ENTOMOPATHOGENIC NEMATODES (NEMATA: RHABDITIDAE) AND NATURAL INSECTICIDES TO CONTROL *ATTA SEXDENS* L. (HYMENOPTERA: FORMICIDAE) IN SUGARCANE

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ABSTRACT

Souza Neto, P. S., A. S. Negrisoli, Jr., and C. R. C. Barbosa Negrisoli. 2017. Entomopathogenic nematodes (Nemata: Rhabditidae) and natural insecticides to control *Atta sexdens* L. (Hymenoptera: Formicidae) in sugarcane. Nematropica 47:135-142.

This study aimed to conduct a laboratory assessment of the association between entomopathogenic nematodes and natural insecticides in the control of *Atta sexdens* L. (Hymenoptera: Formicidae) in sugarcane. The experimental arenas consisted of plastic containers (80 ml) with a fine-mesh cover where the insects were maintained and the treatments were applied. The following insecticides were tested: Derris Rotenona® CE, Pyroligneous Acid Extract®, Pironim Super® WG, Codipirol®, and Pure Neem Oil®. The following nematodes were assessed: *Heterorhabditidis bacteriophora* RS58, *Steinernema glaseri* RS38, and eight isolates *Heterorhabditis* sp., produced *in vivo* in *Galleria mellonella* caterpillars and stored at 15°C. The insecticides Codipirol®, Pure Neem Oil®, and Pyroligneous Extract® caused the highest adult mortality rates in *Atta sexdens*. Codipirol®, Pure Neem Oil®, and Pyroligneous Acid Extract® showed the lowest lethal times (LT_{50}) against *Atta sexdens* adults (62, 66, and 75 h, respectively). LC_{50} of *Heterorhabditis* sp. AL40 was 2.91 IJs/insect in *Atta sexdens* adults. The association between *S. glaseri* RS38 and Codipirol® and between *Heterorhabditis* sp. AL40 and Pyroligneous Acid Extract® had additive effects on the mortality of *Atta sexdens*.

Key words: associated control, *Heterorhabditis* spp., *Steinernema glaseri*

RESUMO

Souza Neto, P.S., A. S. Negrisoli Jr., e C. R. C. Barbosa Negrisoli. 2017. Nematoides entomopatogênicos (Nemata: Rhabditidae) e inseticidas naturais para o controle de *Atta sexdens* L. (Hymenoptera: Formicidae) em cana-de-açúcar. Nematropica 47:135-142.

Este estudo objetivou conduzir avaliações em laboratório com associação entre nematoides entomopatogenicos e inseticidas naturais no controle de *Atta sexdens* L. (Hymenoptera: Formicidae). As arenas experimetais consistiram de recipientes plásticos (80ml) cobertas com tela fina, nas quais os insetos foram mantidos e os tratamentos aplicados. Os seguintes inseticidas foram testados: Derris Rotenona® CE (Agroterra Insumos), Extrato Pirolenhoso® (Agroterra Insumos), Pironim Super® WG (Agroterra Insumos), Codipirol® (Codipa Indústria e Comércio de Agricultura e Pecuária), e Óleo de Nim Puro® (Organix). Os seguintes nematoides foram avaliados: *Heterorhabditidis bacteriophora* RS58, *Steinernema glaseri* RS38, e os isolados *Heterorhabditis* sp. AL39, AL40, AL41, AL42, AL43, AL44, AL46, e AL47, produzidos *in vivo* em lagartas de *Galleria mellonella* L. (Lepidoptera: Pyralidae) e estocados at 15°C. Os inseticidas Codipirol®, Óleo de Nim Puro® e Extrato Pirolenhoso® causaram a maior mortalidade de adultos de *Atta sexdens*. Codipirol®, Óleo de Nim Puro® e Extrato Pirolenhoso® mostraram nos menosres tempos letais medianos (LT_{50}) contra adultos de *Atta sexdens* (62, 66, e 75 horas, respectivamente). A CL50 de *Heterorhabditis* sp. AL40 foi de 2,91 JIs por inseto em adultos de *Atta sexdens*. A associação entre *S. glaseri* RS38 e Codipirol® teve um efeito aditivo na mortalidade de *Atta sexdens*. A associação entre *Heterorhabditis* sp. AL40 e Extrato Pirolenhoso® teve um efeito aditivo a este nematoide na mortalidade de *Atta sexdens*.

Palavras-chave: controle associado, *Heterorhabditis* spp., *Steinernema glaseri*

INTRODUCTION

Many factors limit the yield potential of sugarcane varieties, including diseases and insect attacks (Costa, 2007). Sugarcane forms an agricultural ecosystem that is home to numerous insect species, some of which (depending on the season and the region) may cause serious economic damage (Mendonça *et al.,* 2005). According to Long and Hensley (1972), there may be more than 1,500 species of these insect pests of sugarcane around the world. On the other hand, sugarcane plantations serve as a shelter for numerous beneficial insects (predators and parasitoids) that feed on both pest and non-pest insects (Macedo and Araújo, 2000).

In Brazil, the genera *Atta* and *Acromyrmex*, leaf-cutting ants (Hymenoptera: Formicidae), are economically significant insects. These species are popularly known in Portuguese as sauvas and quenquéns, respectively (Zanetti, 2003; Nilton, 2008; Dinardo-Miranda *et al.*, 2008). In general, their nests contain hundreds of underground cavities (*Atta*), most of them filled with fungi (Pagnocca, 2001). These ants cause great damage to crops, because they attack virtually all cultivated plants and are spread throughout the country, foraging during all seasons of the year (Loeck and Grützmacher, 2001). In addition to the direct damage they cause by cutting leaves, *Atta* ants also mechanically impede the growth of grasses; they remove large quantities of soil to excavate their fungus-farming cavities, and this soil accumulates in the area outside the nest (Forti, 1985).

According to Unnithan and Paye (1991), the most suitable pest control system is based on integrated management to keep insect pests below an economically impactful level of damage. Cultural, mechanical, biological, and chemical control methods have been developed in an attempt to minimize pest damage to native and cultivated plants. Moreover, a result of bans on certain pesticides, and in an attempt to manage agriculture more consistently with ecological and public health principles, there is a need for new research to find products that have less environmental impact, such as insect growth regulators, pheromones, repellents or attractants, and bioinsecticides with targeted action (Nakano *et al.*, 2005). However, in sugarcane, chemical controls are used as the only control method for suitable pests, leading to a series of negative impacts on the environment (Machado and Habib, 2009).

Entomopathogenic nematodes (EPNs) are used in North America, Europe, Asia, and Australia to control pests in the soil and in cryptic environments. These organisms are considered promising in biological pest control because they can be produced on a large scale, be applied with conventional equipment, and they affect a wide range of hosts while being innocuous to the environment (Grewal *et al.*, 2001). Because of the need to find alternatives to the use of chemical pesticides in soil, nematodes were extensively used as bioinsecticides against soil pests (Kaya and Gaugler, 1993).

Another pest-control alternative that has been well studied, is the use of secondary substances present in "plant insecticides." Substances such as rotenoids, pyrethroids, alkaloids, and terpenoids are intermediate or final products of plant secondary metabolism that are found as roots, leaves, and seeds. These substances can severely affect the metabolism of other organisms, causing variable impacts such as repellency and sterilization, feeding or egg-laying deterrence, metabolism, and developmental disorder without necessarily causing death (Medeiros, 1990; Lancher, 2000). Biologically active substances have been created from essential oils extracted from various plant species (Simas *et al.*, 2004). The objective of this study was to evaluate the combination of entomopathogenic nematodes and natural insecticides in the control of *Atta sexdens* in sugarcane.

MATERIALS AND METHODS

The experiments were performed in the Entomology Laboratory at Embrapa Tabuleiros Costeiros/UEP Rio Largo-AL, repeated twice, and the results presented were the last bioassay. The insects were collected from an area where sugarcane was being cultivated for the Paisa Ltd. factory, located in the municipality of Penedo, Alagoas, Brazil. Identification of the species of leaf-cutting ants was based on the taxonomic key from Gallo *et al.* (2002).

The following EPNs species and isolates were evaluated: *Heterorhabditidis bacteriophora* RS58 (corn, Lagoa Vermelha, RS), *Steinernema glaseri* RS38 (corn, Passo Fundo, RS) and isolates of *Heterorhabditis* sp. AL39 (coconut palm, São Miguel dos Campos, AL), *Heterorhabditis* sp. AL40 (guava tree, Pé Leve Velho Village, Arapiraca, AL), *Heterorhabditis* sp. AL41(guava tree, Pé Leve Velho Village, Arapiraca, AL), *Heterorhabditis* sp. AL42 (graviola tree/coconut palm, Pé Leve Velho Village, Arapiraca, AL), *Heterorhabditis* sp. AL43 (orange tree, Arapiraca, AL), *Heterorhabditis* sp. AL44 (sugarcane, Arapiraca, AL), *Heterorhabditis* sp. AL44 (opuntia cactus/squash, Arapiraca, AL), and *Heterorhabditis* sp. AL47 (sugarcane, Arapiraca, AL) and multiplied according to the methodology of Voss *et al.* (2009).

After *in vivo* production in *Galleria mellonella* L.

(Lepidoptera: Pyralidae) caterpillars, the nematodes in water were stored in zip-closure plastic bags (29 × 27 cm) and kept in a climate-controlled room at a temperature of 15 ± 1°C, relative humidity of 70 ± 10%, and a 12-h photophase for a maximum of 1 week before the bioassays.

Before beginning the bioassays, the ants collected from the field were brought to a cold chamber (15°C) to reduce their mobility and facilitate handling, since these ants have powerful jaws with which they defend themselves. Next, the leaf-cutting ants (n = 30) were separated into 500-ml plastic containers containing sterile moist sand (5% v/v).

Determination of lethal concentrations and times ($LC_{50,90}$) of entomopathogenic nematodes on Atta *ants*

Entompathogenic nematodes were inoculated at concentrations of 0, 62, 125, 250, 500, and 1,000 IJs (infective juveniles)/container in 1 ml in separate plastic containers for each nematode tested. Insect mortality was assessed every 24 h starting from the beginning of the experiment and ending on the sixth day (144 h), via observation of the presence of EPNs in the ants' head and abdomen. The insects were dissected to confirm the presence of the nematodes inside each insect and thus quantify insect percentage mortality.

Selection and lethal time ($LT_{50,90}$) of natural insecticides on Atta *ants*

The methodology for selecting and determining the lethal times of natural insecticides on ants was similar to the method described above. Derris Rotenona® CE (Agroterra Insumos), Pyroligneous Acid Extract® (Agroterra Insumos), Pironim Super® WG (Agroterra Insumos), Codipirol® (Codipa Indústria e Comércio de Agricultura e Pecuária), and Pure Neem Oil® (Fig. 5) were applied to the sand at a concentration of 1% (v/v) of active ingredient according to the manufacturers' recommendations; distilled water was used as a control treatment. Insect mortality was assessed every 24 h from the beginning of the experiment as described above.

Combining entomopathogenic nematodes and natural insecticides against Atta *ants*

Entomopathogenic nematodes and natural insecticides used were those selected in the previous tests. The treatments used were as follows: EPNs at a concentration of 3,000 IJs/ml combined with each of the following natural insecticides, all at a 3% concentration, in addition to the distilled water control. The surfactant Will Fix® (as recommended by the manufacturer) was used in all the treatments at a concentration of 0.1%.

The treatments (2 ml of EPN + 2 ml of product) were applied manually using a graduated pipette and were maintained under the same conditions as described above.

Daily insect mortality was assessed for 6 d; the dead insects were transferred to a dry chamber (filter paper in a Petri dish) for dissection to confirm the death by EPNs using a stereoscopic microscope.

Experimental design and analyzed variables

To determine lethal concentration and times, the experimental design was completely randomized with 30 repetitions per treatment, determined by Probit analysis ($P < 0.05$) using PoloPlus software, version 1.0 (LeOra Software Company).

To select the product that caused highest insect mortality, the data were subjected to analysis of variance (ANOVA) and mean difference testing (Tukey, $P < 0.05$) using the SISVAR program.

To assess the effect of the interaction between the products and nematodes, the binomial test was used along with comparison of observed and estimated percent mortality according to Robertson and Preisler (1992), modified by Nishimatsu and Jackson (1998). The percentage of expected mortality was obtained by the formula:

$$\mathbf{P_e = P_o + (1-P_o) + (1-P_o)(1-P_1)(P_2)},$$ where

P_e: expected mortality in combining EPNs and the products;

P_o: mortality in the control (natural mortality of the insect);

P_1: mortality after treatment with the product alone;

P_2: mortality after treatment with the nematodes alone

The chi-squared value (X^2), was calculated using the formula:

$$\mathbf{X^2 = (L_o-L_e)^2/L_e + (P_o-P_e)^2/P_e},$$ where

L_o: observed number of living insects (P_o observed mortality for the insecticide/EPN combination);

L_e: expected number of living insects;

To determine the value of $X^2 = 3.84$ in the table, one degree of freedom (n−1) and $P = 0.05$ were considered, with additive interaction indicated by $X^2 < 3.84$. Antagonism was indicated by $X^2 < 3.84$ and

$P_o < P_e$ and synergism was indicated by $X^2 < 3.84$ and $P_o > P_e$.

To assess the effect of the treatments over time, polynomial regression was performed ($P < 0.05$) using the SISVAR program.

RESULTS

Selection of natural insecticides on Atta sexdens

Atta sexdens adults exposed to the various insecticides for 48 h showed 29.11% mortality (Codipirol®), 27.09% (Pure Neem Oil®), 25.74% (Pyroligneous Acid Extract®), 20.22% (Pironim Super® WG), and 15.32% (Derris Rotenona® CE). There was no statistical difference between the treatments except for the control (Fig. 1).

Similar to the results obtained at 48 h, there was statistical difference between the treatments. *Atta sexdens* adults exposed to the various products for 72 h showed 50.31% mortality (Codipirol®), 45% (Pure Neem Oil®), 34.66% (Pyroligneous Acid Extract®), 27.27% (Pironim Super® WG), and 24.5% (Derris Rotenona® CE) (Fig. 1).

Mortality of adult *Atta* sp. after 96 h of exposure to the various products was as follows: 73.99% (Codipirol®), 71.99% (Pure Neem Oil®), 62.33% (Pyroligneous Acid Extract®), 60.72% (Pironim Super® WG), and 53.38% (Derris Rotenona® CE) (Fig. 1). There was no significant difference between the treatments.

Lethal Times ($LT_{50,90}$) of natural insecticides on Atta sexdens

Atta sexdens adults exposed to different products at a 1% concentration for 144 h exhibited the following lethal times, in ascending order (LT_{50} and LT_{90}, respectively): 62.81 and 153.89 h (Codipirol®), 66.35 and 177.06 h (Pure Neem Oil®), 75.71 and 200.80 h (Pyroligneous Acid Extract®), 82.86 and 212.07 h (Pironim Super® WG), and 95.32 and 269.41 (Derris Rotenona® CE) (Table 1).

Lethal concentrations and times (LC and LT) of isolates of entomopathogenic nematodes on Atta sexdens

The lowest lethal concentration (LC_{50} and LC_{90}) was obtained with *Heterorhabditis* sp. AL40: 3 and 162 IJs per *A. sexdens* adult, respectively. Mortality curves of adult *A. sexdens* for the nematodes *Steinernema glaseri* RS38, *H. bacteriophora* RS58, and *Heterorhabditis* sp. AL43 did not adjust to the Probit model (Table 2).

Lethal times LT_{50} and LT_{90} of *Heterorhabditis* sp. AL39 were 148.14 and 13,530 h for *Atta sexdens* adults, respectively. Mortality curves for adult *Atta sexdens* for the remaining species and isolates did not fit the Probit model (Table 3).

Combination of entomopathogenic nematodes and natural insecticides against Atta sexdens *ants*

Adult *A. sexdens* were exposed to mixtures of different entomopathogenic nematodes and products for 144 h. The best combinations were *S. glaseri* RS38 + Codipirol®, *Heterorhabditis* sp. AL40 + Codipirol®, *Heterorhabditis* sp. AL40 + Pyroligneous Acid Extract® and *Heterorhabditis* sp. AL40 + Pure Neem Oil®. The best treatments did not differ from each other using the Tukey test ($P < 0.05$) (F = 233; gl = 11; P = 0.00; CV% = 8.05; Fig. 2).

The effect of combining natural insecticides and nematodes varied according to the combination (Table 4). Considering the mortality caused by *S. glaseri* RS38 as the main effect, combining this EPN with Codipirol® had an additive effect on the mortality of *A. sexdens*, but the nematode was an antagonist when mixed with other products. Combining *Heterorhabditis* sp. AL40 and Pyroligneous Acid Extract® produced an additive effect on pest mortality.

DISCUSSION

Most of the work using entomopathogenic nematodes for ant control focuses on species of

Table 1. Probit analysis to determine lethal time (LT_{50}, LT_{90}) of natural insecticides on adult *Atta sexdens* ants.

Insecticide	LT_{50} (h)	Conf limit.	LT_{90} (h)	Conf limit.
Codipirol®	62.81	47–77	153.89	115–272
Pure Neem Oil®	66.35	51–82	177.06	130–327
Pyroligneous Acid Extract®	75.71	54–102	200.80	135–591
Pironim Super®	82.86	60–116	212.07	140–727
Derris Rotenona®	95.32	71–144	269.41	166–1,228

Table 2. Probit analysis to determine lethal concentration (LC_{50}, LC_{90}) of entomopathogenic nematodes on adult *Atta sexdens* ants.

Isolates	LC_{50} [y]	Conf limit.	LC_{90} [x]	Conf limit.
S. glaseri RS38	NS[z]	-	NS	-
H. bacteriophora RS58	NS	-	NS	-
Heterorhabditis sp. AL39	161	114–212	3561	1857–11000
Heterorhabditis sp. AL40	3	0-13	162	85–284
Heterorhabditis sp. AL41	82	54–110	1070	717–2015
Heterorhabditis sp. AL42	198	67-195	895	507-3231
Heterorhabditis sp. AL43	NS*	-	NS*	-
Heterorhabditis sp. AL44	34	11–61	1406	775–4628
Heterorhabditis sp. AL46	157	87–245	653	379–2581
Heterorhabditis sp. AL47	186	102–296	1767	831–12504

[y]The data are considered to fit the model when heterogeneity was less than 4, although χ^2 was high.
[z]NS (not significant), the data are not adjusted to the Probit model.

Table 3. Probit analysis to determine lethal time (LT_{50}, LT_{90}) of entomopathogenic nematodes on adult *Atta sexdens* ants

Isolates	LC_{50} [y] (h)	Conf limit.	LC_{90} [x] (h)	Conf limit.
S. glaseri RS38	NS*[y]	-	NS*	-
H. bacteriophora RS58	NS*	-	NS*	-
Heterorhabditis sp. AL39	148.14	99-490	13530	1870–355000
Heterorhabditis sp. AL40	NS*	-	NS*	-
Heterorhabditis sp. AL41	NS*	-	NS*	-
Heterorhabditis sp. AL42	NS*	-	NS*	-
Heterorhabditis sp. AL43	NS*	-	NS*	-
Heterorhabditis sp. AL44	NS*	-	NS*	-
Heterorhabditis sp. AL46	NS*	-	NS*	-
Heterorhabditis sp. AL47	NS*	-	NS*	-
Heterorhabditis sp. AL47	185.99	101.60–295.57	1766.5	831–12504

[y]The data are considered to fit the model when heterogeneity was less than 4, although χ^2 was high.
[z]NS (not significant), the data are not adjusted to the Probit model.

Table 4. Analysis of the effect of mixing entomopathogenic nematodes and natural insecticides on the mortality of *Atta sexdens* adults.

Steinernema glaseri RS38 + products	Mort. Obs. (%)	Mort. Exp. (%)	X^2	Effect[x]
Pure Neem Oil®	74.07	95.27	95.03	antagonist
Pyroligneous Acid Extract®	66.74	94.45	138.28	antagonist
Codipirol®	95.96	96.31	0.03	additive
Pure Neem Oil®	90.89	95.62	5.11	antagonist
Pyroligneous Acid Extract®	90.94	94.45	2.21	additive
Codipirol®	90.52	96.31	9.06	antagonist

[x]Additive interaction was indicated by $X^2 < 3.84$. Antagonism was indicated by $X^2 < 3.84$ and $P_o < P_e$ and synergism was indicated by $X^2 < 3.84$ and $P_o > P_e$.

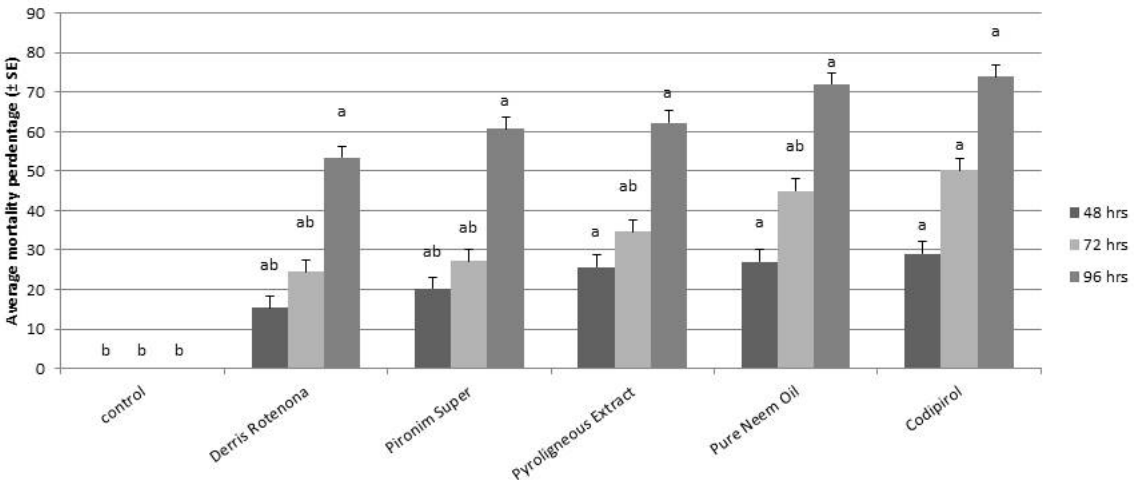

Fig. 1. Mean percentage mortality (± EP) of *Atta sexdens* 48, 72 and 96 h after exposure to natural insecticides. Means followed by the same letter do not differ using the Tukey test ($P < 0.05$).

Solenopsis spp. (Gouge, 2005). The same authors stress that ant physiology is very important to the success or failure of EPN infection; the anatomy of the mouth, the spiracles, and the anus, which have adaptations to conserve water, may also serve as a structure for defense against invading parasites. One strategy that can be adopted is the use of stationary traps that are commonly used with chemical insecticides. In this case, EPNs can be placed within these traps, which have small openings and contain bait. However, many EPNs have low mobility, so studies should be conducted in this area. Georgis (1987) showed that dried or dehydrated IJs of *S. carpocapsae* All. placed in a sugary bait solution were infectious to *Pogonomyrmex* sp. ants.

The additive effect of combinations of nematodes and natural insecticides observed in the bioassay should be further investigated both in the field and with regard to the economic viability of this strategy as an agricultural operation.

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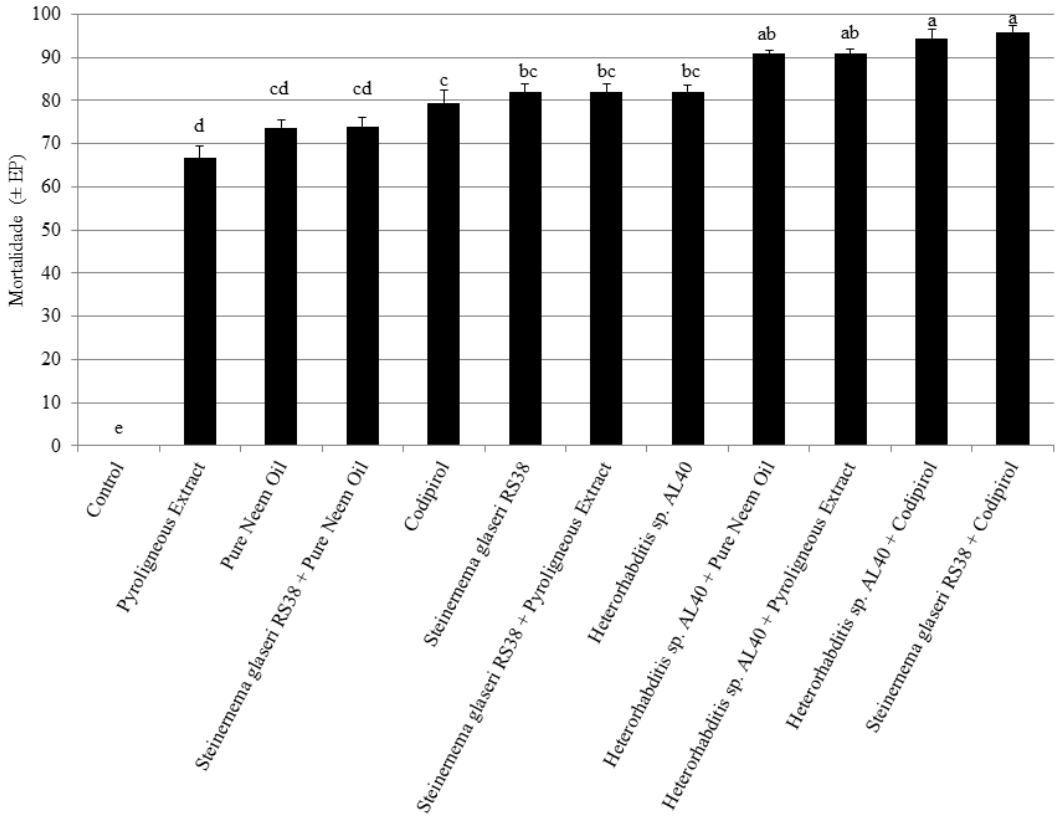

Fig. 2. Percentage mortality (± EP) of adult *Atta sexdens* after 144 h of exposure to isolates/species of entomopathogenic nematodes and natural insecticides (Tukey, 5% probability).

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