Compatibility between entomopathogenic nematodes and crop protection products used in maize seed treatment

Compatibilidade entre nematoides entomopatogênicos e produtos fitossanitários utilizados no tratamento de sementes de milho

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Abstract

Chemical insecticides are widely used to control soil pests but not always effective. Entomopathogenic nematodes (NEPs) are found in the soil and depend on host insects to complete their life cycle, and therefore have the potential to control soil pests. Thus, we aimed to investigate the possible joint use of these control methods by assessing the compatibility of two nematodes (*Heterorhabditis amazonensis* GL and *Heterorhabditis amazonensis* MC01) with five crop protection products used for maize seed treatment (Maxim®, Cruiser 350 FS®, Fortenza 600 FS®, Avicta 500 FS®, and Amulet®), as well as one neem-based product (NeenMax®). The experimental design was completely randomized with five replicates, six treatments, and one control, in which only distilled water was added to nematode suspension. Each replicate consisted of a test tube containing 1 mL suspension with 2,000 infective juveniles (IJs) and 1 mL of diluted product, following the manufacturer's recommendation. The evaluated parameters were viability, infectivity on *Tenebrio molitor* larvae and IJs production after exposure to products. Both nematodes were compatible with NeenMax® and Fortenza 600 FS® since they did not differ from the control and were classified as innocuous. Cruiser 350 FS ® was also compatible with the nematodes since the effect value of the product was lower than 30%. Amulet® was classified as slightly noxious, reducing *H. amazonensis* MC01 and *H. amazonensis* GL infectivity by 17.5% and 28.5%, and production by 18.2% and 22.3%, respectively. Despite not having reduced viability, Avicta 500 FS® and Maxim® were considered harmful. This is because Avicta 500 FS® and Maxim® reduced productivity by 70.0% and 72.5% and production by 66.1% and 65.4% for *H. amazonensis* MC01, respectively. For *H. amazonensis* MC01, both Avicta 500 FS® and Maxim® reduced infectivity by 76.19%, and production by 63.7% and 62.3%, respectively.
Key words: Biological control. Cornstalk borer. *Heterorhabditis*. Integrated pest management. Seed treatment.

Resumo

Os inseticidas químicos apesar de serem os mais utilizados para controle de pragas de solo, nem sempre são eficazes. Os nematoides entomopatogênicos (NEPs) são encontrados no solo e utilizam insetos como hospedeiros para completarem seu ciclo de vida, assim, apresentam potencial no controle de pragas com hábito subterrâneo. Com isso, destaca-se a importância de se avaliar a possibilidade de uso conjunto desses métodos de controle, objetivando-se avaliar a compatibilidade dos nematoides *Heterorhabditis amazonensis* GL e *Heterorhabditis amazonensis* MC01 com cinco produtos fitossanitários utilizados no tratamento de sementes de milho, Maxim®, Cruiser 350 FS ®, Fortenza 600 FS®, Avicta 500 FS®

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e Amulet®, além de um produto à base de nim, NeenMax®. O ensaio foi conduzido em delineamento inteiramente casualizado com cinco repetições, totalizando seis tratamentos e o controle, no qual foi adicionada apenas água destilada à suspensão do nematoide. Cada repetição foi constituída de um tubo de ensaio contendo 1 mL de suspensão com 2.000 juvenis infectantes (JIs) e 1 mL do produto diluído de acordo com a recomendação do fabricante. Foram avaliados os parâmetros viabilidade, infectividade sobre larvas de *Tenebrio molitor* e produção de JIs após o contato com os produtos. Verificou-se que para ambos os nematoides os produtos NeenMax® e Fortenza 600 FS® foram considerados compatíveis, pois não diferiram do controle e foram classificados como inócuos. O produto Cruiser 350 FS® também foi compatível aos nematoides testados, já que o valor de efeito do produto obtido foi menor que 30%. O produto Amulet® foi classificado como levemente nocivo, causando uma redução de 17,5% na infectividade e de 18,2% na produção de *H. amazonensis* MC01 e de 28,5% e 22,3 % para *H. amazonensis* GL. Avicta 500 FS® e Maxim® foram considerados nocivos, pois apesar de não reduzirem a viabilidade dos nematoides, diminuíram em 70,0% e 72,5% a infectividade e 66,1% e 65,4% a produção de *H. amazonensis* MC01, respectivamente; enquanto para *H. amazonensis* GL causaram o mesmo valor de redução de infectividade, 76,19%, e causaram 63,7% e 62,3% de redução da produção, respectivamente.
Palavras-chave: Broca-do-colmo. Controle biológico. *Heterorhabditis*. Manejo integrado de pragas. Tratamento de sementes.

Introduction

Maize production chain is an important pillar of the Brazilian agribusiness, corresponding to approximately 40% of the national grain production (CONAB, 2018). Both international demand and domestic consumption of maize have increased in recent years, because of its use for livestock feed production and due to the growth of the meat market, mainly poultry and pork (PAVÃO; FERREIRA FILHO, 2011).

Despite the large area under maize cultivation in Brazil, crop yields are among the lowest worldwide, on account of several factors that may contribute to such low productivities (CONAB, 2018; USDA, 2019). One of these factors is a large number of difficult-to-manage insect pests damaging the crop, such as *Elasmopalpus lignosellus* (Zeller) (Lepidoptera: Pyralidae), which is found near or inside maize stems or even lodged into the soil, becoming a hard target for chemical applications (ZORZETTI et al., 2017).

Data on damage by soil insect pests are scarce, but it is estimated that damages by *E. lignosellus* may increase losses in more than 20% to total crop destruction when under high infestation levels (VIANA, 2004). Such infestation index (20%) in

seedlings may correspond to reductions of 2.8 and 2.4% in grain and silage productions, respectively (ALL et al., 1982).

Pest control strategies in maize crops are based on the use of insecticides. Seed treatment is the most used chemical control due to practicality, cost, and efficiency. However, in areas with severe drought records and consequently low soil moisture, insecticides show reduced effectiveness. This happens because insecticides require moisture to provide effective caterpillar control, and besides that, dry soils are favorable to a lesser cornstalk borer development (VIANA; MENDES, 2011).

The inappropriate use of chemical control methods tends to select resistant insect populations, causing harmful effects to the environment, animal health, and high production costs (DALVI et al., 2011; ZORZETTI et al., 2017). Thus, it is necessary to develop alternative control methods such as biological control.

Entomopathogenic nematodes (EPNs) of the families Steinernematidae and Heterorhabditidae (Nematoda: Rhabditida) have the potential to be used as biological control agents, due to their capability to develop an intimate and specific association with bacteria responsible for disease establishment and

consequently insect death (NGUYEN; HUNT, 2007).

Studies have demonstrated that NEPs are effective in controlling pests of different crops (FOELKEL et al., 2016; GIOMETTI et al., 2011; MANACHINI et al., 2013). Feaster and Steinkraus (1996) and Andaló et al. (2010) assessed pupae control of *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae) and *Spodoptera frugiperda* (Smith) (Lepidoptera: Noctuidae) in maize using NEPs; both pests had mortality rates higher than 90%. Lepidoptera, such as *E. lignosellus*, pupate in the soil and become reachable targets for NEPs, which are found in the soils of several biomes worldwide, completing their life cycle hosting in insects (LAWRENCE et al., 2006).

The aim of this study was to assess the compatibility of crop protection products recommended for maize seed treatment and a neem-based insecticide with two entomopathogenic nematodes, *Heterorhabditis amazonensis* GL and *H. amazonensis* MC01.

Material and Methods

The nematodes used in the experiments were cultured in larvae of *Tenebrio molitor* L. (Coleoptera: Tenebrionidae), reared according to the method of Potrich et al. (2007) and collected according to the method of Molina and López (2001).

Five crop protection products recommended for maize seed treatment (Maxim®, Cruiser 350 FS®, Fortenza 600 FS®, Avicta 500 FS®, and Amulet®) and one neem-based insecticide (NeenMax®) were tested for compatibility with two entomopathogenic nematodes (*H. amazonensis* GL and *H. amazonensis* MC01). There were, therefore, a total of six treatments (products) plus one control treatment (distilled water). Each treatment had five replicates.

The experiment was conducted following the IOBC/WPRS protocol, proposed by Vainio (1992). Working solutions were prepared for each product (Table 1), using double the dose recommended by the manufacturer per water mL. Thus, the used volumes were 0.31 mL of Maxim®, 0.60 mL of Cruiser 350 FS®, 0.87 mL of Fortenza 600 FS®, 0.15 mL of Avicta 500 FS®, 0.5 mL of Amulet®, and 0.25 mL of NeenMax®.

Table 1. Phytosanitary products used in the compatibility study with entomopatogenic nematodes (AGROFIT, 2018).

Trademark	Active ingredient	Description	Dose
Maxim®	Fludioxonil 25 g L^{-1}; Inert ingredients 1018 g L^{-1}	Fungicide	150 mL cp*/100 kg seeds
Cruiser 350 FS®	Thiametoxam 350 g L^{-1}; Other ingredients 820 g L^{-1}	Insecticide	120 mL/60.000 seeds
Fortenza 600 FS®	Cyantraniliprole 600 g L^{-1}; Other Ingredients 630 g L^{-1}	Insecticide	350 mL/100 kg seeds
Avicta 500 FS®	Abamectin 500 g L^{-1}; Other ingredients 508 g L^{-1}	Nematicide e Insecticide	60-70 mL/60.000 seeds
Amulet®	Fipronil 250 g L^{-1}; Other ingredients 850 g L^{-1}	Insecticide	50-200 mL ha^{-1}
NeenMax®	Azadirachtin (1,200 ppm) 1.0%; Inerts 99.0%	Insecticide	200 mL L^{-1} of water

*Commercial product.

An aliquot of 1 mL of each working solution was placed into a glass tube (8 cm high x 2.5 cm in diameter), together with 1 mL of distilled water containing 2,000 infective juveniles (IJs). Thus, each tube contained a total volume of 2 mL (1 mL of product + 1 mL of EPN suspension). For the control, 1 mL of distilled water was added to 1 mL of the EPN suspension. The tubes were sealed with Parafilm® and kept in a chamber under the following controlled conditions: 24 ± 1˚C temperature, 70 ± 10% relative humidity, and scotophase.

The assessed parameters were viability, infectivity, and IJs production after exposure to products. Due to the strong pigmentation of products, the JIs cleaning preceded the viability evaluation. Cleaning was done by passing the nematode suspensions through a 500-mesh sieve, using water for washing the IJs, which were retained without the products. Afterward, the initial volume of 2 mL was adjusted by adding distilled water. This procedure was carried out for all treatments in order to standardize the method for all tested products.

Viability assessment was done after 48 h, removing 0.1 mL of suspension from each tube, observing the IJs under a stereo microscope and counting the total number of live and dead juveniles in the aliquot. Those that remained inert, following the addition of 50 μL of NaOH, were considered dead (CHEN; DICKSON, 2000).

Juvenile infectivity was assessed by adding 3 mL of distilled water into the tubes, which were left for 30 minutes in the refrigerator (10°C) for IJs decantation. After this period, supernatant (about 3 mL) was discarded. This washing procedure was repeated three times.

Once the washing was completed, 1.0 mL was withdrawn from the bottom of each replicate (each tube) and placed in a glass Petri dish (9 cm in diameter) containing two filter paper sheets and ten *T. molitor* larvae. The Petri dishes were kept for five days in a chamber, under the same conditions previously described. After this period,

the percentage of larvae killed by the nematodes was determined by counting the number reddish-brown larvae, which is characteristic death symptomatology caused by *Heterorhabditis* (KAYA et al., 1993).

To determine IJs production, the larvae killed by nematodes during the infectivity test were transferred to White's traps and kept in a chamber under the aforementioned conditions. The IJs produced were collected from the White's traps for five days and counted under a stereo microscope.

Normality and homoscedasticity assumptions were tested by the Shapiro-Wilk and Levene's tests, respectively. Subsequently, the IJs viability and insect mortality data were submitted to analysis of variance, and nematode mortality values were corrected by the Abbott (1925) formula:

$$Mc\% = \frac{Mo\% - Mt\% \, x \, 100}{100 - Mt\%}$$

Mc = Corrected mortality; Mo = Observed mortality; and Mt = Control mortality.

Infectivity was obtained from *T. molitor* larvae mortality percentage. The infectivity reduction caused by the treatments was determined by the formula:

$$Rinf\% = \left(1 - \frac{It\%}{Ic\%}\right) x \, 100$$

Rinf% = Infectivity reduction; It% = Treatment infectivity; and Ic% = Control infectivity.

Production was determined by counting the number of IJs obtained from *T. molitor* larvae. The production reduction caused by the treatments was determined by the formula:

$$Rfec\% = \left(1 - \frac{Ft}{Fc}\right) x \, 100$$

Rfec% = Production reduction; Ft = Treatment production; and Fc = Control production.

The Peters and Poullot (2004) modified formula was used in order to determine the insecticide effect (E%).

$$E\% = 100 - (100 - Mcorr\% - Rinf\% - Rfec\%)$$

The aforementioned formula follows the IOBC standards (International Organization for Biological and Integrated Control of Noxious Animals and Plants). For E% calculation, a zero (0) was assigned to the factors Mc%, Rinf%, and Rfec%, when they presented negative values.

The effect values of insecticides were classified as: innocuous (E% < 30), slightly noxious (E% entre 30 a 79), moderately noxious (E% entre 80 a 99) and noxious (E% > 99).

Results and Discussion

Regarding viability, the survival of IJs exceeded 95% for most of the products. The highest mortality for both nematodes was caused by Maxim®, with 7.63% for *H. amazonensis* MC01 and 7.21% for *H. amazonensis* GL (Tables 2 and 3).

Table 2. Compatibility of *Heterorhabditis amazonensis* MC01 after 48 hours of exposure to phytosanitary products (protocol IOBC/WPRS), Vainio (1992).

Treatment	Viability (%)	Infectivity (%)	Mc%	Rinf%	Rfec%	E%	IOBC Classificarion
Control	99.6 ± 0.55 a	80.0 ± 7.07 b	0.0	0.0	0.0	0.0	Innocuous
NeenMax®	99.2 ± 1.10 a	88.0 ± 8.37 a	0.40	0.0	0.0	0.0	Innocuous
Fortenza 600 FS®	99.0 ± 1.22 a	96.0 ± 5.48 a	0.60	0.0	0.0	0.0	Innocuous
Cruiser 350 FS®	97.0 ± 2.00 b	70.0 ± 7.07 c	2.61	12.50	0.0	13.50	Innocuous
Avicta 500 FS®	96.8 ± 0.84 b	24.0 ± 5.48 d	2.81	70.00	66.1	138.89	Harmful
Amulet®	95.6 ± 3.29 b	66.0 ± 8.94 c	4.02	17.50	18.2	39.68	Slightly harmful
Maxim®	92.0 ± 1.58 c	22.0 ± 8.37 d	7.63	72.50	65.4	145.56	Harmful
CV (%)	1.79	11.56					

Means followed by the same letter are not significantly different (P < 0.05).
Mean ± Standard Error
Mc% = Corrected mortality
Rinf% = Infectivity reduction
Rfec% = Production reduction
E% = Insecticide effect.

The viability for both nematodes did not differ after exposure to Cruiser 350 FS®, Avicta 500 FS®, and Amulet®. When exposed to Cruiser 350 FS® and Avicta 500 FS®, the IJs of *H. amazonensis* MC01 and of *H. amazonensis* GL had mortality rates of 2.61% and 2.81%, respectively. Yet, when exposed to Amulet®, *H. amazonensis* MC01 had its viability reduced by 4.02% and *H. amazonensis* GL by 3.01%. On the other hand, NeenMax® and Fortenza 600FS® did not differ from the control in terms of viability (Tables 2 and 3).

The infectivity of *H. amazonensis* MC01 to *T. molitor* larvae increased after exposure to NeenMax® and Fortenza 600 FS® when compared to the control (Table 2). Similarly, Abdel-Rasek and Gowen (2002) and Mahmoud (2007) also showed the compatibility of nematodes of the genus *Heterorhabditis* with neem extract, indicating that the combined use of the nematode and neem may lead to a synergistic effect on the control of *Plutella xylostella* L. (Lepidoptera: Plutellidae) and *Bactrocera zonata* (Saunders) (Diptera: Tephritidae).

Table 3. Compatibility of *Heterorhabditis amazonensis* GL after 48 hours of exposure to phytosanitary products (protocol IOBC/WPRS). Vainio (1992).

Treatment	Viability (%)	Infectivity (%)	Mc%	Rinf%	Rfec%	E%	IOBC Classification
Control	99.8 ± 0.45 a	84.0 ± 5.48 a	0.0	0.0	0.0	0.0	Innocuous
NeenMax®	99.6 ± 0.55 a	82.0 ± 8.37 a	0.20	2.38	3.7	6.32	Innocuous
Fortenza 600 FS®	98.6 ± 1.34 a	86.0 ± 5.48 a	1.20	0.0	6.9	5.7	Innocuous
Cruiser 350 FS®	97.2 ± 0.84 b	76.0 ± 5.48 a	2.61	9.52	2.8	14.97	Innocuous
Avicta 500 FS®	97 ± 1.58 b	20.0 ± 7.07 c	2.81	76.19	63.7	142.67	Harmful
Amulet®	96.8 ± 0.84 b	60.0 ± 7.07 b	3.01	28.57	22.3	53.85	Slightly harmful
Maxim®	92.6 ± 1.52 c	20.0 ± 7.07 c	7.21	76.19	62.3	145.74	Harmful
CV (%)	1.13	10.88					

Means followed by the same letter are not significantly different (P < 0.05).
Mean ± Standard Error
Mc% = Corrected mortality
Rinf% = Infectivity reduction
Rfec% = Production reduction
E% = Insecticide effect.

Cruiser 350 FS® (12.5%) and Amulet® (17.5%) reduced *H. amazonensis* MC01 infectivity, which did not differ from each other (Table 2). Despite that, Cruiser 350 FS® was classified as innocuous while Amulet® as slightly noxious. Such a difference may be because the IOBC protocol takes into account all the analyzed parameters for the classification of products (E%) (Table 2).

Tavares et al. (2009) exposed the nematodes *H. indica* IBCBn5 and *Steinernema brasilense* IBCBn6 to thiamethoxam, which is the active ingredient in Cruiser 350 FS®, and demonstrated the compatibility of both nematodes with the insecticide. Other studies have already reported the compatibility of *thiamethoxam with other nematode species, e.g., H. bacteriophora, S. carpocapsae, S. glaseri,* and *S. arenarium* (KOPPENHÖFER; KAYA, 1998; KOPPENHÖFER et al., 2003; ALUMAI; GREWAL, 2004; ANDALÓ et al., 2004).

Maxim® and Avicta 500 FS® caused similar effects on both nematodes, reducing both infectivity and production of the IJs. Maxim® reduced infectivity and production by 72.5% and 65.4% for *H. amazonensis* MC01 and by 76.19% and 62.3% for *H. amazonensis* GL, respectively. Avicta 500 FS®

reduced infectivity and production by 70.0% and 66.1% for *H. amazonensis* MC01 and by 76.19% and 63.7% for *H. amazonensis* GL respectively. Thus, Maxim® and Avicta 500 FS® were considered incompatible with both nematodes after exposure for 48 hours (Tables 2 and 3).

When exposed to Amulet®, *H. amazonensis* MC01 reduced its infectivity by 17.5% and production by 18.2%, while *H. amazonensis* GL exposure to Amulet® reduced its infectivity by 28.57% and production by 22.3%. Therefore, Amulet® was considered to be slightly noxious to IJs according to the IOBC classification (Table 3).

Infectivity reduction may be related to the fact that some chemicals reduce the amount of lipids in nematodes, which are an important energy source for these organisms, as observed by Andaló et al. (2009) for *H. amazonensis* RSC5 when applied on caterpillars [*Galleria mellonella* (L.) (Lepidoptera: Pyralidae)] treated with the herbicides Ranger® and Topeze®, for 5 days.

Sabino et al. (2014) found similar results regarding infectivity reduction when the IJs of *H. amazonensis* JPM4 were exposed to abamectin, which is the active ingredient in Avicta 500 FS®.

Likewise, such a reduction in infectivity was also associated with a decrease in lipid reserves.

In general, the products Cruiser 350 FS®, Fortenza 600 FS®, and NeenMax® were compatible with both nematodes. This is because the effect of the product (E%) was lower than 30 (Tables 2 and 3), which shows the potential for the combined use of these products with the nematodes. Amulet®, Avicta 500 FS®, and Maxim® were noxious since the E% was higher than 30%, indicating the non-compatibility of the products with the nematodes under the tested conditions.

Field tests are still needed to assess the effects of products considered to be harmful to nematodes because, under laboratory conditions, the EPNs are subjected to maximal insecticide exposure, whereas in the field there are other factors that may influence nematode responses.

Conclusions

According to the IOBC classification, Amulet® was classified as slightly noxious to *H. amazonensis* MC01 and *H. amazonensis* GL, while Avicta 500 FS® and Maxim® were considered noxious, indicating that treating seeds with these products may interfere with nematode survival in the soil. NeenMax®, Fortenza 600 FS®, and Cruiser 350 FS® were considered innocuous to both nematodes.

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