

ENTOMOPATHOGENIC NEMATODES ON CONTROL OF MEDITERRANEAN FRUIT FLY (DIPTERA: TEPHRITIDAE)¹

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ABSTRACT - Mediterranean fruit fly (*Ceratitits capitata*) is one of the world's major pests infesting fruit crops, causing significant losses. However, this insect has a soil phase and is a potential target for entomopathogenic nematodes. The objective of this work was to test the efficiency of the nematodes *Steinernema carpocapsae* ALL and *Heterorhabditis amazonensis* JPM4 in controlling larvae and pupae of *Ceratitits capitata*. Petri dishes with filter paper or pots with 200 g of soil were used to assess different nematode concentrations; 20 individuals (larvae or pupae) were placed in each container and the nematode suspension was applied at concentrations of 0, 140, 180, 220, 260, and 300 IJs (infective juveniles) cm⁻². The nematode horizontal movement was tested using pots with soil and a metal screen at every 10 cm to define different distances (0, 10, 20, and 30 cm from the application site). Ten larvae and ten pupae were transferred to every distance; 10 mL of the nematode suspension (220 IJs cm⁻²) were applied at the distance zero; 10 mL of the nematode suspension with 220 IJs cm⁻² was applied to each pot, using ten larvae and ten pupae for each evaluation time (0, 10, 20, and 30 days after application of nematodes) to study the effectiveness of these nematodes over time. Both nematodes killed *C. capitata* larvae and pupae, but *S. carpocapsae* ALL was the most effective. However, this nematode showed no ability for horizontal movement, which was found in *H. amazonensis* JPM4 nematodes. Both nematodes showed decreasing efficiency over time.

Keywords: *Ceratitits capitata*. *Steinernema carpocapsae*. *Heterorhabditis amazonensis*. Biological control.

NEMATÓIDES ENTOMOPATOGÊNICOS PARA O CONTROLE DA MOSCA-DO-MEDITERRÂNEO (DIPTERA: TEPHRITIDAE)

RESUMO - A mosca-das-frutas (*Ceratitits capitata*) é considerada uma das principais pragas da fruticultura, acarretando perdas significativas para este setor. Este inseto passa uma fase de sua vida no solo, sendo alvo em potencial para nematoides entomopatogênicos. O objetivo desse trabalho foi avaliar a eficiência de *Steinernema carpocapsae* ALL e *Heterorhabditis amazonensis* JPM4 para o controle de *Ceratitits capitata* (Diptera: Tephritidae). Para a avaliação de diferentes concentrações foram utilizadas placas com papel filtro ou vasos com 200g de solo. Para cada recipiente foram transferidos 20 indivíduos (larvas ou pupas) e aplicada a suspensão dos nematoides nas concentrações 140, 180, 220, 260 e 300 IJs/cm². Para avaliação do deslocamento horizontal, foram utilizados vasos contendo solo e a cada 10cm de distância foi colocada uma tela de metal, para delimitar as diferentes áreas (0, 10, 20 e 30cm do local de aplicação do nematoide). Para cada área foram transferidas 10 larvas e 10 pupas. Na distância zero foram aplicados de 10 mL de suspensão dos nematoides contendo 220IJs/cm². Para o estudo da eficiência desses nematoides ao longo do tempo, em cada vaso foram aplicados 10mL de suspensão de nematoide com 220IJs/cm², e em cada período (0, 10, 20 e 30 dias pós-aplicação do nematoide) foram transferidas 10 larvas e 10 pupas. Verificou-se que os dois nematoides causaram mortalidade de *C. capitata*, sendo que *S. carpocapsae* ALL foi mais eficiente. Porém, esse nematoide não apresentou capacidade de deslocamento horizontal, característica essa observada para *H. amazonensis* JPM4. Ambos os nematoides tiveram sua eficiência reduzida ao longo do tempo.

Palavras-chave: *Ceratitits capitata*. *Steinernema carpocapsae*. *Heterorhabditis amazonensis*. Controle biológico.

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INTRODUCTION

Mediterranean fruit fly (*Ceratitis capitata*) is one of the world's major pests infesting fruit crops, causing significant losses due to direct and indirect damages. Females cause direct damage because of the oviposition during fruit ripening; the larvae forage on the fruit pulp, causing rot and premature fall of fruits. Indirect damages also occur, since this insect is a quarantine pest. The presence of this pest species limits the access of fruits to international markets due to quarantine restrictions imposed by importing countries (MURIITHI et al., 2016).

Ceratitis capitata (Wiedemann) (Diptera: Tephritidae) is one of the most important fruit fly species because it is the most cosmopolitan and invasive species, occurring in all biogeographic regions of the world and, consequently, causing the most damages to fruit production (EGARTNER et al., 2019). The control of fruit flies usually requires more than one method. It can be achieved through cultural, behavioral, legislative, biological, and chemical methods, the latter being the most used (DIAS et al. 2018). However, chemical control using toxic sprays or baits contributes to an imbalance in the ecosystem, affecting natural enemies and non-target organisms. In addition, pesticides are expensive and can leave toxic residues on fruits, which are prejudicial to the fresh fruit marketing and consumption (MURIITHI et al., 2016).

Entomopathogenic nematodes belonging to the Heterorhabditidae and Steinernematidae families are efficient biological control agents because they present selectivity and harmlessness to the environment and other living organisms, including humans; thus, they are a great resource for integrated pest managements (LABAUDE; GRIFFIN, 2018). These entomopathogens have potential to control insect pests that live in the soil, such as fruit flies, whose larvae fall to the soil to develop into pupae, allowing the action of nematodes (GRIFFIN et al., 2005).

Laboratory and field studies have been developed to determine the effects of species and strains of entomopathogenic nematodes on different developmental stages of *C. capitata* insects and showed high susceptibility for this insect (MALAN; MANRAKHAN, 2009; ROHDE et al., 2010; ROHDE et al., 2012; ROHDE et al., 2013; MINAS et al., 2016; JAMES; MALAN; ADDISON, 2018; CHERGUI et al., 2019).

However, further studies are needed to determine the best conditions and application strategies to achieve maximum efficiency of these entomopathogens in controlling fruit flies. Thus, the objectives of this study were to evaluate the efficiency of applications with different concentrations of the nematodes *Steinernema carpocapsae* ALL (Rhabdita: Steinernematidae) and *Heterorhabditis amazonensis* JPM4 (Rhabditida:

Heterorhabditidae) in controlling larvae and pupae of *C. capitata* in laboratory and greenhouse assays, and to determine the horizontal movement and efficiency of these nematodes over time.

MATERIAL AND METHODS

Ceratitis capitata insect rearing

C. capitata pupae from the Center for Nuclear Energy in Agriculture (CENA; Piracicaba, SP, Brazil) were used at the beginning of the breeding; they were maintained under controlled temperature (25 ± 2 °C), relative humidity ($70\pm 10\%$), and photoperiod (12 hours), as proposed by Minas et al. (2016).

Obtention of entomopathogenic nematodes

The bioassays were carried out using the *H. amazonensis* JPM4 isolate, from Minas Gerais, Brazil; and *S. carpocapsae* ALL isolate, from North Carolina, USA. The nematodes were multiplied in vivo, using a method adapted from Woodring and Kaya (1988), using last instar larvae of *Galleria mellonella* (Linnaeus) (Lepidoptera: Pyralidae).

Entomopathogenic nematode concentration on larvae and pupae of *C. capitata* in Petri dishes

Bioassays were carried out in a randomized design with seven replications. The plots consisted of Petri dishes with 20 individuals. The larva trial was carried out using standardized individuals at the end of the third instar; and the pupa bioassay tested two ages the effect of the nematodes was tested on young (0- to 2-day-old) and old (6- to 8-day-old) pupae. The individuals were transferred to Petri dishes (9 cm diameter) with two sheets of filter paper, where 1 mL of the nematode suspension (*H. amazonensis* JPM4 or *S. carpocapsae* ALL) was added at concentrations of 0 (control treatment), 140, 180, 220, 260, and 300 IJs (infective juveniles) cm⁻²; 1 mL of distilled water was used for the control treatment. These Petri dishes were kept in an incubator (25 ± 1 °C, relative humidity of $70\pm 10\%$, and photoperiod of 12 hours). The insects were evaluated at 5 days after application, and the dead ones were analyzed for typical symptoms of death by *Steinernema* sp. (dark brown color) or *Heterorhabditis* sp. (red color). In addition, the dead ones were dissected and the presence of nematodes confirmed their death by nematodes.

Entomopathogenic nematode concentration on larvae and pupae of *C. capitata* in soils under laboratory and greenhouse conditions

Bioassays were carried out in a randomized

design with seven replications. The plots consisted of 1-liter plastic pots with 200 g of sterilized soil (Typic Hapludox with initial standardized moisture of 25%) and 20 *C. capitata* insects. The laboratory experiment was carried out using 20 third-instar larvae and twenty 6- to 8-day-old pupae. The greenhouse experiment was carried out using a mix of ten third-instar larvae and ten 6- to 8-day-old pupae; 10 mL of nematode suspension (*H. amazonensis* JPM4 or *S. carpocapsae* ALL) at concentrations of 0 (control treatment), 140, 180, 220, 260, and 300 IJs cm⁻² were applied, using 10 mL of distilled water for the control treatment. The pots were kept in an incubator (25±1 °C, relative humidity of 70±10%, and photoperiod of 12 hours) or in a greenhouse (10 to 33 °C). The soil moisture in the pots was corrected when necessary. The same evaluation procedure of the previous bioassay was used at 5 days after application.

Horizontal movement of entomopathogenic nematodes

Bioassays were carried out in a randomized design with six replications. The plots consisted of plastic pots (40 cm long × 13 cm wide × 11 cm high) with sterilized soil (Typic Hapludox with initial standardized moisture of 25%). A 60-mesh metal screen was placed every 10 cm to allow the nematodes and prevent *C. capitata* larvae to pass through; this procedure defined different distances (10, 20, 30, and 40 cm from the nematode application site). A mix of ten third-instar larvae and ten 6- to 8-day-old pupae was applied to each distance; 10 mL of the nematode suspension (*H. amazonensis* JPM4 or *S. carpocapsae* ALL) at 220 IJs cm⁻² were applied to the distance zero, using 10 mL of distilled water for the control treatment. The pots were kept in a greenhouse (10 to 33 °C) and watered evenly when necessary. The insects were evaluated at 10 days after application, following the same procedure of the previous bioassay.

Efficacy of entomopathogenic nematodes in controlling larvae and pupae of *C. capitata* over time

Bioassays were carried out in a randomized design with four treatments for each nematode, with seven replications. The plots consisted of 1-liter plastic pots with 200 g of sterilized soil (Typic Hapludox with initial standardized moisture of 25%); 10 mL of nematode suspension (*H. amazonensis* JPM4 or *S. carpocapsae* ALL) at 220 IJs cm⁻² were applied to each pot, using 10 mL of

distilled water for the control treatment. The pots were kept in a greenhouse (7 to 34 °C) and watered evenly when necessary. A mix of ten third-instar larvae and ten 6- to 8-day-old pupae was transferred to each pot at each evaluation time (0, 10, 20, and 30 days after application of nematodes). The insects were evaluated at 10 days after application, following the same procedure of the previous bioassay. The samples were analyzed and then discarded after each evaluation time.

Statistical analysis

The data were subjected to the Shapiro-Wilk normality test and did not showed normality; thus, they were subjected to non-parametric analyses by the Kruskal Wallis Test ($p \leq 0.05$), and the values were compared by the Simes-Hochberg test, using the Action Stat Program.

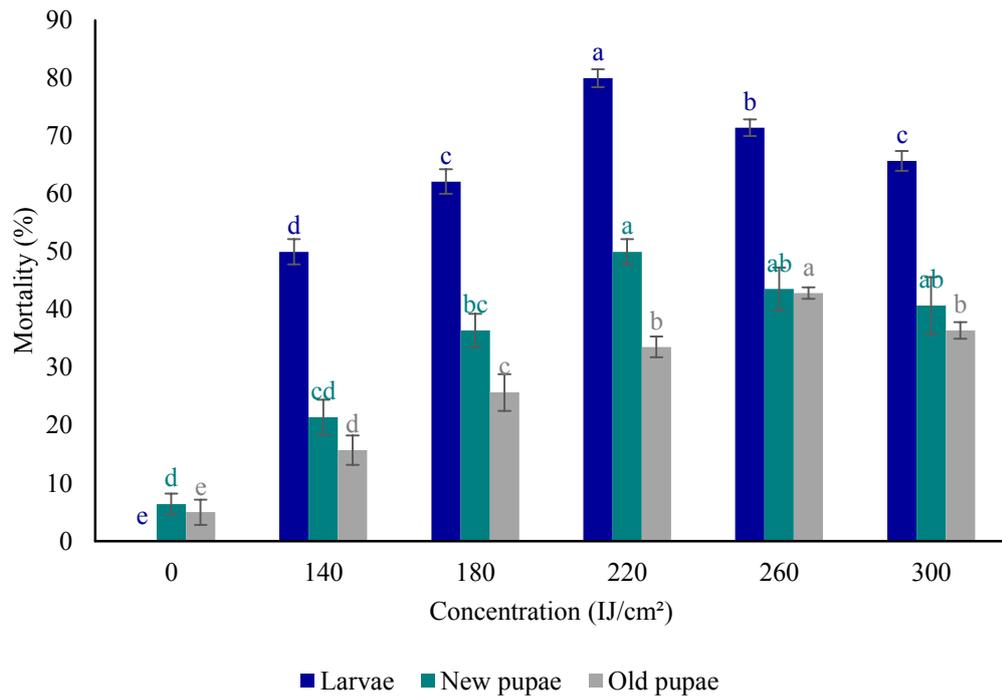
RESULTS AND DISCUSSION

Entomopathogenic nematode concentration on larvae and pupae of *C. capitata* in Petri dishes under laboratory conditions

All evaluated concentrations of *S. carpocapsae* ALL nematodes killed *C. capitata* larvae and pupae at different ages; the larval stage was the most susceptible, showing mortality rates of 50% to 80%. This nematode killed 21% to 50% young pupae and 15% to 43% old pupae (Figure 1).

The *C. capitata* larvae mortality rate increased proportionally to the nematodes concentration up to 220 IJs cm⁻², and showed relative decreases when using concentrations higher than 220 IJs cm⁻². The three highest concentrations used were the best for young pupae. Old pupae showed higher mortality rate for the concentration of 260 IJs cm⁻².

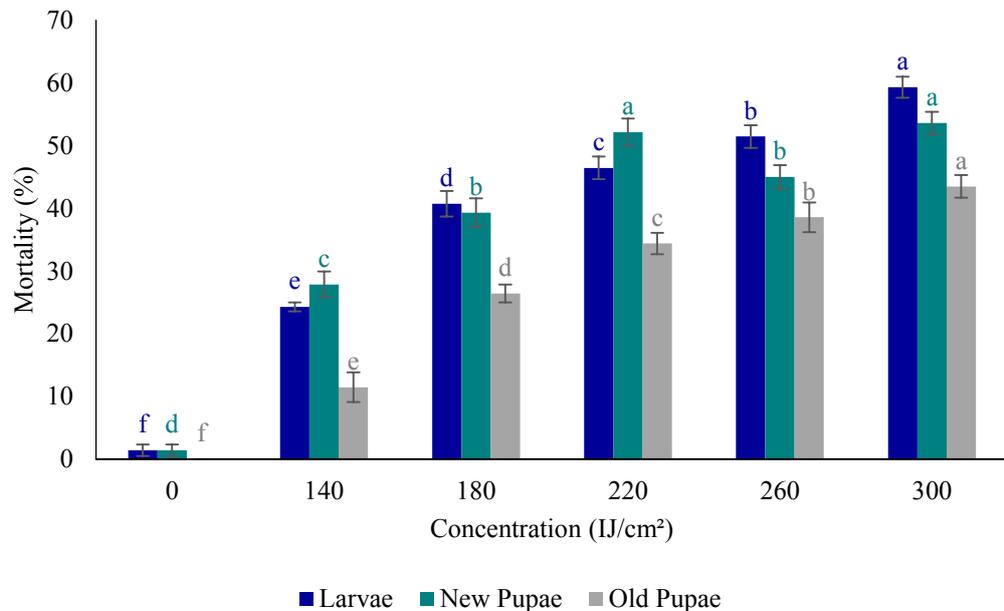
The decreases in mortality rate when using concentrations of *S. carpocapsae* ALL nematode greater than 220 IJs cm⁻² for *C. capitata* larvae or 260 IJs cm⁻² for pupae were probably due to intraspecific competition of the nematode. According to Gaugler, Wang and Campbell (1994), when a host is infected by entomopathogenic nematodes, a small number of nematodes is required to overcome the host immune system and colonize it until its death. However, when the number of nematodes is very large, intraspecific competition occurs, affecting their survival, development, and reproduction, which reduces their virulence (SELVAN; CAMPBELL; GAUGLER, 1993).



Bars with the same color and letter are not different by the Simes-Hochberg's test ($p \leq 0.05$). **Figure 1.** Mortality rates (mean \pm standard error) of larvae, young pupae, and old pupae of *Ceratitis capitata* evaluated at five days after exposure to different concentrations of *Steinernema carpocapsae* ALL nematodes in Petri dishes under laboratory conditions (25 ± 1 °C, relative humidity of $70 \pm 10\%$, and photoperiod of 12 hours).

The all evaluated concentrations of *H. amazonensis* JPM4 nematodes also killed *C. capitata* larvae and pupae at different ages. The larval stage was also the most susceptible to *H. amazonensis*

JPM4 nematodes, presenting mortality rates of 24% to 60%, with 28% to 54% for young pupae and 11% to 44% for old pupae (Figure 2).



Bars with the same color and letter are not different by the Simes-Hochberg's test ($p \leq 0.05$). **Figure 2.** Mortality rates (mean \pm standard error) of larvae, young pupae, and old pupae of *Ceratitis capitata* evaluated at five days after exposure to different concentrations of *Heterorhabditis amazonensis* JPM4 nematodes in Petri dishes under laboratory conditions (25 ± 1 °C, relative humidity of $70 \pm 10\%$, and photoperiod of 12 hours).

The higher efficiency of *S. carpocapsae* ALL nematodes in controlling *C. capitata* larvae may be due to the relatively small size of infective juveniles (438 to 650 μ) (ADAMS; NGUYEN, 2002), which facilitates the penetration of Steinernematidae species through natural openings of the host (spiracles, mouth, and anus). Thus, the lower efficiency of *S. carpocapsae* ALL nematodes in controlling *C. capitata* pupae was because of the behavior of the nematode, which is an ambusher species (SHAPIRO ILAN; HAZIR; GLAZER, 2017). Lewis et al. (2006) point out that ambusher nematodes, which wait for the best moment to attack the host, are more effective in finding high-mobility hosts, whereas cruiser nematodes, which move in search for hosts, are more likely to find sedentary hosts.

Infective juveniles of *H. amazonensis* nematodes have body sizes of 567 to 618 μ (ANDALÓ; MOREIRA; MOINO Jr, 2009), larger than *S. carpocapsae* ones. This explains the lower success of using them for the control of *C. capitata* larvae. Studies have shown that *H. amazonensis* are cruiser nematodes (ANDALÓ; MOREIRA; MOINO Jr, 2014), which increases their success in searching for low-mobility preys and may have contributed to a higher success in controlling young and old pupae when compared to *S. carpocapsae* nematodes.

Efficiency of *S. carpocapsae* ALL nematodes in controlling *C. capitata* larvae under laboratory conditions has been found in other studies. Rohde et al. (2012) found mortality rates of *C. capitata* larvae higher than 90%. Similar results were found by Foelkel, Monteiro and Voss (2016) for *Anastrepha fraterculus* (Wiedemann) (Diptera: Tephritidae); by Heve et al. (2017) for *Anastrepha suspensa* (Loew) (Diptera: Tephritidae); and by Sirjani, Lewis and Kaya (2009) for *Bactrocera oleae* (Gmelin) (Diptera: Tephritidae). Rohde et al. (2012) tested the efficiency of *S. carpocapsae* ALL nematodes in controlling *C. capitata* pupae and found mortality rate of 20% when using 200 IJs per insect, equivalent to 100 IJs cm^{-2} .

Efficiency of *H. amazonensis* JPM4 nematodes in controlling *C. capitata* insects was also found by Rohde (2012), who reported mortality rates of 40% and 41.3% for larvae and pupae, respectively, when using 200 IJ per insect (equivalent to 100 IJs cm^{-2}). Foelkel, Monteiro and Voss (2016) tested *H. amazonensis* JPM4 nematodes for the control of *A. fraterculus* larvae and found mortality rates of approximately 50% when applying the nematodes in the soil, in laboratory tests.

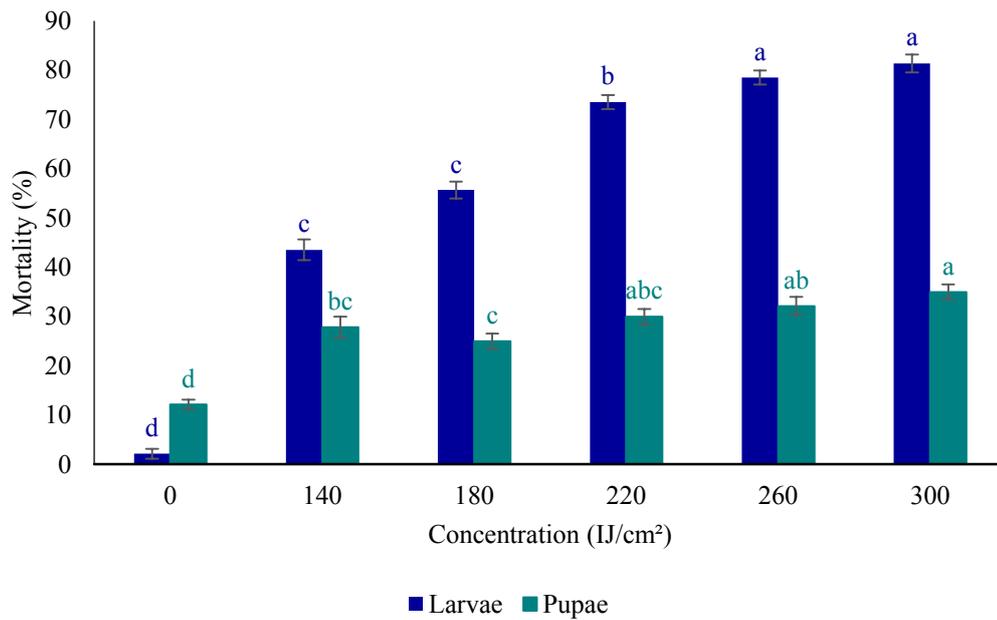
Both nematodes present greater virulence to *C. capitata* larvae than to young and old pupae. This may be due to the greater susceptibility of larvae to entomopathogenic nematodes because of their great locomotion at this stage and great release of CO_2 , which attracts nematodes (SHAPIRO-ILAN; HAZIR; GLAZER, 2017). The large natural openings in the body of *Steinernema* and *Heterorhabditis* larvae and less sclerotized tegument (compared to pupal stage) of *Heterorhabditis* facilitate the infection of the insect by the nematode. The lower susceptibility of pupae can be related to the small openings of their spiracles, which hinders the penetration of the nematode, or it is due to the protection of the pupa by a sclerotized puparium that hinders the penetration (MINAS et al., 2016).

Similar studies with *C. capitata* insects also reported higher susceptibility of larvae than pupae (ROHDE et al., 2012; MINAS et al., 2016); studies with insects of the species *C. capitata*, *Ceratitis rosa* Karsch (Diptera: Tephritidae) (MALAN; MANRAKHAN, 2009), *Rhagoletis cerasi* Linnaeus (Diptera: Tephritidae) (KEPENEKCI; HAZIR; ÖZDEM, 2015), *A. suspensa* (HEVE et al., 2017), *Bactrocera oleae* Rossi (Diptera: Tephritidae) (TORRINI et al., 2020), and *Bactrocera dorsalis* Handel (Diptera: Tephritidae) (GODJO et al., 2018) exposed to different *Steinernema* and *Heterorhabditis* nematode species and isolates found no or low susceptibility of pupae to the nematodes.

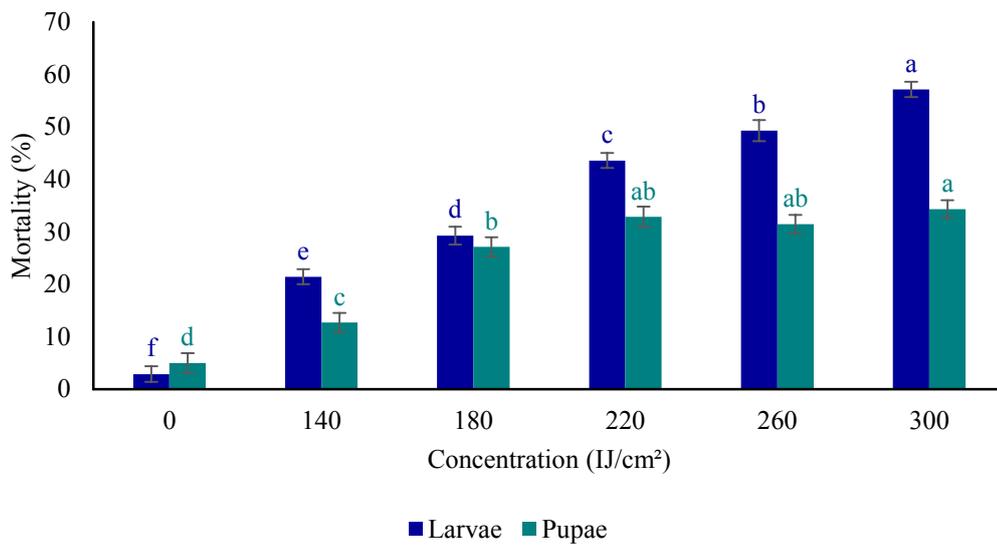
Young pupae were more susceptible than old pupae to both nematodes, as also found by Chergui et al. (2019), who evaluated the effect of entomopathogenic nematodes on *C. capitata* insects. The tegument of young pupae is under sclerotization process and, thus, more susceptible to nematodes (GODJO et al., 2018).

Entomopathogenic nematode concentration on larvae and pupae of *C. capitata* in soils under laboratory and greenhouse conditions

The mortality rates of larvae and pupae of *C. capitata* in the soil under laboratory conditions increased proportionally to the nematode concentration for both evaluated nematode species. The mortality rates of *C. capitata* insects caused by *S. carpocapsae* ALL nematodes were 43% to 81% for larvae, and 25% to 35% for pupae (Figure 3). The mortality rates of *C. capitata* insects caused by *H. amazonensis* JPM4 nematodes were 21% to 57% for larvae and 13% to 34% for pupae (Figure 4).



Bars with the same color and letter are not different by the Simes-Hochberg's test ($p \leq 0.05$). **Figure 3.** Mortality rates (mean \pm standard error) of larvae, young pupae, and old pupae of *Ceratitis capitata* evaluated at five days after exposure to different concentrations of *Steinernema carpocapsae* ALL nematodes in soil under laboratory conditions (25 ± 1 °C, relative humidity of $70 \pm 10\%$, and photoperiod of 12 hours).



Bars with the same color and letter are not different by the Simes-Hochberg's test ($p \leq 0.05$). **Figure 4.** Mortality rates (mean \pm standard error) of larvae, young pupae, and old pupae of *Ceratitis capitata* evaluated at five days after exposure to different concentrations of *Heterorhabditis amazonensis* JPM4 nematodes in soil under laboratory conditions (25 ± 1 °C, relative humidity of $70 \pm 10\%$, and photoperiod of 12 hours).

The infectivity of both nematodes to larvae was similar when they were inoculated in Petri dishes or in the soil under laboratory conditions. However, when considering pupae in the soil, the mortality rate was 16% lower when exposed to *S. carpocapsae* nematodes and 14% lower when exposed to *H. amazonensis* nematodes.

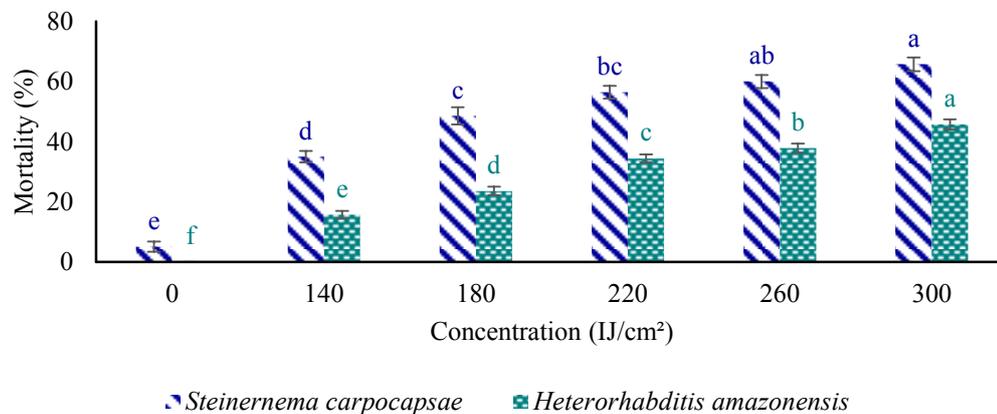
Minas et al. (2016) also found that the

mortality rate of larvae and pupae of *C. capitata* increases proportionally to the nematode concentration used (*Heterorhabditis* sp. LPP17, *Heterorhabditis* sp. LPP14 and *Heterorhabditis baujardi* LPP7), and found higher susceptibility of larvae, with mortality rates of 80% to 100% when exposed to concentrations of 237 and 553 IJ cm⁻²; they found high mortality rate of pupae

(approximately 80%), but using concentrations higher than 800 IJ cm⁻² (2.6-fold higher than the concentration used in the present study).

In greenhouse conditions, the results found for both nematodes were similar; the mortality rates

of *C. capitata* insects increased proportionally to the nematode concentration (Figure 5). *S. carpocapsae* ALL nematodes presented greater virulence than *H. amazonensis* JPM4, confirming the results found under laboratory conditions.



Bars with the same color and letter are not different by the Simes-Hochberg's test ($p \leq 0.05$).
Figure 5. Mortality rates (mean \pm standard error) of larvae, young pupae, and old pupae of *Ceratitis capitata* evaluated at five days after exposure to different concentrations of *Steinernema carpocapsae* ALL and *Heterorhabditis amazonensis* JPM4 nematodes in soil under laboratory conditions (25 \pm 1 °C, relative humidity of 70 \pm 10%, and photoperiod of 12 hours).

The efficiency of these nematodes in greenhouse conditions was approximately 20% lower than that found for larvae in soil under laboratory conditions. This difference was because the *C. capitata* larvae and pupae were evaluated separately in laboratory bioassays, but in greenhouse conditions, the effect of the entomopathogens was assessed together for the two stages. Therefore, the pupal stage is less susceptible, showing lower mortality than the larval stage evaluated in the bioassay.

Another factor that may have contributed to this lower mortality is the air temperature, which affects soil temperature. Temperature variations were observed inside the greenhouse throughout the experiment period, with minimum of 10 °C and maximum of 33 °C.

In general, *S. carpocapsae* nematodes show higher virulence when kept at temperatures between 20 and 30 °C (ROHDE et al., 2010; MASTORE et al., 2019). El-Sadawy (2001) found higher virulence of species of the genus *Heterorhabditis* when they were kept at 25 °C.

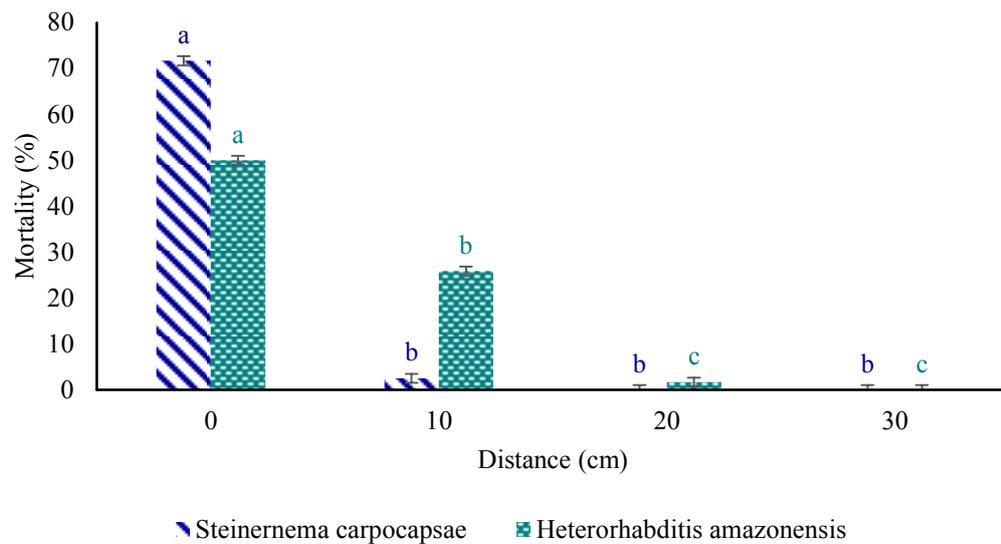
The nematodes *S. carpocapsae* ALL and *H. amazonensis* JPM4 did not reach 100% mortality rate of *C. capitata* insects in any of the evaluated concentrations, showing the need for assessing the effect of higher concentrations. However, the use of higher concentrations is economically unviable, mainly for large agricultural areas, because of the

product availability; thus, the use of low rates of the product combined with other control methods in integrated pest management is more viable. The concentration of 220 IJs cm⁻² used in the present work caused considerable reduction in pest population and was used in the following bioassays.

Horizontal movement of entomopathogenic nematodes

The *S. carpocapsae* nematodes killed *C. capitata* insects only in the site where they were applied (0 to 10 cm). *H. amazonensis* nematodes killed *C. capitata* insects in the site where they were applied (0 to 10 cm) and at the distance of 20 cm away (Figure 6).

The low mortality rate of *C. capitata* insects caused by *S. carpocapsae* nematodes in the other distances showed that this nematode had no ability to move horizontally to reach the insects at those distances. These nematodes are ambushers, they wait the hosts to approach to attack them; they have less mobility and great effectiveness against high-mobility hosts (LEWIS et al., 2006). Morton and García-del-Pino (2009) tested the vertical movement ability of this nematode and found higher concentration of IJs in the soil surface than in deeper layers, confirming the low migration capacity of this entomopathogen.



Bars with the same color and letter are not different by the Simes-Hochberg's test ($p \leq 0.05$).
Figure 6. Mortality rates (mean \pm standard error) of *Ceratitis capitata* insects evaluated at 10 days after exposure to *Steinernema carpocapsae* ALL and *Heterorhabditis amazonensis* JPM4 nematodes, at different distances from the nematode application site in greenhouse conditions.

Contrastingly, *H. amazonensis* are cruiser nematodes (ANDALÓ; MOREIRA; MOINO Jr, 2014), which is confirmed by the results found for the migration ability of this nematode. Andaló et al. (2012) found that this nematode can kill larvae of *G. mellonella* and *Spodoptera frugiperda* (Smith) (Lepidoptera: Noctuidae) at up to 60 cm away, in sand columns.

Efficacy of entomopathogenic nematodes in controlling larvae and pupae of *C. capitata* over time

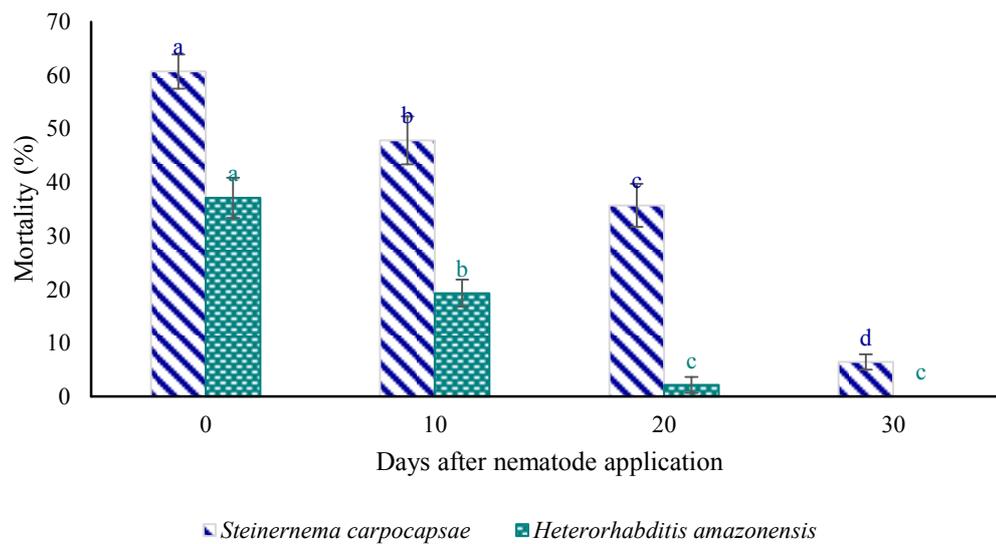
Both nematodes (*S. carpocapsae* and *H. amazonensis*) presented a decreasing virulence over time. *S. carpocapsae* nematodes killed *C. capitata* insects in all evaluation times, but the mortality rates decreased 21%, 41%, and 89% at 10, 20, and 30 days after the nematode application, respectively, when compared to the mortality rate of insects in contact with nematodes on the same day they were applied (day 0). The *H. amazonensis* nematodes presented decreases in mortality rates of approximately 58% and 94% at 10 and 20 days after the nematode application, respectively, when compared to those at day 0, and presented mortality rate of zero for insects at 30 days after the nematode application (Figure 7).

S. carpocapsae nematodes remained virulent to *C. capitata* insects even at 20 days after the nematode application, a longer time than that found for *H. amazonensis* nematodes, which maintained the

same virulence from day 0 only until 10 days after application. These results denote that the control of *C. capitata* insects in soil using *S. carpocapsae* nematodes requires fewer applications than that using *H. amazonensis* nematodes. However, further studies with these nematodes under field conditions and with presence of host insects are needed to evaluate the nematode maximum persistence and virulence, and determine the adequate interval between applications to control fruit flies.

Several factors can affect the persistence, mobility, development, and reproduction of entomopathogenic nematodes over time, thus affecting their infectivity. Temperature, humidity, and ultraviolet radiation are the most important of these factors, because their extremes can reduce the nematode survival, mainly of infective juveniles, and affect other parameters.

The optimum temperature, humidity, and ultraviolet radiation ranges for the activity of entomopathogenic nematodes vary according to the species and isolates. These entomopathogens, in general, remain viable in areas with temperatures between 15 and 35 °C (ULU; SUSURLUK, 2014; KHOURY et al., 2018), soil moistures that allow for the formation of a free water film between soil particles to prevent desiccation and allow the nematodes to move and survive (SHAPIRO-ILAN; HAN; DOLINSKI, 2012), and low exposure to ultraviolet radiation (SHAPIRO-ILAN; HAN; DOLINSKI, 2012).



Bars with the same color and letter are not different by the Simes-Hochberg's test ($p \leq 0.05$).
Figure 7. Virulence of *Steinernema carpocapsae* ALL and *Heterorhabditis amazonensis* JPM4 nematodes to *Ceratitidis capitata* insects evaluated at different days after the nematode application in the soil, under greenhouse conditions.

Soil moisture was probably not a factor responsible for the decreases in nematode virulence, since the soil had its initial moisture content standardized to 25%, which was maintained throughout the bioassay through irrigation. This initial soil moisture of 25% was used because it was the moisture in which *S. carpocapsae* ALL nematodes had higher efficiency in controlling *C. capitata* larvae in bioassays carried out by Rohde et al. (2010).

Temperature may have affected the nematode virulence because of its high variation in the greenhouse throughout the bioassay, with minimum of 7 °C and maximum of 34 °C. Rohde et al. (2010) found that infectivity is proportional to increases in temperature, with maximum mortality rates of 86.7% and 80.0% caused by *S. carpocapsae* and *Heterorhabditis* species, respectively, at 31 °C. The high exposure to ultraviolet radiation may have also affected the performance of the entomopathogen. Shapiro-Ilan, Hazir and Leite (2015) found that *S. carpocapsae* nematodes are less susceptible to desiccation and ultraviolet radiation than *Heterorhabditis* species, which may explain the greater decrease in gradual virulence of *S. carpocapsae* ALL when compared to that of *H. amazonensis* JPM4 nematodes.

CONCLUSION

The nematodes *Steinernema carpocapsae* ALL and *Heterorhabditis amazonensis* JPM4 killed larvae and pupae of *Ceratitidis capitata*, and the first stage of development of the insect was the most susceptible. *S. carpocapsae* ALL was the most

virulent nematode species, reducing the *C. capitata* population by 66% when applied to the soil, under greenhouse conditions, and at a concentration of 300 IJs cm⁻². Under these same conditions, the nematodes *H. amazonensis* JPM4 killed nearly 46% of the *C. capitata* population. The nematodes *S. carpocapsae* ALL were not capable of horizontal migration, a characteristic found for the nematodes *H. amazonensis* JPM4 which migrated to distances of up to 20 cm. The efficiency of the nematodes *S. carpocapsae* ALL and *H. amazonensis* JPM4 in controlling *C. capitata* insects decreased over time, and *H. amazonensis* JPM4 presented the highest decreases.

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