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Selection of entomopathogenic nematodes for the control of the fruit fly *Ceratitis capitata* (Diptera: Tephritidae)

ABSTRACT

Entomopathogenic nematodes are considered excellent biological control agents, with greater potential against soil insect pests and pests of cryptic environments. The fruit fly *Ceratitis capitata* is considered one of the main fruit crop pests worldwide. This insect stays in the soil during a phase of life, where it becomes a target for entomopathogenic nematodes. The objective of this study was to evaluate the pathogenicity and virulence of entomopathogenic nematodes on *C. capitata*. The bioassays were organized with four replications, containing 10 individuals; 1 mL of a nematode suspension containing 200 IJ/insect was applied. The most virulent isolates against *C. capitata* larvae were selected and applied at concentrations of 0, 50, 100, 150, 200, 250, 300, 350 and 400 IJ/insect. All isolates were pathogenic for *C. capitata*. The *S. carpocapsae* ALL and *Heterorhabditis* sp. RSC01 isolates were the most virulent against the larval stage, with mortalities of over 85%. As to the pupal stage, isolates *Heterorhabditis* sp. PI, *Heterorhabditis* sp. JPM4, *H. bacteriophora* HP88, *S. feltiae* and *S. glaseri* were the best, with mortalities ranging between 35 and 44%.

Key words: biological control, mediterranean fruit fly, pathogenicity

Seleção de nematoides entomopatogênicos visando ao controle da mosca das frutas *Ceratitis capitata* (Diptera: Tephritidae)

RESUMO

Nematoides entomopatogênicos são considerados excelentes agentes de controle biológico demonstrando maior potencialidade em insetos-praga de solo e de ambientes crípticos. A mosca das frutas *Ceratitis capitata* é considerada uma das principais pragas para a fruticultura mundial. Este inseto passa uma fase de sua vida no solo, tornando-se alvo em potencial para esses entomopatógenos. Assim, teve-se como objetivo avaliar a patogenicidade e a virulência de nematoides entomopatogênicos sobre *C. capitata*. Os bioensaios foram realizados com quatro repetições contendo 10 indivíduos e aplicado 1 mL de suspensão de nematoides com 200 IJ/inseto. Os isolados que se apresentaram mais virulentos para larvas de *C. capitata* foram selecionados e aplicados nas concentrações de 0, 50, 100, 150, 200, 250, 300, 350 e 400 IJ/inseto. Verificou-se que todos os isolados foram patogênicos para *C. capitata*. Os isolados *S. carpocapsae* ALL e *Heterorhabditis* sp. RSC01 foram os mais virulentos para a fase larval causando mortalidade superior a 85%. Em relação à fase de pupa os isolados *Heterorhabditis* sp. PI, *Heterorhabditis* sp. JPM4, *H. bacteriophora* HP88, *S. feltiae* e *S. glaseri* se destacaram com mortalidade variando entre 35 e 44%.

Palavras-chave: controle biológico, mosca-do-mediterrâneo, patogenicidade

INTRODUCTION

Brazil is among the three largest world fruit producers, with a yield of over 45 million t year⁻¹. This sector is responsible for generating 15% of Brazil's agricultural production value and directly employs six million workers, corresponding to 27% of all agricultural labor generated in the country (IBRAF, 2011). However, these business sectors could be optimized if fresh fruit exports would increase, since these exports are still very low in relation to the sector's production capacity. One of the most important reasons for low exports is the presence of pests, especially the Mediterranean fruit fly *Ceratitis capitata* (Wiedemann, 1824) (Diptera: Tephritidae).

Among all species in the family Tephritidae, the Mediterranean fruit fly is the most cosmopolitan and invasive of them, since it occurs in all biogeographic regions, and is consequently the species that causes the most damage to fruit crop worldwide (Zucchi, 2001).

Damages occur because females oviposit in ripening fruit, which opens a door for microorganisms, resulting in fruit contamination and decay (Carvalho, 2005). The larvae also cause damage, since they feed on fruit pulp, resulting in local decay and early fruit drop (Saldanha & Silva, 1999).

In addition, the Mediterranean fruit fly is considered a quarantine pest, with losses to the fruit industry due to phytosanitary barriers raised by importing countries and a requirement that the area of origin of fruits be declared fruit fly-free (Bueno, 2000; Malavasi, 2000).

Until the end of the 1980's, fruit fly control was based exclusively on chemical control. However, due to indiscriminate use of chemical products, problems arisen such as the resurgence of pests, resistant populations, environmental contamination, and residues in the final sales product, which required the adoption of other control practices to fight the pest.

Entomopathogenic nematodes in the genera *Steinernema* and *Heterorhabditis* are promising for the control of *C. capitata*, as the pest's behavior of leaving the fruit and penetrating the soil for pupal development allows nematode action.

In this respect, studies were conducted under laboratory and field conditions to determine the effectiveness of entomopathogenic nematodes on different *C. capitata* developmental stages; observations showed high susceptibility of this insect (Lindegren, 1990; Gazit et al., 2000; Laborda et al., 2003; Malan & Manrakhan, 2009; Rohde et al., 2010).

The results of these studies demonstrated great variability in the susceptibility of *C. capitata* larvae to the various species and isolates tested, emphasizing the need for selection studies. Consequently, the objective of this study was to evaluate the pathogenicity and virulence of entomopathogenic nematodes on *C. capitata* larvae and pupae under laboratory conditions.

MATERIAL AND METHODS

Ceratitis capitata rearing

The colony was started with pupae obtained from the Entomology Laboratory at Centro Nacional de Pesquisa de Mandioca e Fruticultura Tropical, Empresa Brasileira de

Pesquisa Agropecuária - Embrapa. The rearing was maintained under controlled temperature (25 ± 2 °C), relative humidity ($70 \pm 10\%$), and photophase (12 h) conditions, according to the methodology proposed by Silva (1990).

Obtaining entomopathogenic nematodes

In the bioassays native and exotic isolates of entomopathogenic nematodes were used; the same were stored at the Pathogen Bank, Insect Pathology Laboratory of Universidade Federal de Lavras - UFLA. The nematodes were multiplied by the *in vivo* method, adapted from Woodring & Kaya (1988), using last-instar larvae of *Galleria mellonella* Linnaeus, 1758 (Lepidoptera: Pyralidae) from a stock rearing maintained in the same laboratory.

Pathogenicity of *Steinernema* and *Heterorhabditis* isolates on *C. capitata* larvae and pupae

The pathogenicity of different native and exotic isolates in the genera *Heterorhabditis* and *Steinernema* was evaluated on *C. capitata* larvae and pupae (Table 1). Isolate *H. bacteriophora* HP88 was not evaluated in the pathogenicity study on larvae, while isolate *H. bacteriophora* was not evaluated on pupae because these nematodes could not be multiplied during the period when the bioassays were conducted.

Table 1. Native and exotic isolates of entomopathogenic nematodes (Rhabditida: Heterorhabditidae and Steinernematidae) used in the bioassays

Tabela 1. Isolados nativos e exóticos de nematoides entomopatogênicos (Rhabditida: Heterorhabditidae e Steinernematidae) utilizados nos bioensaios

Isolates	Place of origin
<i>Heterorhabditis bacteriophora</i>	Unknown
<i>Heterorhabditis bacteriophora</i> HP88	New Jersey/USA
<i>Heterorhabditis</i> sp. JPM3	Lavras/MG/Brazil
<i>Heterorhabditis</i> sp. JPM3.1	Lavras/MG/Brazil
<i>Heterorhabditis</i> sp. JPM4	Lavras/MG/Brazil
<i>Heterorhabditis</i> sp. PI	Teresina/Piauí/Brazil
<i>Heterorhabditis</i> sp. RSC01	Benjamin Constant/AM/Brazil
<i>Heterorhabditis</i> sp. RSC02	Benjamin Constant/AM/Brazil
<i>Heterorhabditis</i> sp. RSC03	Benjamin Constant/AM/Brazil
<i>Heterorhabditis amazonensis</i> RSC05	Benjamin Constant/AM/Brazil
<i>Steinernema arenarium</i> (= <i>anomali</i>)	Voronezh/Russia
<i>Steinernema carpocapsae</i> All	North Carolina/USA
<i>Steinernema feltiae</i> Sn	Florida/USA
<i>Steinernema glaseri</i> NA	Florida/USA
<i>Steinernema riobrave</i> 355	Texas/USA

The bioassays were organized in a completely randomized experimental design with four replications, each consisting of two Petri dishes containing 10 individuals each (larvae or pupae). The larvae were standardized at the end of the 3rd instar, while the pupae had a standard age between five and eight days after pupate.

The individuals were transferred to Petri dishes (5 cm diameter) containing three sheets of filter paper that received 1 mL of a nematode suspension with 200 IJ/insect, applied with a pipette. The control treatment received 1 mL of distilled water.

The bioassays were maintained in an incubator (25 ± 2 °C, RH $70 \pm 10\%$, 12 h photophase). The evaluation was made five days later, and mortality was confirmed by means of symptom observations and corpse dissections.

The data were submitted to analysis of variance (F test) and the means were compared by the Scott-Knott test ($P \leq 0.05$), using the Sisvar statistical software (Ferreira, 2000).

Effect of isolate concentrations of *Steinernema* and *Heterorhabditis* on *C. capitata* larvae

The isolates that proved the most virulent to *C. capitata* larvae were selected for the concentration study. Serial dilutions were prepared at standard concentrations of 0, 50, 100, 150, 200, 250, 300, 350, and 400 IJ/insect. The bioassay was conducted under the same experimental procedure and design adopted in the pathogenicity bioassays.

The data were submitted to analysis of variance (F test) and the means were compared by polynomial regression ($P \leq 0.05$), using the Sisvar statistical software (Ferreira, 2000).

RESULTS AND DISCUSSION

Study on the pathogenicity of *Steinernema* and *Heterorhabditis* isolates on *C. capitata* larvae and pupae

Larvae: *C. capitata* larvae were susceptible to all entomopathogenic nematode isolates and species studied, with mortalities ranging between 32.5 and 87.5% (Table 2). Among the 14 isolates evaluated, only two caused mortalities lower than 41%, 10 had mortality rates varying between 46 and 63%, and two caused mortalities higher than 86%, namely *S. carpocapsae* ALL and *Heterorhabditis* sp. RSC01.

Table 2. Confirmed mortality (\pm SE) of *Ceratitis capitata* larvae and pupae by entomopathogenic nematodes (Rhabditida: Heterorhabditidae and Steinernematidae)

Tabela 2. Mortalidade confirmada (\pm EP) de larvas e pupas de *Ceratitis capitata* por nematoides entomopatogênicos (Rhabditida: Heterorhabditidae e Steinernematidae)

Species/Isolates	Confirmed mortality (%) ¹	
	Larvae	Pupae
Control	0.0 \pm 0.00 d	0.0 \pm 0.00 c
<i>Heterorhabditis bacteriophora</i> HP88	---	35.0 \pm 6.12 a
<i>Heterorhabditis bacteriophora</i>	63.8 \pm 6.25 b	---
<i>Heterorhabditis</i> sp. PI	55.0 \pm 6.45 b	43.8 \pm 11.25 a
<i>Heterorhabditis</i> sp. JPM3	48.8 \pm 3.15 b	7.5 \pm 5.95 c
<i>Heterorhabditis</i> sp. JPM3.1	56.3 \pm 5.15 b	13.8 \pm 7.18 b
<i>Heterorhabditis</i> sp. JPM4	40.0 \pm 2.89 c	41.3 \pm 7.74 a
<i>Heterorhabditis</i> sp. RSC01	87.5 \pm 4.33 a	21.3 \pm 5.15 b
<i>Heterorhabditis</i> sp. RSC02	58.8 \pm 7.74 b	3.8 \pm 2.39 c
<i>Heterorhabditis</i> sp. RSC03	46.3 \pm 8.00 b	7.5 \pm 4.33 c
<i>Heterorhabditis amazonensis</i> RSC05	53.8 \pm 6.25 b	13.8 \pm 5.54 b
<i>Steinernema anomali</i>	63.8 \pm 4.27 b	21.3 \pm 10.08 b
<i>Steinernema carpocapsae</i>	86.3 \pm 3.75 a	20.0 \pm 6.45 b
<i>Steinernema feltiae</i>	51.3 \pm 7.18 b	41.2 \pm 9.66 a
<i>Steinernema glaseri</i>	61.3 \pm 5.91 b	43.8 \pm 10.48 a
<i>Steinernema riobrave</i>	32.5 \pm 4.33 c	17.5 \pm 9.24 b
	CV = 20.29%	CV = 48.82%

¹ Means followed by the same letter are not different by the Scott-Knott test ($P \leq 0.05$ significance)

The high efficiency of *S. carpocapsae* ALL on *C. capitata* larvae could be related to the relatively small size of their infective juveniles, ranging between 438 - 650 μ m (Adams & Nguyen, 2002), thus facilitating the penetration mode of the steinernematids, which takes place through the natural openings of the host (spiracles, mouth, and anus).

With reference to isolate *Heterorhabditis* sp. RSC01, identification studies are still needed and no comparisons can be

made with regard to the morphological and behavioral aspects of this nematode. However, the specificity of an entomopathogen to a host cannot be explained based on a single trait, since a variety of factors act upon that relationship. Consequently, specificity is directly associated with the entomopathogen's efficiency in locating, infecting, developing, and reproducing without being recognized by the host's immune system.

Coevolutionary processes may determine the specificity of an entomopathogen to its host, providing the development of host recognition mechanisms by means of chemical perception or the development of specific physical structures that aid the infection process (Tol et al., 2001).

Most *C. capitata* larvae exposed to the nematode died during the pupal stage. This fact was also observed by Rohde et al. (2010) while studying the susceptibility of *C. capitata* larvae to the entomopathogen, and Yee & Lacey (2003), who investigated the pathogenicity of nematodes on *Rhagoletis indifferens* Curran, 1932 larvae.

The genera studied were not different from each other, since one isolate in the genus *Heterorhabditis* (RSC01) and another isolate in the genus *Steinernema* (*S. carpocapsae* ALL) were the most virulent to *C. capitata* larvae. In addition, the lowest mortalities were also caused by an isolate in the genus *Heterorhabditis* (JPM4) and one isolate in the genus *Steinernema* (*S. riobrave*). Also, no relationship was observed between native or exotic isolates and entomopathogen efficiency, since *Heterorhabditis* sp. RSC01 and *Heterorhabditis* sp. JPM4 are native, while *S. carpocapsae* ALL and *S. riobrave* are exotic.

The results demonstrated great variability in the susceptibility of *C. capitata* larvae to the various species and isolates tested, emphasizing the need for selection studies.

Gazit et al. (2000) also observed great variability in the mortality of *C. capitata* larvae while studying the pathogenicity of different isolates from the genera *Steinernema* and *Heterorhabditis*. Among the nematodes evaluated, *S. riobrave* had one of the highest mortality rates (above 80%). The paper by these authors is in disagreement with the results found in our study, since this species was one of the least efficient. Such divergence is probably related to the high genetic variability that exists among isolates of a given nematode species. Several studies have demonstrated variability in the morphology (Stock et al., 2000), genetics (Gaugler et al., 1989), physiology (Fitters et al., 1999), infectivity (Griffin & Downes, 1991), and climatic adaptation (Solomon et al., 1999) of different isolates of the same species.

Studies carried out by Lindegren (1990) produced similar results as our research, with *S. carpocapsae* ALL causing mortalities higher than 90% under laboratory conditions and above 85% under field conditions for *C. capitata* larvae.

The susceptibility of *C. capitata* larvae to a product based on the entomopathogenic nematode Biorend C (a mixture of *Steinernema* spp. and chitosan, Idebio/ABF, Spain) was also demonstrated. The product caused a mortality index higher than 90% (Laborda et al., 2003).

Studies under field and laboratory conditions have also demonstrated the pathogenicity of different isolates and species of *Steinernema* and *Heterorhabditis* on larvae of *Anastrepha ludens* Loew, 1873 (Toledo et al., 2005; Toledo et al., 2006),

Bactrocera zonata Saunders, 1842 (Attalla et al., 2002), *Dacus curcubitae* and *D. dorsalis* (Lindgren, 1990), *Rhagoletis indifferens* (Yee & Lacey, 2003), and *R. cerasi* Linnaeus, 1758 (Koppler et al., 2003).

Pupae: There was great variability in pupal mortality caused by the various isolates and species of entomopathogenic nematodes (Table 2). Among the 14 isolates evaluated, only three caused mortalities lower than 7.5%, six caused mortalities between 13 and 21%, and five caused mortalities higher than 35%.

Just like for larvae, there were no important differences between the genera studied against *C. capitata* pupae, since the isolates that caused the highest mortality rates belonged to both *Steinernema* (*S. feltiae* and *S. glaseri*) and *Heterorhabditis* (*H. bacteriophora* HP88, PI, and JPM4). However, the lowest mortalities were caused by isolates exclusively in the genus *Heterorhabditis* (JPM3, RSC02, and RSC03).

Among all isolates, one native (*Heterorhabditis* sp. JPM4) and four exotic (*H. bacteriophora* HP88, *Heterorhabditis* sp. PI, *S. feltiae*, and *S. glaseri*) had particularly good performances, while the least efficient isolates were all natives (*Heterorhabditis* sp. JPM3, *Heterorhabditis* sp. RSC01, and *Heterorhabditis* sp. RSC03).

Attalla et al. (2002) also observed great variability in the pupal mortality of *B. zonata* when exposed to different isolates of *S. carpocapsae* ALL, *S. riobrave*, and *H. bacteriophora*, with mortalities ranging between 16.67 and 93.70%. The pupal stage was as susceptible as the larval stage to the isolates tested, with a maximum mortality of 91.49%; this fact was not observed in our study, since the maximum mortality obtained for the pupal stage was 43.8%, with a maximum mortality of 87.5% for the larval stage.

Studies conducted with *A. suspensa*, *R. indifferens*, and *C. capitata* also demonstrated low susceptibility of the pupal stage to different isolates and species of the genera *Steinernema* and *Heterorhabditis* (Yee & Lacey, 2003; Laborda et al., 2003).

According to Yee & Lacey (2003), the factors that cause differences in the susceptibility of different growth stages of insect and in the efficiency of different nematode isolates and species need to be determined. In this respect, several hypotheses have attempted to explain such differences.

Thus, the higher susceptibility of larvae to entomopathogenic nematodes could be related to greater locomotion at that stage, with higher release of CO₂, a chemical compound that plays a role in attracting the entomopathogenic nematode. In addition, the large natural openings of the larva's body, in the case of *Steinernema* and *Heterorhabditis*, and the poorly sclerotized larval integument (when compared to the pupal integument) in the case of *Heterorhabditis* facilitate insect infection by the nematode.

The low pupal susceptibility, however, could be related to spiracle opening size, which could be very small for nematode penetration, or could result from the fact that the pupa is protected by the puparium, making penetration difficult (Toledo et al., 2005).

The high virulence of *H. bacteriophora*, *S. glaseri*, and *S. feltiae* against *C. capitata* pupae could be related to the strategy embraced by these entomopathogens to find the host, since the

first two are cruiser strategists, while the latter is an intermediate strategist between cruiser and ambusher. With reference to the isolates *Heterorhabditis* sp. PI and *Heterorhabditis* sp. JPM4, identification studies are still needed and no comparisons can be made with regard to their morphological and behavioral aspects.

In this respect, Lewis et al. (2006) emphasized that cruiser strategist nematodes (that actively search for the host) have a greater probability of finding hosts that have cryptic or sedentary habits, while ambusher's strategists (i.e., those that sit and wait for the host in order to attack) are more effective in finding high mobility hosts.

S. carpocapsae ALL, which was highly virulent to *C. capitata* larvae, was probably little effective against the pupal stage because it is an ambusher strategist, making encounters more difficult between pupae (sedentary) and infective juveniles.

Due to the low susceptibility of the pupal stage to the entomopathogenic nematodes, the concentration study was conducted for the larval stage only.

Study on the concentration of entomopathogenic nematode isolates of the genera *Steinernema* and *Heterorhabditis* on *C. capitata* larvae

Both *S. carpocapsae* ALL and *Heterorhabditis* sp. RSC01 caused mortality on *C. capitata* larvae at all concentrations applied, except in the control treatment. The *C. capitata* larval mortality caused by *S. carpocapsae* ALL varied between 57.5 and 96.3%; mortality by *Heterorhabditis* sp. RSC01 varied between 37.8 and 67.5% (Figure 1).

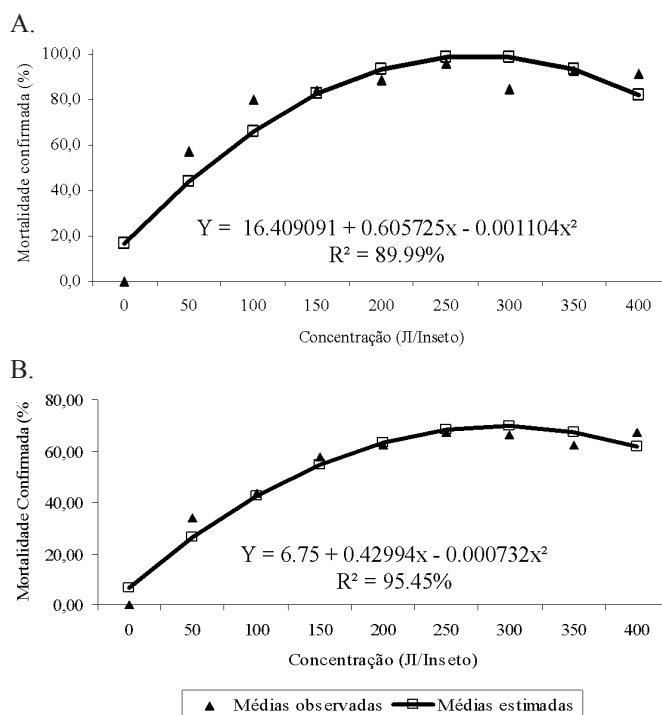


Figure 1. Regression analysis for confirmed mortality of *Ceratitis capitata* larvae submitted to different concentrations of (A) *Steinernema carpocapsae* ALL and (B) *Heterorhabditis* sp. RSC01

Figura 1. Análise de regressão para mortalidade confirmada de larvas de *Ceratitis capitata* submetidas a diferentes concentrações de (A) *Steinernema carpocapsae* ALL e (B) *Heterorhabditis* sp. RSC01

For both nematodes, mortality was directly proportional to the increase in concentration up to a maximum, after which mortality decreased as concentration increased.

From the mortality curve for *C. capitata* larvae caused by *S. carpocapsae* ALL, a maximum mortality of 99.49% was obtained at a concentration of 274 IJ/insect or 137 IJ/cm², while for *Heterorhabditis* sp. RSC01 the maximum was 69.88% at a concentration of 293 IJ/insect or 147 IJ/cm².

According to Gaugler et al., (1994), when a host is infected by entomopathogenic nematodes a minimum number of IJs is required to overcome the host's immune system, colonize the host, and cause mortality. However, intraspecific competition will occur when that number is exceeded, compromising nematode survival, development, and reproduction, reducing their virulence (Selvan et al., 1993).

CONCLUSION

All species and strains of entomopathogenic nematodes tested were pathogenic to larval and pupal stages of *C. capitata*. The larval stage of *C. capitata* was the most susceptible to entomopathogenic nematodes. The results found in this study indicate the great potential of the *S. carpocapsae* ALL and *Heterorhabditis* sp. RSC01 isolates against larvae and pupae of the Mediterranean fruit fly. Further studies are still required to define application strategies for these entomopathogens in order to control this insect pest.

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