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Susceptibility of the Asian citrus psyllid *Diaphorina citri* to entomopathogenic nematodes

Susceptibilidade do psilídeo asiático dos citros *Diaphorina citri* à nematoides entomopatogênicos

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Highlights

The activity of entomopathogenic nematodes were evaluated against a citrus psyllid.
Entomopathogenic nematodes caused high mortality of the citrus psyllid *Diaphorina citri.*
Heterorhabditis amazonensis isolates were the most effective to control the citrus psyllid.

Abstract

The Asian citrus psyllid, *Diaphorina citri* Kuwayama, 1908, (Hemiptera: Psylloidea: Liviidae), is one of the most important pests for citrus production, as it is the main vector of '*Candidatus* Liberibacter spp.' bacteria, the causal agents of Huanglongbing (HLB). Applications of chemical insecticides are currently the primary method for psyllid control, but have shown low efficiency and problems such as the development of resistant insect populations, and increased risks for the human health and the environment. Thus, the aim of this study was to assess the use of entomopathogenic nematodes (EPNs) as an alternative and sustainable strategy for the control of *D. citri.* Initially, the activity of 10 EPN isolates of the genera *Heterorhabditis* and *Steinernema* was tested against *D. citri.* Assessments were carried out on *D. citri* adults under laboratory conditions. Next, the efficiency of the concentrations of 50, 100, 150, and 200 Infective Juveniles (IJs)/cm² of the UENP 06 and NEPET 11 EPN isolates in causing the death of *D. citri* adults was tested. The NEPET 11 and UENP 06 isolates, both *Heterorhabditis amazonensis,* were the most effective in controlling psyllids, achieving confirmed mortality rates of 68.25 and 53.00%, respectively. In all IJ concentrations tested, these isolates caused confirmed mortality of *D. citri* adults, with no significant differences between concentrations and EPN isolates.

Key words: Biological control. HLB. Greening. Asian citrus psyllid. *Heterorhabditis. Steinernema.*

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Resumo

O psilídeo asiático dos citros, *Diaphorina citri* Kuwayama, 1908, (Hemiptera: Psylloidea: Liviidae), é uma das pragas mais importantes da citricultura, por ser o principal vetor das bactérias '*Candidatus* Liberibacter spp.', agentes causais do Huanglongbing (HLB). Atualmente, aplicações de inseticidas químicos tem sido a base para o controle do psilídeo. Entretanto, esse método de controle tem apresentado baixa eficiência, além de gerar diversos problemas, como a resistência de populações do inseto à produtos químicos e aumento dos riscos para a saúde humana e para o meio ambiente. Assim, o objetivo deste estudo foi avaliar a utilização de nematóides entomopatogênicos (NEPs) como uma estratégia alternativa e sustentável para o controle do psilídeo *D. citri.* Inicialmente, foram testadas a atividade de 10 isolados de NEPs dos gêneros Heterorhabditis e Steinernema contra a *D. citri.* As avaliações foram realizadas sobre adultos de *D. citri* em condições de laboratório. Posteriormente, foi testada a eficiência das concentrações de 50, 100, 150 e 200 de Juvenis Infectantes (JIs)/cm² dos isolados UENP 06 e NEPET 11 em causar mortalidade de adultos de *D. citri.* Os isolados NEPET 11 e UENP 06, ambos *Heterorhabditis amazonensis,* foram os mais eficientes no controle do psilídeo asiático dos citros, atingindo mortalidade confirmada de 68,25 e 53,00%, respectivamente. Em todas as concentrações de JIs testadas, os isolados UENP 06 e NEPET 11 apresentaram mortalidade confirmada sobre adultos de *D.citri,* não apresentando diferenças significativas entre concentrações e isolados de NEPs.
Palavras-chave: Controle biológico. HLB. Greening. Psilídeo asiático dos citros. *Heterorhabditis. Steinernema.*

Citrus production (*Citrus* spp.) plays an essential role in the Brazilian agribusiness, a leading global producer of sweet oranges [*Citrus sinensis* (L.) Osbeck]. Further, Brazil is one of the world's largest producers and exporters of citrus products (Companhia Nacional de Abastecimento [CONAB], 2022; Food Agricultural Organization [FAO], 2019). However, Brazilian citrus industry faces significant challenges due to the occurrence of diseases and pests that affect citrus orchards. One of the main concerns is greening or Huanglongbing (HLB), a disease caused by the gram-negative bacteria '*Candidatus* Liberibacter spp.' (Bové, 2006; Li et al., 2006). Already present in Brazil's main citrus-producing regions, this disease causes substantial economic losses (Dala-Paula et al., 2019).

HLB bacteria are transmitted by insect vectors, primarily by the Asian citrus psyllid *Diaphorina citri* (Bové, 2006; Li et al., 2006). Currently, chemical psyllid control is widely used in citrus orchards to prevent HLB. This control strategy involves sequential and preventive applications of different chemical insecticides on the citrus plants in order to eliminate the psyllid (Bassanezi et al., 2010). However, the application of such insecticides has several limitations, including high costs for the citrus growers, environmental contamination, the development of resistant insect populations, and damage to the natural enemies of the psyllid (Tiwari et al., 2011).

Thus, there is a growing need to develop alternative methods to chemicals for the control of agricultural pests, including the Asian citrus psyllid *D. citri,* and biological control is an important option. In this respect, the use of entomopathogenic nematodes (EPNs) is an alternative to chemical products, as they have already shown with high potential in controlling a number of important agricultural pests (Grewal et al., 2001). Fernandes et al. (2021) selected native EPN isolates that exhibited pathogenicity and virulence for *Alphitobius diaperinus* control in poultry litter. Silva (2011) used EPNs to control *Quesada gigas* nymphs (Hemiptera: Cicadidae) and found that *Heterorhabditis* sp. JPM4 was the most efficient, remaining in the environment up to 60 days after application.

EPNs distributed in the genera *Heterorhabditis* and *Steinernema* are obligate insect parasites symbiotically associated with bacteria. EPNs and their symbiotic bacteria work together to cause the death of their hosts. While EPNs of the genus *Steinernema* are associated with *Xenorhabdus* spp. bacteria and colonize the insect's intestinal vesicle, *Heterorhabditis* isolates are associated with *Photorhabdus* spp. bacteria and colonize the insect's gut (Ciche & Ensign, 2003).

The aim of this study was to evaluate EPNs as an alternative and sustainable strategy for controlling the Asian citrus psyllid *D. citri,* an insect vector of the *'Candidatus* Liberibacter spp.' bacteria that cause HLB in citrus.

The *D. citri* specimens used in the present study were obtained from colonies maintained in the Insect Biological Control Laboratory of the Instituto de Desenvolvimento Rural do Paraná – IAPAR/Emater (IDR-Paraná), Londrina, PR, Brazil. The EPN isolates were obtained from the entomopathogen bank at the Laboratory of Natural Enemy Testing at the Universidade Estadual Norte do Paraná (UENP), Cornélio Procópio, PR, Brazil (Table 1).

In tests to select the pathogenic and most aggressive EPNs against the Asian citrus psyllid (*D. citri*), ten isolates of the genera *Heterorhabditis* and *Steinernema* from different origins were assessed (Table 1). Each EPN isolate was considered a treatment, and the experimental unit consisted of a 9 cm diameter Petri dish containing ten psyllids and two filter paper sheets. The EPNs were applied at a concentration of 100 IJs/cm² in distilled water suspension, using a micropipette. The control treatment consisted of applying only distilled water. After the applications, the Petri dishes were sealed with plastic film and kept in a B.O.D. chamber at 25 ± 1 °C, with a 12-hour photoperiod. After five days, the treatments were assessed, and the psyllid mortality was confirmed by dissecting the specimens. The experiment was in a randomized experimental design with ten treatments and four repetitions. Data were submitted to analysis of variance, and means compared using the Scott-Knott test at 5% significance, and SISVAR 5.4 software (Ferreira, 2011).

Table 1
Entomopathogenic nematode (EPN) isolates and their origins, and mortality (%) of *Diaphorina citri* adults under controlled conditions at 25 ± 1 °C, 70 ± 10% relative humidity, and a 12-hour photoperiod.

Isolate	Species	Origin	Mortality (%)
NEPET 11	*Heterorhabditis amazonensis*	Palmeira das Missões, RS, Brazil	68.25 ± 5.6 Aa
UENP 06	*Heterorhabditis amazonensis*	Ribeirão Claro, PR, Brazil	53.00 ± 8.1 B
UENP 03	*Heterorhabditis* sp.	Ribeirão Claro, PR, Brazil	42.75 ± 7.4 C
UEL 07	*Heterorhabditis amazonensis*	Londrina, PR, Brazil	40.50 ± 5.0 C
UEL 08	*Heterorhabditis amazonensis*	Londrina, PR, Brazil	40.25 ± 5.6 C
MC 01	*Heterorhabditis amazonensis*	Monte Carmelo, MG, Brazil	30.75 ± 5.8 D
All	*Steinernema carpocapsae*	Florida, EUA	30.75 ± 3.1 D
HB	*Heterorhabditis bacteriophora*	New Jersey, EUA	28.25 ± 3.5 D
UENP 02	*Heterorhabditis amazonensis*	Ribeirão Claro, PR, Brazil	23.00 ± 2.1 D
IBCB 02	*Steinernema carpocapsae*	Florida, EUA	13.75 ± 2.9 E
Control			0 ± 0 F
C.V.			18.47

[a]Means followed by the same uppercase letter in the column did not differ according to the Scott-Knott test at p> 0.05.

In the studies to determine the maximum lethal concentration, two EPN isolates (UENP 06 and NEPET 11) were included. These isolates performed best in the EPN selection tests, causing the highest mortality of *D. citri* psyllids (Table 1). To determine the maximum lethal concentration, four concentrations were assessed: 50, 100, 150, and 200 IJs/cm². Each treatment was repeated four times, and each plot consisted of a 9 cm diameter Petri dish containing ten psyllids and two filter paper sheets. Based on the results obtained, the maximum lethal concentration was estimated based on the derived regression equation, determining the desired value within the range evaluated. Regression analyses and mean comparison by Tukey's test were performed using the SISVAR 5.4 statistical software (Ferreira, 2011).

The ten EPN isolates included in the selection study showed activity against *D. citri,* with confirmed individual mortality ranging from 13.75 up to 68.25% (Table 1). The isolates with the highest efficiency for controlling adults of *D. citri* were NEPET 11 and UENP 06, both belong to the *H. amazonensis* species, with confirmed mortality rates above 50% and differing significantly from the other isolates (Table 1). Further, NEPET 11 was the most effective in controlling adult *D. citri,* causing 68.25% mortality (Table 1), followed by UENP 06, which also showed significant results in the *D. citri* control, resulting in 53% confirmed mortality (Table 1).

The IBCB 02 isolate of the species *Steinernema carpocapsae* showed the lowest adult *D. citri* mortality rate, reaching only 13.75% (Table 1). In previous studies

by Giometti et al. (2011), this same EPN isolate also did not perform well when tested against the sugarcane weevil (*Sphenophorus levis*), resulting in only 17.1% mortality. It is important to point out that this EPN isolate is not native from Brazil, but from Florida, USA (Table 1). This origin may explain its low activity against both insect pests, and be related to factors such as the specific characteristics of the susceptibility of the insect species to nematodes. On the other hand, previous studies have underscored the effectiveness of NEPET 11 in insect control. Fernandes et al. (2021) demonstrated that NEPET 11 was efficient in controlling larvae of the lesser mealworm *Alphitobius diaperinus,* reaching 100% mortality. Magnabosco et al. (2020) observed that NEPET 11 significantly reduced *Elasmopalpus lignosellus* pupae, a pest in corn crops. These results are consistent with our findings and highlight the promising potential of EPN isolates in controlling agricultural pests.

In the inoculum concentration tests, NEPET 11 and UENP 06 showed significant efficacy in adult *D. citri* mortality at all concentrations tested (50, 100, 150, and 200 IJs/cm²), although with different performance between them (Figure 1). For NEPET 11, D. citri mortality ranged from 62.5 up to 92.5% at different concentrations, while mortality for UENP 06 was 75.0 up to 95.0%, showing consistent results.

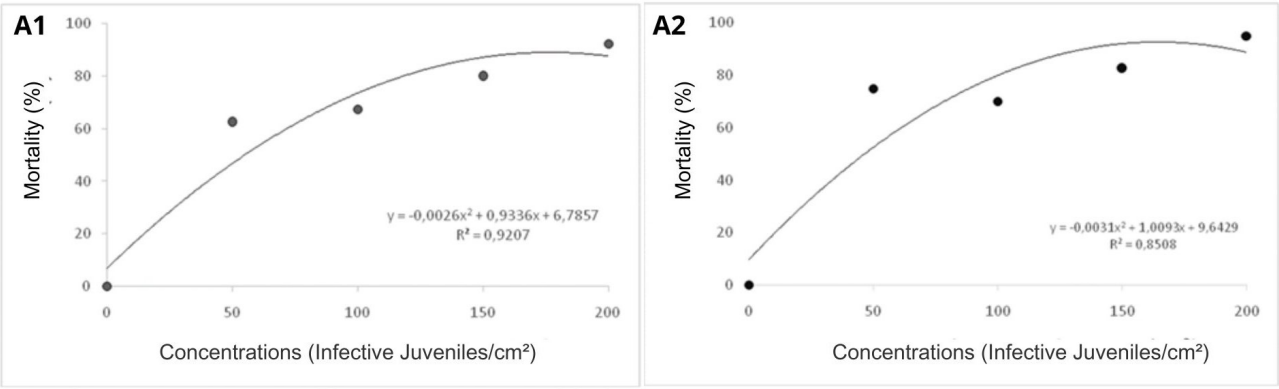

Figure 1. Regression curve of the mortality (%) of *Diaphorina citri* adults as a function of the concentration of the entomopathogenic nematodes (EPNs) *Heterorhabditis amazonensis* NEPET 11 (1A) and UENP 06 (1B).

Significant differences were observed in psyllid mortality between the four concentrations tested. In the case of NEPET 11, 200 IJs/cm² and 150 IJs/cm² were the most effective concentrations, with 92.5 and 80.0% mortality, respectively. For UENP 06, 200, 150, and 100 IJs/cm² showed no statistical differences, and were the most effective concentrations against the adult psyllids.

A slight stabilization trend in *D. citri* mortality efficiency was observed for both isolates from 150 IJs/cm² to higher concentration (Figure 1). It is important to note that the two EPNs have different geographic origins. The NEPET 11 isolate was collected in Rio Grande do Sul state, while UENP 06 was obtained in Paraná (Table 1). These differences in origin may influence the performance of EPNs in different environments and against different pests.

In conclusion, EPNs demonstrated pathogenicity and caused adult mortality of the Asian citrus psyllid *D. citri.* The *H. amazonensis* isolates NEPET 11 and UENP 06 were the most efficient EPNs for the control of this psyllid. On the other hand, no significant differences in adult *D. citri* mortality were observed in the four infective juvenile (IJ) concentrations assessed for the isolates NEPET 11 and UENP 06. Thus, the results obtained in this study emphasize the potential of EPNs as a biological control strategy for *D. citri.* However, further studies are needed to assess EPN activity against the Asian citrus psyllid *in vivo,* to explore application methods in the citrus plants, and to better understand the activity of these organisms under field conditions.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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