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Scientific paper

Effect of *Heterorhabditis* sp. and *Steinernema carpocapsae* applied in different periods of soil infestation with larvae of *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae)

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Abstract

The Mediterranean Fruit Fly, (*Ceratitis capitata*) (Wiedemann) is considered one of the main pests of fruit culture around the world, causing significant losses in this sector. This insect stays in the soil during a phase of life, where it becomes a target for entomopathogenic nematodes. Therefore, this work aimed to evaluate the effect of *Heterorhabditis* sp. RSC01 and *Steinernema carpocapsae* All, applied alone or combined, and in different periods of soil infestation with larvae of *C. capitata*. For the first bioassay the treatments were: *S. carpocapsae*; *Heterorhabditis* sp.; *S. carpocapsae* + *Heterorhabditis* sp. (both applied immediately after the transfer of larvae); *S. carpocapsae* (applied immediately after the transfer of larvae) + *Heterorhabditis* sp. (applied 12 hours after the transfer of larvae); and *S. carpocapsae* (applied 12 hours after the transfer of larvae) + *Heterorhabditis* sp. (applied immediately after the transfer of larvae). For the second bioassay the treatments were: application of the nematodes, and then the soil infested with larvae; application of the nematode and after 24 hours soil infested with the larvae; soil infested with the larvae and then 24 hours after, application of the nematode. Ten *C. capitata* larvae were transferred to plastic jars (12 cm × 6 cm) containing 100 g soil, followed by the application of 3 mL of an aqueous suspension containing 125 JI cm^{-2}. In control treatment was applied 3 mL of distilled water. The mortality's evaluation was performed after five days later and was confirmed by symptom observations and corpse dissections. It was observed that *Heterorhabditis* sp. and *S. carpocapsae* were effective in controlling larvae of *C. capitata* when applied on the soil surface, alone or combined, with mortality rates ranging in 26 and 74%. For the range of application, *S. carpocapsae* was more efficient when applied immediately after the transfer of larvae to the soil, and 24 hours before to infestation (80 and 90% mortality, respectively). However, *Heterorhabditis* sp. was more efficient only when applied 24 hours before to infestation of the soil (90% mortality).

Keywords: Biological control, fruit flies, mediterranean fruit fly.

Efeito de *Heterorhabditis* sp. e *Steinernema carpocapsae* aplicados de forma isolada ou combinada e em diferentes períodos de infestação do solo com larvas de *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae)

Resumo

A mosca-do-Mediterrâneo (Ceratitis capitata) (Wiedemann) é considerada uma das principais pragas da fruticultura mundial acarretando perdas significativas nesse setor. Esse inseto passa uma fase de sua vida no solo, sendo um alvo em potencial para nematóides entomopatogênicos. Assim, os objetivos deste trabalho foram avaliar a eficiência de *Heterorhabditis sp.* RSC01 e *Steinernema carpocapsae* All, aplicados de forma isolada ou combinada, e em diferentes períodos de infestação do solo com larvas de *C. capitata*. Para o primeiro bioensaio os tratamentos foram: *S. carpocapsae; Heterorhabditis* sp.; *S. carpocapsae* + *Heterorhabditis* sp. (ambos aplicados logo após a transferência das larvas); *S. carpocapsae* (aplicado logo após a transferência das larvas) + *Heterorhabditis* sp. (aplicado 12 horas após a transferência das larvas) e *S. carpocapsae* (aplicado 12 horas após a transferência das larvas) + *Heterorhabditis* sp. (aplicado logo após a transferência das larvas). Para o segundo bioensaio os tratamentos foram: aplicação do nematóide e, em seguida, infestação do solo com as larvas; aplicação do nematóide e, após 24 horas, infestação do solo com as larvas; infestação do solo com as larvas e, 24 horas após, aplicação do nematóide. Foram transferidas dez larvas de

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C. capitata para potes plásticos contendo 100 g de solo e aplicados 3 mL de suspensão com 125 JI cm^{-2}. No tratamento controle foram aplicados 3 mL de água. A avaliação da mortalidade foi realizada após cinco dias e a confirmação foi feita através da observação dos sintomas característicos do ataque de nematóides e da dissecação dos cadáveres. Verificou-se que *Heterorhabditis* sp. e *S. carpocapsae* foram eficientes no controle de larvas de *C. capitata* quando aplicados na superfície do solo, de forma isolada ou combinada, com mortalidade variando entre 26 e 74%. Em relação ao intervalo de aplicação, *S. carpocapsae* foi mais eficiente quando aplicado no momento da infestação do solo e 24 horas antes da infestação (80 e 90% de mortalidade, respectivamente). Por outro lado, *Heterorhabditis* sp. apresentou maior eficiência somente quando aplicado 24 horas antes da infestação do solo (90% de mortalidade**).**

Palavras-chave: Controle biológico, mosca-das-frutas, mosca-do-mediterrâneo.

Efecto de *Heterorhabditis* sp. y *Steinernema carpocapsae* aplicados en diferentes períodos de infestación del suelo con larvas de *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae)

Resumen

La mosca del Mediterráneo (*Ceratitis capitata*) (Wiedemann) es considerada una de las principales plagas de la fruticultura mundial, causando pérdidas significativas en este sector. Este insecto tiene una fase de su vida en el suelo, siendo un blanco potencial para los nematodos entomopatógenos. Así, los objetivos de este trabajo fueran evaluar la eficiencia de Heterorhabditis sp. RSC01 y Steinernema carpocapsae All, aplicados aislados o combinados, y en diferentes períodos de infestación del suelo con larvas de C. capitata. Para el primero bioensayo los tratamientos fueran: *S. carpocapsae; Heterorhabditis sp.; S. carpocapsae* + *Heterorhabditis sp.* (ambos aplicados inmediatamente después de la transferencia de las larvas); *S. carpocapsae* (aplicado logo después de la transferencia de las larvas) + *Heterorhabditis sp.* (aplicado 12 horas después de la transferencia de las larvas) y *S. carpocapsae* (aplicado 12 horas después de la transferencia de las larvas) + *Heterorhabditis sp.* (aplicado logo después de la transferencia de las larvas). Para el segundo bioensayo los tratamientos fueran: aplicación de nematodos y luego infestación del suelo con larvas; aplicación de los nematodos, y después de 24 horas, infestación del suelo con larvas; la infestación del suelo con larvas, y 24 horas después aplicación de los nematodos. Se transfirieron Diez larvas de *C. capitata* a macetas de plástico con 100 g de suelo y se aplicó 3 ml de suspensión con 125 JI cm^{-2}. En el tratamiento control se aplicó 3 ml de agua. La evaluación de la mortalidad se realizó cinco días después y la confirmación se realizó mediante la observación de los síntomas característicos del ataque de nematodos y disección de los cadáveres. Se encontró que *Heterorhabditis sp.* y *S. carpocapsae* fueron eficaces en el control de larvas de *C. capitata* cuando aportados en la superficie del suelo, ya sea aislado o combinado con tasas de mortalidad que oscilaran entre 26 y 74%. Para el intervalo de aplicación, *S. carpocapsae* fue más eficaz cuando aplicado en el momento de la infestación del suelo y 24 horas antes de la infestación (80 y 90% de mortalidad, respectivamente). Además, *Heterorhabditis sp.* Presentó más alta eficiencia sólo cuando se aplicó 24 horas antes de la infestación del suelo (90% de mortalidad).

Palabras clave: Control biológico, moscas de la fruta, mosca del mediterráneo.

Introduction

The fruit fly is considered a major pest of fruit production worldwide causing significant losses for this sector, due to direct and indirect damages it causes. The species *Ceratitis capitata (Wiedemann)* (Mediterranean-Fly) is considered one of the most important, because it is cosmopolitan, invasive and with occurrence in all biogeographical regions of the world, and consequently the species that cause most damage to fruit production throughout the world (ZUCCHI, 2001).

Entomopathogenic nematodes belonging to the families *Heterorhabditidae* and *Steinernematidae* are considered excellent biological control agents, demonstrating greater potential for soil insect pests and of cryptic environment, which is the case of the fruit fly, which has the behavior of leaving the fruit and penetrate the soil for development of pupae, allowing the action of the pathogen.

In this sense studies were developed on laboratory and field conditions to determine the efficiency of different species and strains of entomopathogenic nematodes on different development stages of *C. capitata* being observed high susceptibility of this insect (LINDEGREN and VAIL, 1986; LINDEGREN et al. 1989; LINDEGREN, 1990, GAZIT et al. 2000; LABORDA et al. 2003).

As an alternative to increasing the efficiency of control against insect pests, many studies have proposed the combination of entomopathogenic agents. According to KAYA et al. (1993), the combination of two or more isolated or species of nematodes to a single insect pest may have a synergistic effect or additive. This combination

may be more effective when using nematodes with different behavior, to control an insect pest that has more than one stage of development in the same environment, but occupying different soil depths.

Another way to increase the efficiency of control is the synchronization between product application and the stage most susceptible of the insect pests. Regarding the fruit fly, several studies show that the larval stage is most susceptible in relation to pupal stage (BEAVERS and CALKINS, 1984; YEE and LACEY, 2003; LABORDA et al. 2003).

Thus the objectives of this study were to evaluate the virulence of *Steinernema* and *Heterorhabditis* applied in isolation and combined on larvae of C. *capitata* and applied in different periods of soil infestation with larvae of C. *capitata.*

Material and methods

Creation of *Ceratitis capitata*

For the beginning of the creation were used pupae derived from the Laboratory of Entomology of the National Research Center for Cassava and Tropical Fruits of the Brazilian Company of Research and Animal Agriculture - Embrapa. The creation was maintained under controlled conditions of temperature of 25 ± 2 ° C, relative humidity of 70 ± 10% and photoperiod of 12 hours, according to the methodology proposed by Silva (1990).

Obtainment of the entomopathogenic nematodes

For the realization bioassays were used isolated Heterorhabditis sp. RSC01 (originating in Amapá, Brazil) and Steinernema carpocapsae All (originating in North Carolina, USA), stored in the Bank of Pathogens of the Laboratory of Insect Pathology in the Federal University of Lavras - UFLA. The multiplication of nematodes was performed through the in vivo method, adapted from WOODRING and KAYA (1988), using larvae of last instar Galleria mellonella (Lepidoptera: Pyralidae) Linnaeus.

Bioassay 1 - Effect of *Heterorhabditis sp.* and *Steinernema carpocapsae* applied isolated or combined on larvae of *C. capitata*

Was evaluated the efficiency of Heterorhabditis sp. and S. carpocapsae, inoculated isolated or combined in different periods of application in relation to the release of the larvae of *C. capitata,* being the treatments: 1) distilled water (applied shortly after the transfer of the larvae) 2) *S. carpocapsae* (applied shortly after the transfer of the larvae) 3) *Heterorhabditis sp.* (applied shortly after the transfer of larvae), 4) *S. carpocapsae* + *Heterorhabditis sp.* (both applied shortly after the transfer of the larvae); 5) *S. carpocapsae* (applied shortly after the transfer of the larvae) + *Heterorhabditis sp.* (applied 12 hours after the transfer of larvae), and 6) *S. carpocapsae* (applied 12 hours after the transfer of larvae) + *Heterorhabditis sp.* (applied shortly after the transfer of the larvae).

The bioassay was performed according to the completely randomized design with three replications of 10 larvae each, standardized in the late third instar. The larvae were transferred to plastic pots (12 cm × 6 cm) containing 100 g of soil (Oxisol with humidity standardized at 25%) and, later, received 3 mL of suspension of the treatment with total concentration of 125 infective juveniles (JI) / cm^2 (in treatments where the nematodes were applied in a combined way, where were applied to 62.5 JI/cm^2 of each isolate), being that in the control treatment was applied 3 mL of distilled water.

The plastic pots were capped and kept in an incubator (25 ± 2 ° C, RH 70 ± 10%, 12h photophase). The evaluation was performed after five days and confirmation of death was performed by observing the symptoms and the dissection of corpses.

Data were subjected to analysis of variance (F test) and the averages compared by the Scott-Knott test (P ≤ 0.05), using the statistical program Sisvar (FERREIRA, 2000).

Bioassay 2 - Effect of application of the nematode in different periods of soil infestation with larvae of *C. capitata*

Was evaluated the effect of the interval between the application of the nematode (*Heterorhabditis sp.* and *S. Carpocapsae*) and the soil infestation with larvae of *C. capitata*, being the treatments: 1) application of distilled water and then soil infestation with larvae, 2) application of the nematode and then infestation with larvae in the soil, 3) application of nematodes, and after 24 hours, soil infestation with larvae and 4) soil infestation with the larvae, and 24 hours later application of the nematode.

This bioassay was performed following the same procedure and experimental design of the bioassay 1.

Results and discussion

In the bioassay 1 was found that *Heterorhabditis sp*. and *S. carpocapsae* were effective in controlling larvae of *C. capitata* when applied to the soil surface, isolated or combined with mortality rates ranging between 26.7 and 74.5% (Table 1).

Table 1. Mortality confirmed (± SE) of larvae of *Ceratitis capitata* by *Steinernema carpocapsae* and *Heterorhabditis sp.* isolated or combined in different periods of application of nematodes in relation to the release of the insect.

Treatment	Mortality (%)*
Control (1h)**	0.0 ± 0.00 d
S. carpocapsae (1h)	74.5 ± 9.85 a
Heterorhabditis sp. (1h)	41.3 ± 5.32 b
S. carpocapsae (1h) + *Heterorhabditis* sp. (1h)	66.7 ± 8.82 a
S. carpocapsae (1h) + *Heterorhabditis* sp. (12h)	66.7 ± 3.33 a
S. carpocapsae (12h) + *Heterorhabditis* sp. (1h)	26.7 ± 8.82 c
CV = 26.41%	

** Means followed by the same letter do not differ by Sott-Knott test (P ≤ 0.05).*

*** distilled or of the nematode after releasing the insect.*

When applied in an isolated way, *S. carpocapsae* was more efficient than *Heterorhabditis sp*., causing mortality of 74.5 and 41.3%, respectively. When applied in a combined way, the treatment in which both the isolates were applied in the first hour and the treatment in which the *S. carpocapsae* was applied in the first hour and *Heterorhabditis sp*., after 12 hours, presented the same efficiency, with 66.7% mortality. Now for the treatment in which *Heterorhabditis sp.* was applied for the first hour and *S. carpocapsae*, applied after 12 hours occurred a lower mortality (26.7%).

Although not been done to identify the species of nematode which caused the mortality and which colonized and reproduced better in the host, these results indicate that the major effect of the combined application of nematodes is mainly due to the action of *S. carpocapsae*, since only the treatments in that this nematode were applied during the first hour caused higher mortality of larvae of fruit-fly, with results similar to the isolated application pg the same. Probably, when *S. carpocapsae* was inoculated after 12 hours, the larvae of *C. capitata* had already passed to the pupal stage, hindering the process of infection by this nematode and, consequently, reducing the mortality rate in this treatment.

Besides morphological and physiological factors natural of the nematode, this increased virulence of *S. carpocapsae* may be related to the same behavior to remain on the soil surface (YEE and LACEY, 2003), ie in the same environment of the larvae of *C. capitata*.

In a similar study, ALATORRE-ROSES and KAYA (1991) observed the effect of the combined application of *S. carpocapsae* and *H. bacteriophora*, noting that *S. carpocapsae* infected and reproduced more successfully than *H. bacteriophora*. However, when *S. carpocapsae* was applied in a combined way with *S. glaseri* it had the production of infective juveniles reduced (KONDO, 1989).

According ALATORRE-ROSES and KAYA (1991), in a combined application of two species of nematodes occurs competition within the host, preventing or hindering the development of one of them. This competition occurs mainly when only one species of symbiotic bacteria colonizes the host, allowing only the reproduction of the nematode species associated thereto.

In this sense, BOEMARE (2002) emphasizes that bacteria belonging to the genus *Xenorhabdus* are known to produce bacteriocins lethal to the genus *Photorhabdus*. This fact may explain the prevalence of *S. carpocapsae* on *Heterorhabditis sp*.

Due to the possibility of only one nematode species predominate in the host, ALATORRE-ROSES and KAYA (1990) consider the combined use of these agents of control to a single insect pest as not advantageous. Moreover, for KAYA et al. (1993), this combination can be effective with the use of nematode species which exhibit different behavior search of the host and for the control of different stages of insect pests that occur in various soil depths, allowing the specific action of each species of nematode in distinct habitats, a situation which occurs with fruit flies, where the larvae of last instar are found in the soil surface and the pupae at greater depths.

In Bioassay 2 observed high susceptibility of *C. capitata* to *S. carpocapsae* and *Heterorhabditis sp*. at different intervals of soil infestation with larvae and application of the nematode, with a mortality rate ranging between 60 and 90% (Table 2).

The greater efficiency of *S. carpocapsae* was detected in treatments in which was inoculated at the moment when the soil was infested with the larvae and when applied 24 hours before the infestation thereof.

This high virulence may be related to the behavior of *S. carpocapsae* of moving horizontally, keeping itself on the soil surface (YEE and LACEY, 2003), which facilitated the infection of the larvae when the soil was infested at the moment or after 24 hours of the application of the nematode. Furthermore, this exposure period, the nematode could infect *C. capitata*, still in the larval stage, which is more susceptible to the entomopathogen compared to the pupal stage (BEAVERS and CALKINS, 1984; LABORDA et al. 2003; YEE and LACEY, 2003).

Table 2. Mortality confirmed (± SE) of larvae of Ceratitis capitata by Steinernema carpocapsae and Heterorhabditis sp. in different periods of application of the nematode in relation to the release of the insect.

Treatment	Mortality (%)*
Control (1h)** + Larva (1h)**	0.0 ± 0.00 c
S. carpocapsae (1h) + Larva (1h)	80.0 ± 5.77 a
S. carpocapsae (1h) + Larva (24h)	90.0 ± 5.77 a
S. carpocapsae (24h) + Larva (1h)	63.3 ± 3.33 b
Heterorhabditis sp. (1h) + Larva (1h)	60.0 ± 5.77 b
Heterorhabditis sp. (1h) + Larva (24h)	90.0 ± 5.77 a
Heterorhabditis sp. (24h) + Larva (1h)	73.3 ± 3.33 b
CV = 14.96%	

** Means followed by the same letter do not differ by Sott-Knott test (P ≤ 0.05).*

*** distilled or of the nematode after releasing the insect.*

Similar data of this work were obtained by YEE and LACEY, (2003) by studying the susceptibility of Rhagoletis indifferens (Diptera: Tephritidae) to entomopathogenic nematodes, checking high virulence of *S. carpocapsae* when inoculated with the larvae or when inoculated 48 hours before the soil infestation with larvae, with mortality at 90 and 92%, respectively.

On the other hand the treatment in which *S. carpocapsae* was inoculated 24 hours after soil infestation with the larvae was less efficient, probably because the larvae had already passed to the pupal stage, which is less susceptible to the entomopathogen, besides occupying the deeper layers of the soil, making it difficult to the nematode encounter with the insect.

Now the isolated *Heterorhabditis sp*. showed greater virulence only in the treatment that was inoculated 24 hours before the soil infestation with larvae. This lower virulence of *Heterorhabditis sp*. when compared to *S. carpocapsae* on larvae of *C. capitata*, should be related to morphological, physiological, genetic and behavioral aspects of the isolated itself, which still need to be studied.

Conclusions

The results of this study demonstrate the effectiveness of entomopathogenic nematodes, especially of *S. carpocapsae*, for control of fruit fly C. capitata. The combined application of this nematode with *Heterorhabditis sp*., Did not increase its efficiency. The period of application of the nematode in relation to the period in which soil infestation with the larvae of the fruit fly interferes with the efficiency of control. Thus, for a more efficient control of *C. capitata*, the application of the nematode must be made before or at the time when the majority of the larvae migrate from the fruit into the ground, for the development of pupae.

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