entomopathogenic nematodes, *Heterorhabditis amazonensis* MC01 and *Steinernema feltiae* during seven days

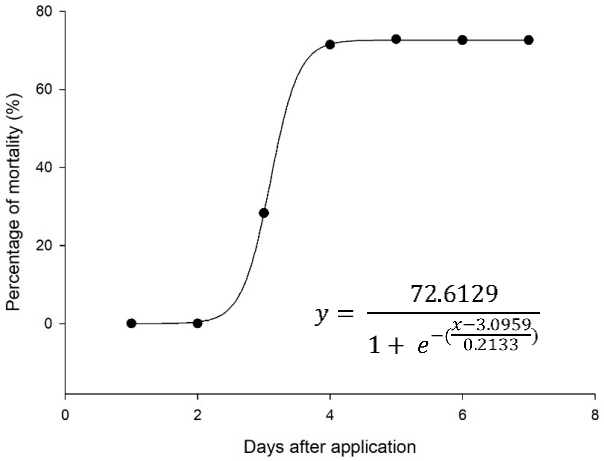

Fig. 6: Average of pupae mortality (%) of *Leucoptera coffeella* promoted by entomopathogenic nematodes, *Heterorhabditis amazonensis* MC01 and *Steinernema feltiae* during seven days

Pino *et al*. (2013) observed that *S. carpocapsae* and *H. bacteriophora* promoted high mortality rates of *T. absoluta* larvae, with rates of 100.0 and 96.7%, respectively. For *L. coffeella*, we showed mortality rates of up to 79% (*H. amazonensis* GL) when the concentration of 80 IJs insect^{-1} was applied, which suggests a higher susceptibility of larvae to entomopathogenic nematodes.

Both pathogenicity and virulence evaluations were used as criteria to select the suitable isolates to be tested along with the study, which are essential tests to evaluate whether such nematode species holds potential for *L. coffeella* control. Studies carried out by Steyn *et al*. (2019), by assessing the potential of *Heterorhabditis* sp. and *Steinernema* sp. in controlling *Holocist capensis* Van Nieukerken & Geertsema (Lepidoptera: Heliozelidae), observed that *H. noenieputensis*, *H. indica* and *H. baujardi* are highlighted in terms of mortality of mining insect, as compared to other nematodes species. It is important to bear in mind that many studies have tested steinernematidae nematodes species (Damme *et al*. 2015; Gözel and Kasap 2015; Kamali *et al*. 2017) and therefore, the outputs from Steyn *et al*. (2019) report with heterohabditids as a promising potential alternative for virulence highlights the importance of carrying out the isolate selection tests.

Kepenekci *et al*. (2013) evaluated nematodes entomopathogenic to *P. operculella* and evidenced that *S. carpocapsae* was the most virulent species; however, *H. bacteriophora* also displayed high mortality rates (80%). In the present study, five isolates are highlighted, among these, four isolates of *Heterorhabditis* sp. and one of *Steinernema* sp., which suggests thus the importance of selecting entomopathogenic nematodes that are better associated with the targeted insect.

Among the most virulent, *H. amazonensis* MC01 was selected because it is a native species and better fitted to local conditions. Ndereyimana *et al*. (2019), for example, observed that native isolates present higher mortality (53.3–96.7%) to *T. absoluta* than exotic species (0–26.7%), thus demonstrating the importance of testing native isolates that can better survive in the environment and promote higher pest mortality rates.

Steinernema feltiae was selected because it is the only species of *Steinernema* sp. among the most virulent and hold different biological and behavioral characteristics from heterorhabditids, such as size, absence of dorsal teeth and intermediate foraging strategists (ambusher and cruiser) (Campbell and Gaugler 1997), which can be more efficient in targeting the host. *Heterorhabditis* displays a cruiser behavior, but the dorsal tooth enables insect's cuticle infection (Griffin *et al*. 2005).

Based on the observed promising results for larvae, we speculate the potential ability to control *L. coffeella* pupae, thereby allowing the targeting of applications to different stages of insect pest development. Thus, studies with pupae were included based on the concentration tests, in which it made it possible to verify that both *H. amazonensis* and *S. feltiae* were also pathogenic to pupae by causing 80% mortality rates.

Hassani-Kakhki *et al*. (2013) showed that the prepupae was the most susceptible development stage of *P. operculella* to entomopathogenic nematodes, highlighting the importance of the difference in susceptibility as a function of insect's developmental stages. However, for *T. absoluta* pupae, Batalla-Carrera *et al*. (2010) and Garcia-del-Pino *et al*. (2013) observed low susceptibility, which is the stage of development least affected by the action of the entomopathogen.

Susceptibility differences may be associated with morphological characteristics of pupae, such as the absence of natural openings (oral cavity and anus) that facilitate the invasion of nematodes in pupae (Kaya and Hara 1980; González-Ramírez *et al*. 2000).

Concerning the concentration of application, Kepenekci *et al*. (2013) observed that there is a positive correlation between concentration and mortality for *P. operculella*, except for *S. feltiae*, which displayed no difference in mortality at the tested concentrations. *Steinernema feltiae* was also considered a nematode with low virulence to *P. operculella* by Hassani-Kakhki *et al*. (2013). The increase in mortality of *L. coffeella* was associated with increases in the concentration of IJs, both for *H. amazonensis* MC01 and for *S. feltiae*.

Damme *et al*. (2015) and Ndereyimana *et al*. (2019) have suggested that this direct relationship between concentration and mortality may occur as a consequence of a higher level of infective juveniles into the insect, thereby leading to a higher number of symbiotic bacteria and consequently, increased rates of both toxins and secreted hydrolytic enzymes by bacteria, thereby causing higher insect mortality during a shorter timeframe.

Yan *et al*. (2020) pointed out the LC_{50} of 181 IJs larva^{-1} *S. carpocapsae* to 4th instar larvae of *P. operculella* as the most susceptible instar, while Hassani-Kakhki *et al*. (2013) observed lower rates of susceptibility with *S. carpocapsae* at concentration of 64 IJs larvae^{-1}. Thus, it is important to note that the trials regarding concentration must be performed to optimize and select the best level of IJs, thus improving the control and reducing expenses - since applications in high concentrations may only not increase insect mortality but also elevate the costs of control.

Regarding lethal time, Ndereyimana *et al*. (2019) observed mortality of *T. absoluta* larvae shortly after 24 h of inoculation, with rates of up to 96.7%, while after 72 h, the values reached up to 100%. In the present study, the onset of larvae and pupae mortality occurred after 72 h and reached a level of stability after 96 h of inoculation. These times can be classified as short by considering other biological control agents, such as entomopathogenic viruses and fungi (Goettel *et al*. 2005; Valicente 2019), being thus a response to released bacteria inside the insect as soon as the infection occurs (Gaugler and Kaya 1990).

The quick infection of entomopathogenic nematodes in

the insect is a characteristic that allows its survival when applied on leaves, thus avoiding long exposures to high temperatures, UV radiation and desiccation (Ndereyimana *et al*. (2019).

Based on the observed results in the control of *L. coffeella*, it can be highlighted the ability of entomopathogenic nematodes to complete their life cycle and reproduce in the insect. It is interesting because it allows a longer period of action of the nematode in the field. Likewise, Yan *et al.* (2020) demonstrated that *S. carpocapsae* is able to reproduce in *P. operculella*, acting in long-term pest management.

Mhatre *et al*. (2020), evaluating the biocontrol potential of *S. cholashanense* in *P. operculella* larva and pupae, also reported a high reproduction rate of *S. cholashanense* in *P. operculella*, with results indicating a good control potential.

The application of entomopathogenic nematodes to control the leaf-miner can reduce the use of chemical insecticides in coffee areas, since in Brazil chemical control is the most used method, including mainly the application of organophosphates, pyrethroids, carbamates, neonicotinoids and more recently the diamides. However, the effectiveness of this inappropriate management, has developed resistance of the insect to some insecticides, also has increased the cost of production and has not been considerate effective to reduce the population of the pest (Almeida *et al.* 2020; Leite *et al*. 2020). The inclusion of entomopathogenic nematodes in a management program can increase the mortality of *L. coffeella*, reduce the chemicals insecticides, consequently reducing environment damage and the impact on natural enemies, providing a more sustainable agriculture. The association of entomopathogenic nematodes with the chemical insecticides should be investigated to avoid nematodes mortality or the reduction of infectivity.

Considering the difficult to control *L. coffeella* by chemical insecticides and the severe economic losses caused by the presence of the insect-pest, the obtained results with entomopathogenic nematodes are suitable and promising, and further tests in the field should be addressed, since the behavior of nematodes and their interaction with the environment can be different in the field, and these factors can affect their effectiveness.

Conclusion

All nematodes tested were pathogenic to *L. coffeella* larvae. LC_{80} values for *L. coffeella* by using *S. feltiae* were 194 IJs $larva^{-1}$ and 195 IJs $pupa^{-1}$; for *H. amazonensis* MC01, LC_{80} were 165 IJs $larva^{-1}$ and 150 IJs $pupa^{-1}$. On the fourth day it was obtained stability in insect mortality. The nematode holds the ability to complete its life cycle in the insect, reproducing and forming next generations, which could allow a persistence of the nematodes in the field and could also reduce the number and the intervals of application. The higher the concentration of applied infective juveniles, the greater the number of nematodes able to infect and reproduce in *L. coffeella*.

Future research related to the compatibility of entomopathogenic nematodes with chemical insecticides; the survivor of the infective juveniles on the leaves; protective products that could extend the viability and infectivity of the nematodes; the impact in pollinators and natural enemies, are tests that can be performed to better understand the effectiveness of entomopathogenic nematodes in controlling *L. coffeella*.

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Author Contributions

Conceptual Idea: AV, AGA de, MTFN de; Methodology design: AGA de, AV, FLS; Data collection: MTFN de, AV, FLS; Data analysis and interpretation: CFJ; Writing and editing: MTFN de, AV, AGA de, CFJ

Conflict of Interest

All authors declare no conflict of interest.

Data Availability

Data presented in this study will be available on a fair request to the corresponding author.

Ethics Approval

Not applicable to this paper.

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