Brazilian Nematological Society

NEMATODA

Compatibility of entomopathogenic nematodes (Nematoda: Rhabditida) with insecticides used in the tomato crop

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HIGHLIGHTS

- The compatibility of entomopathogenic nematodes (EPNs) was tested against eight insecticides used in tomato crops.
- The insecticides abamectin and chlorpyrifos didn't kill the IJs, but reduced their infectivity capacity.
- Abamectin and chlorpyrifos were considered slightly toxic (class 2) for the two species of EPNs tested (*Steinernema carpocapsae* All and *Heterorhabditis amazonensis* JPM4.

ABSTRACT: Entomopathogenic nematodes (EPNs) are agents that can be used for the biological control of pests associated with insecticides in a tank mix. Compatibility studies need to be conducted to analyze which products are compatible with nematodes. The aim of this work was to evaluate the compatibility between EPNs and the insecticides that are most used on the tomato crop, and to correlate the toxicological classification of the chemical products with two species of EPNs that have the potential to control tomato leaf miner, *Tuta absoluta*. Among the products tested, Certero® (triflumuron), Decis® (deltamethrin), Previcur® (dimethylamino-propyl), Ampligo® (lambda-cyhalothrin + chlorantranilprole), Premio® (clorantranilprole), Engeo Pleno® (thiamethoxam + lambda-cyhalothrin) were compatible (IOBC class 1) with both nematode species.

Keywords: associated control, biological control, selectivity.

Cite as
Sabino PHS, Sales FS, Guevara EJ, Moino A Jr, Filgueiras CC. Compatibility of entomopathogenic nematodes (Nematoda: Rhabditida) with insecticides used in the tomato crop. Nematoda. 2014;1:e03014. http://dx.doi.org/10.4322/nematoda.03014

Received: Jan. 1, 2014 **Accepted:** Jul. 15, 2014

INTRODUCTION

The tomato, *Lycopersicon esculentum* Mill is considered one of the main agricultural crops worldwide, being currently distributed in all Brazilian regions, especially the Midwest and the South-East[1]. Among the principal insect pests of the tomato crop the tomato leaf miner, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae), stands out. It is considered a key pest, causing damage to leaves, shoots and fruit, and may cause complete loss of the crop[2]. Chemical control is the most common way to reduce pest populations, but it can often lead to serious problems such as elimination of the population of natural enemies, human and environmental contamination and induced resistance in pest populations[3].

Biological control is an important tool in the reduction of the aforementioned problems. One biological control strategy is the use of entomopathogenic nematodes (EPNs), especially species from the genera *Steinernema* and *Heterorhabditis,* due to the mutualistic association with bacteria from the genera *Xenorhabdus* and *Photorhabdus*, respectively, which cause rapid death to the insect. Parasitism starts when infective juveniles (IJs) enter through natural openings (mouth, anus and spiracle) or

directly in the insect's tegument (especially *Heterorhabditis*), releasing the bacteria through the anus and causing host death[4].

Foliar application of nematodes presents great potential for controlling leaf miner larvae[5, 6]. The nematodes are applied in crops that receive varying agricultural inputs, such as fertilizers and chemical products applied on the leaves; some products may reduce the survival and infectivity of these nematodes[7]. In integrated pest control, selective insecticides are used together with biological control agents, and they may influence the activity of these organisms[8]. It has thus become very important to learn more about which insecticides help the nematodes in integrated control and, in consequence, reduce the establishment of populations with genes that confer resistance to a control agent[9]. Thus, it is vital to evaluate critically the compatibility of insecticides and entomopathogenic nematodes, aiming to introduce these organisms into integrated pest management (IPM).

The aim of this work was to evaluate the compatibility between insecticides that are most used on the tomato crop and correlate the toxicological classification of the products with two species of EPNs that have the potential to control tomato leaf miner.

MATERIALS AND METHODS

The nematodes used in the bioassay were *Steinernema carpocapsae* All (isolated in North Carolina, USA) and *Heterorhabditis amazonensis* JPM4 (isolated in Lavras, MG, Brazil, identified at the University of Florida)[10], which were maintained in an aqueous suspension (500 IJs / mL) at 16 ± 1 °C.

Production of *Galleria mellonella* (L) (Lepidoptera: Pyralidae) was carried out in accordance with the methodology adapted by Dutky et al.[11], using an artificial diet modified by Parra[12]. The entomopathogenic nematodes were multiplied on final-instar larvae of *G. mellonella*, in accordance with Kaya & Stock[13].

The nematodes obtained from *G. mellonella* larvae were kept in aqueous suspension at 16 ± 1 °C and stored for up to one week before being used in the experiment. The concentration of IJs in the final suspension was quantified with the use of polystyrene sheets containing 96 wells as used in serological tests, applying 0.1 mL of IJ suspension per well. At the end, the quantity of IJ per aliquot of 1 mL was obtained, and the mean value of three aliquot counts was taken.

Compatibility bioassay between entomopathogenic nematodes and insecticides

To determine the compatibility of the entomopathogenic nematodes with the insecticides used on tomato crops, the methodology modified by Negrisoli Jr et al.[14] was used, evaluating the viability and infectivity of the IJs after exposure to the products. The bioassay was carried out with eight insecticides normally used on the tomato crop (Table 1). One liter of each formulated product was prepared, at

Table 1. Characteristics of the insecticides used in the bioassay.

Name technical	Name commercial	Formulationa	T.Cb	Chemical group	Concentration/ hac
Abamectin	Vertimec 18®	CE	III	Avermectin	1.2 L
Triflumuron	Certero®	SC	II	Benzoylurea	300 mL
Deltamethrin	Decis 25®	CE	III	Pyrethroid	400 mL
Dimethylamino-propyl	Previcur N®	CS	IV	Carbamate	1.5 L
Chlorpyrifos	Klorpan 480®	CE	I	Organophosphate	800 mL
Lambda-cyhalotrin + Chlorantranilprole	Ampligo®	SC	II	Pyrethroid + Anthranilamide	300 mL
Chlorantranilprole	Premio®	SC	III	Anthranilamide	200 mL
Thiamethoxan + Lambda-cyhalothrin	Engeo Pleno®	SC	III	Neonicotinoids + Pyrethroid	800 mL

[a]EC = Emulsionable concentrate; CS = concentrated suspension; SC = soluble concentrate.
[b]T.C = Toxicological classification.
[c]Highest recommended concentration.

double the recommended dose for application on one hectare. From this solution, one aliquot of 1 mL was taken and placed in each glass tube of each treatment, with each treatment being composed of five tubes (each tube as one repetition), to which 1 mL of suspension containing 2.500 IJs was added and shaken. The bioassay took place in a chamber climatized at 27 ± 1 °C, RH of 70 ± 10%.

The viability of the nematodes was evaluated 48 hours after exposure to the products. For this, one aliquot of 0.1 mL (which corresponds to 125 IJs) was removed from the suspension and the IJs were observed under stereoscopic microscope to verify the effect caused by the tested products on nematode viability. Those that did not respond to stimulation with scalpel were considered dead. Soon after evaluating viability, the infectivity test was carried out; for this 3 mL of distilled water was added to the glass tubes, which were then left to decant for 30 minutes in a chamber climatized at 27 ± 1 °C, RH of 70 ± 10%. The supernatant (about 3 mL) was discarded and washing was repeated three times. After the last wash, 0.2 mL (about 250 IJs) was removed from the bottom of each tube and pipetted on to five Petri dishes per treatment (9 cm in diameter), each containing one sheet of filter paper, previously moistened with 1.8 mL of distilled water. Each dish received 10 final-instar *G. mellonella* larvae and was kept in a chamber climatized under the same conditions as the previous test, for three days. After this period, the dead larvae were transferred to Petri dishes (9 cm in diameter) containing filter paper, and maintained in a chamber climatized at 27 ± 1 °C, RH of 70 ± 10% for three more days. After this period they were observed under stereoscopic microscope and submitted to dissection to verify the presence of nematodes. The experimental design was completely random, and the mortality values of nematodes and larvae were submitted to analysis of variance.

The effect of the insecticides was analyzed for each nematode. The differences in the viability and infectivity of the EPN species were analyzed using Tukey test ($p < 0.05$), with the SISVAR program[15]. The effects of the treatments on EPN infectivity in *G. mellonella* were classified according to Peters & Poullot[16], based on the IOBC guide and the formula:

$$E\% = 100 - (100 - \%\ \text{corrected mortality}) \times (100 - Red)$$

In which Red = percentage reduction in infectivity in the treatment

The corrected mortality was equal to zero for all treatments, and was thus not considered in the calculation of E%.

The percentage reduction in EPN infectivity was calculated by the formula:

$$Red = (1 - It / Ic) \times 100$$

It = mortality of *G. mellonella* in each treatment
Ic = mortality of *G. mellonella* in control treatment

Based on the value of E% the products were classified as: 1 – non-toxic (< 30%), 2 – slightly toxic (30 – 79%), 3 – moderately toxic (80 – 99%) and 4 – toxic (> 99%).

For the treatments that presented a percentage of dead larvae that was greater than in the control treatment, E% was considered equal to zero and the product was considered non-toxic.

RESULTS AND DISCUSSION

In relation to the viability of the IJs exposed to different insecticides, all provoked low mortality in the IJs from the two nematode species (Table 2). In relation to *S. carpocapsae* All, the control treatment was statistically equal to the products Vertimec®, Decis® and Klorpan®. The highest mortality registered for *H. amazonensis* JPM4 was with Previcur®, causing 24.6% of IJ mortality; this was the only product that differed statistically from the control treatment. Other products caused mortality lower than 20% on *H. amazonensis* JPM4 IJs.

S. carpocapsae All obtained higher mean infectivity in comparison to *H. amazonensis* JPM4 (Table 3). The products that most affected *H. amazonensis* JPM4 were Vertimec® and Klorpan®, provoking 30 and 26% mortality of *G. mellonella* larvae, respectively, being the only ones that differed statistically from the control treatment. The same happened with *S. carpocapsae* All, with Vertimec® and Klorpan® causing 20 and 50% of mortality of *G. mellonella* larvae, respectively.

In the present work, the two species of EPNs *S. carpocapsae* All and *H. amazonensis* JPM4 were not affected in relation to their viability when exposed to the insecticide Vertimec®, but a reduction in IJ infectivity took place. This result may have been observed due to the nematicidal effect of this product, as abamectin can involve a toxic effect directly on the phytopathogenic nematodes by damaging their sensorial organs, making it possible to recognize the penetration site[17]. Work carried out by Head et al. [18] also demonstrated that Dynamec® caused low infectivity (0.1%) of IJs in *G. mellonella* larvae.

Table 2. Percentage of mortality (mean ± SE) of *Heterorhabditis amazonensis* JPM4 and *Steinernema carpocapsae* All exposure to the insecticides used on the tomato crop (27 ± 1 °C, RH of 70 ± 10%).

Heterorabditis amazonensis		*Steinernema carpocapsae*	
Control	9.0[a] ± 0.20 a	Control	2.0 ± 0.20 a
Vertimec	13.2 ± 0.19 ab	Vertimec	13.6 ± 0.17 ab
Certero	9.2 ± 0.20 a	Certero	18.2 ± 0.64 b
Decis	19.2 ± 0.25 ab	Decis	9.2 ± 0.44 ab
Previcur	24.6 ± 0.42 b	Previcur	22.8 ± 0.37 b
Klorpan	16.2 ± 0.29 ab	Klorpan	12.8 ± 0.20 ab
Ampligo	8.0 ± 0.12 a	Ampligo	21.2 ± 0.35 b
Premio	8.4 ± 0.17 a	Premio	21.6 ± 0.31 b
Engeo Pleno	6.2 ± 0.19 a	Engeo Pleno	17.6 ± 0.36 b
CV(%)	7.6		7.8

[a]Means followed by the same letter do not significantly differ according to Tukey's test at a 5% significance level.

Table 3. Mortality of *Galleria mellonella* larvae (mean ± SE) for *Heterorhabditis amazonensis* JPM4 and *Steinernema carpocapsae* All exposure to the insecticides used on the tomato crop (27 ± 1 °C, RH of 70 ± 10%).

	Heterorabditis amazonensis			*Steinernema carpocapsae*		
	% de Mortalidade[a]	E%[c]	C[d]	% de Mortalidade[a]	E%[c]	C[d]
Control	86.0[b] ± 0.60 a	–		96.0[b] ± 0.40 a	–	
Vertimec	30.0 ± 0.40 b	65.1	2	20.0 ± 0.54 c	79.1	2
Certero	76.0 ± 0.50 a	11.6	1	82.0 ± 0.50 a	14.5	1
Decis	70.0 ± 0.31 a	18.6	1	96.0 ± 0.40 a	0	1
Previcur	82.0 ± 0.37 a	4.6	1	80.0 ± 0.70 a	16.6	1
Klorpan	26.0 ± 0.40 b	69.7	2	50.0 ± 0.54 b	47.9	2
Ampligo	76.0 ± 0.24 a	11.6	1	100.0 ± 0.00 a	0	1
Premio	80.0 ± 0.70 a	6.9	1	100.0 ± 0.00 a	0	1
Engeo Pleno	90.0 ± 0.44 a	0	1	94.0 ± 0.40 a	2.0	1
CV(%)	13.8			14.1		

[a]Dead *Galleria mellonella* larvae.
[b]Means followed by the same letter do not significantly differ according to Tukey's test at a 5% significance level.
[c]Treatment effect: E% = 100 – (100 – % corrected mortality) × (100 – Red). % of corrected mortality was null in all treatments and therefore not considered for calculating E.
[d]IOBC toxicological classification of the insecticides: 1—harmless (< 30%), 2—slightly harmful (30-79%)

In the present work, the two species of EPNs presented insensitivity to the chitin-inhibiting insecticide Certero®. This result may be due to the absence of chitin in the cuticular structure of the nematodes, as their primary constitution is formed by collagens, cuticulins and other proteins[19]. In addition, the insecticide with action similar to that of Certero®, chitin-inhibitor diflubenzuron (unknown commercial product), did not provoke any inhibition in the reproduction and development of *S. carpocapsae* in *in vitro* tests carried out by Hara & Kaya[20]. Chitin-inhibiting insecticides had previously been observed not affecting the viability of *Heterorhabditis bacteriophora*[21], ratifying the results observed by Rovesti & Deseö[22] on *S. carpocapsae* and *Steinernema feltiae* and by De Nardo & Grewal[23] with the insecticide Adept IGR® on *S. feltiae*, also agreeing with the results obtained in the present work.

Studies carried out by other authors also show low IJ mortality for *S. carpocapsae* when exposed to chlorpyrifos[24, 25, 26]. One hypothesis that may explain this insensitivity in the EPNs involves the presence

of butyrylcholinesterase in the synapse of parasitic nematodes, protecting the acetylcholinesterase, and thus acting as a frontline defense against such compounds[27]. Negrisoli Jr et al.[14] observed that Pyrinex® caused low mortality in *H. bacteriophora* (2.8%) and *S. carpocapsae* (2.2%), however, it caused a reduction in the infectivity of the IJs in *G. mellonella* larvae. The same result was found in the present study, and this may be related to the reduction in lipids in the EPNs after contact with insecticides. The quantity of lipids present in the IJs directly influences host infectivity[28].

Observing the action of pyrethroids, Negrisoli Jr et al.[19] found low EPN mortality (below 10%) when exposed to the product Decis®. Similar results were found in this study, which broadens the results related to the safety of this product for EPNs. The safety of other insecticides based on pyrethroids was also proved by Rovesti et al.[21] and Rovesti & Deseö[22], who verified high viability of *H. bacteriophora* and *S. carpocapsae*, after exposure to these products.

In this study the EPNs presented viability and infectivity similar to the control treatment when exposed to the product Engeo Pleno®. Koppenhöfer et al.[29] evaluated the effect of Merit® and Meridian® combined with *H. bacteriophora* and *Steinernema glaseri*, obtaining synergic effects from both nematodes with each of the products on the same hosts. Actara® also maintained high viability in *Steinernema arenarium* (83%), *S. carpocapsae* (83.3%), *S. glaseri* (85.1%) and *H. bacteriophora* (83.4%) in a work carried out by Andaló et al.[30]. These authors also found high infectivity (over 80%) in these nematodes when exposed to Actara®. They found high infectivity (over 60%) in four species of nematodes tested when exposed to the insecticide Furadan®; the same was found with Previcur® for the two species of EPNs used in the present study.

From the results presented in this work it can be observed that the association of certain products increases the infectivity of EPNs, so that synergy occurs and integrated pest management works better.

The products that possess more than one active ingredient, such as Ampligo® and Engeo Pleno®, did not cause damage to the two species of nematodes, confirming the safety of these products in integrated control. Products with two active ingredients were also analyzed by Negrisoli Jr et al.[14], and the product Verdadero® reduced infectivity of *H. bacteriophora*, not causing an effect on *S. carpocapsae*. The active ingredients of the chemical group anthranilamide did not present any difference in the viability and infectivity of the nematodes, but the active ingredients of the chemical group pyrethroid did present different effects on the viability of the two nematode species. This difference may be due to the formulation of the products, which may contain surfactants that are more or less toxic to the nematodes[31, 32].

There was a correlation in the toxicological classification of the products Decis®, Premio®, Engeo Pleno® (class III) and Previcur® (class IV), causing a low effect on the viability and infectivity of the IJs, except for Vertimec® (class III), which caused a loss in the infectivity of the IJs when compared to the control treatment. The same occurred with Klorpan®, reducing the infectivity of the IJs; however, correlating with its toxicological classification (class II), the other products did not present a toxicological correlation with the nematodes, in agreement with the findings of Rovesti et al.[21].

Vertimec® and Klorpan® were considered slightly toxic (class 2 – slightly toxic) for the two species of EPNs, but the toxicity of a product *in vitro* does not always represent its toxicity in the field[8], since in the laboratory the contact is extreme and guaranteed for 48 hours. One way of using the incompatible nematodes and insecticides would be applying them at different moments after the period of persistence of the product, or vice versa[19]. Foliar applications have been severely limited due to environmental obstacles such as ultraviolet radiation, high temperatures and low humidity, reducing the survival and efficacy of the nematodes[33]. Thus, the applications should be carried out at night or in the early morning when unfavorable environmental conditions can be avoided[34]. The other products used in the bioassay were considered compatible (class 1 – non-toxic) with the two species of nematodes.

In the present work, it was confirmed that the nematodes *Heterorhabditis amazonensis* isolate JPM4 and *Steinernema carpocapsae* All are considered compatible with most of the products tested for the tomato crop.

ACKNOWLEDGMENTS

The authors thank the Minas Gerais Research Foundation (Fundação de Amparo a Pesquisa do Estado de Minas Gerais - FAPEMIG) for financial support.

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