ENTOMOPATHOGENIC NEMATODES AND THEIR INTERACTION WITH CHEMICAL INSECTICIDE AIMING AT THE CONTROL OF BANANA WEEVIL BORER, *COSMOPOLITES SORDIDUS* GERMAR (COLEOPTERA: CURCULIONIDAE).

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ABSTRACT

The banana weevil borer (*Cosmopolites sordidus*) is the main pest of banana crops, causing significant losses in productivity, being recommended control by chemical insecticides which cause several environmental impacts. On the other hand, entomopathogenic nematodes can be an alternative to the pest control, mainly because of their habits. Thus, this study aimed at evaluating isolated entomopathogenic nematodes under laboratory conditions and also their interaction with a chemical insecticide (carbofuran), aiming at their use for the weevil borer control. Sixteen Sterinernematidae and Heterorhabditidae isolates were evaluated, applied over the banana tree pseudo stem (100 JIs/cm^2) and they were compared to one another concerning mortality caused in adults individual of *C. sordidus*. The most infective isolates were subjected to in vivo multiplication at the host *Galleria mellonella* and interaction with the insecticide carbofuran, including in this case, viability and infectivity analysis of the entomopathogenic nematodes exposed to the product, as well as the effect of the insecticide on the symbiotic bacteria of the entomopathogenic nematodes. The experiments at this stage were conducted in completely randomized design and the data were subjected to ANOVA, with application of the Tukey test ($p < 0.05$). The most virulent isolates were IBCBn24 and IBCBn40 (respectively 33.3% and 36.7% of confirmed mortality), which also showed high multiplication in corpses. The insecticide did not affect the viability of the isolate, but it really harmed its infectivity, although it did not affect the development of the symbiotic bacterium.

KEY WORDS: Heterorhabditis, Steinernema, biological control, chemical insecticide.

RESUMO

NEMATOIDES ENTOMOPATOGÊNICOS E SUA INTERAÇÃO COM INSETICIDA QUÍMICO VISANDO AO CONTROLE DA BROCA-DA-BANANEIRA *COSMOPOLITES SORDIDUS* GERMAR (COLEOPTERA: CURCULIONIDAE). A broca-da-bananeira (*Cosmopolites sordidus*) é a principal praga dos cultivos de banana, acarretando perdas significativas na produtividade da cultura, sendo recomendados inseticidas químicos para seu controle, os quais causam impacto ambiental. Por outro lado, os nematoides entomopatogênicos podem ser uma alternativa para o controle da praga, principalmente devido aos seus hábitos. Assim, este trabalho teve como objetivo avaliar isolados de nematoides entomopatogênicos em condições de laboratório e a interação com inseticida químico (carbofurano), visando a sua utilização no controle da broca. Foram testados 16 isolados das famílias Sterinernematidae e Heterorhabditidae, aplicados sobre pseudocaule de bananeira (100 JIs/cm^2) e comparados entre si quanto à mortalidade causada em indivíduos adultos de *C. sordidus*. Os isolados mais infectivos foram submetidos a experimentos de multiplicação *in vivo* no hospedeiro *Galleria mellonella* e de interação com o inseticida carbofurano incluindo, neste caso, análise de viabilidade e infectividade dos nematoides expostos ao produto, bem como o efeito do inseticida sobre as bactérias simbiontes dos nematoides entomopatogênicos. Os experimentos dessa fase foram conduzidos em delineamento inteiramente casualizado, sendo os dados submetidos à ANOVA, com aplicação do teste de Tukey ($p < 0,05$). Os isolados mais virulentos foram o IBCBn24 e IBCBn40 (respectivamente 33,3% e 36,7% de mortalidade confirmada), os quais também apresentaram elevada multiplicação em cadáveres. O inseticida não afetou a viabilidade

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do isolado IBCBn40, mas prejudicou sua infectividade, porém, não afetou o desenvolvimento da bactéria simbionte.

PALAVRAS-CHAVE: *Heterorhabditis, Steinernema,* controle biológico, inseticida químico.

INTRODUCTION

The culture of banana occupies a remarkable degree of importance for populations of tropical countries. In Brazil, banana is not only highly consumed, but it is also an exported product. Brazil is the fifth largest fruit producer in the world context and the second in relation to the American continent, producing a little less than Ecuador (FOOD..., 2011). However, it still occupies a low representation in the most demanding markets due to the low level of technology in banana production chain in Brazil, resulting in loss of organoleptic properties and low productivity (Matthiesen; Boteon, 2003; FOOD..., 2010).

Among the problems affecting the crop plant, with reference to pests, there is the banana weevil borer *Cosmopolites sordidus* (Germar) (Coleoptera, Curculionidae). Insect larvae pierce the rhizome, making plants weak and more susceptible to tipping and low productivity. In addition, the galleries built facilitate the entry of phytopathogens such as fungus that causes the Panama disease, *Fusarium oxysporum* f. sp. *cubense* (Suplicy Filho; Sampaio, 1982; Arleu et al., 1984; Batista Filho et al., 2002, Gallo et al., 2002; Messiaen, 2002; Mesquita, 2003).

The control of this pest consists primarily of the application of carbofuran-based insecticides in baits of banana, which attract and kill adult insects (Agrofit, 2003). However, due to the observation of borer populations development resistant to chemical insecticides (Suplicy Filho; Sampaio, 1982; Raga; Oliveira, 1996), it is necessary to search for alternatives of management and control, such as using traps containing aggregation pheromone, resistant varieties and biological control based on entomopathogenic fungi and nematodes (EPNs) (Gold et al., 2001; Grewal et al., 2001; Batista Filho et al., 2002; Messiaen, 2002; Leite, 2006).

Several studies in the laboratory showed the EPNs potential against the banana weevil borer (Treverrow; Bedding, 1993; Rosales; Suárez, 1998; Grewal et al., 2001; Sepúlveda-Cano et al., 2008), and proved their efficiency in the field (Treverrow et al., 1991, Schimitt et al., 1992; Treverrow; Bedding, 1993).

It is also emphasized that many EPNs are tolerant to many pesticides, there being cases where their performance were increased when associated with such products (Rovesti et al., 1988; Kermarrec; Mauléon, 1989, Gordon et al., 1996; Koppenhöfer; Kaya, 1998; Koppenhöfer *et al*., 2002).

Since the potential insecticide of an EPN to a particular insect depends on several factors and relationships involving the target insect, the entomopathogenic nematode specificity and the pathogenicity of their symbiotic bacteria, and the environment, the search for more virulent species or isolates should be constant in pest control programs as well as additional studies about the interactive effects of pesticides on the performance of the selected pathogen. Therefore, this work aimed to evaluate new isolates of EPNs against adults of *C. sordidus* and their interaction with a carbofuran-based chemical insecticide.

MATERIAL AND METHODS

Adults of *C. sordidus* were collected with pseudostem traps (roof tile-type baits) installed in a commercial production area consisting of banana trees of *nanicão* cultivar, in São Miguel do Iguaçu, west of Paraná State (5.47° S, 35.37° W), Brazil. Insects were transferred t o laboratory, kept in plastic containers with moistened sand and bits of banana tree pseudostem, covered with voile and kept at 26 ± 1º C and natural photoperiod, where remained until their use in experiments. Every week, there was exchange of sand and pseudostem.

Nematodes multiplication was performed by the in vivo method on *Galleria mellonella* (L.) (Lepidoptera: Pyralidae) larvae (Poinar Junior, 1979; modified by Woodring; Kaya, 1988; Parra, 1998).

Nematodes used were supplied by Instituto Biológico, Campinas, Brazil and Laboratório de Patologia de Insetos, Universidade Federal de Lavraslates belonging to the genera *Heterorhabditis* and *Steinernema* (Table 1).

Nematodes in infective juveniles phase (IJ) were kept in aqueous suspensions, plastic containers with perforated cover and at 25 ± 2º C until the experiment start, which occurred within seven days after IJ emergence.

Selection of isolates

Insecticidal Activity: Experiments were performed in transparent and colorless plastic containers; 8.5 cm in diameter, in which 100 g of moistened sterile sand (10% moisture) and five adult individuals of *C. sordidus* were placed. Subsequently, pieces of pseudostem (7.5 cm long) were cut lengthwise and 1 mL of nematode suspension (100 IJ/cm^2 or 1.134 IJ/insect) in distilled water was applied at the cut face with the support of a manual pipette. In control was applied 1 mL of distilled water. Thereafter,

each piece was placed inside the containers with the treated surface in contact with sand.

Containers were then closed with a perforated lid and kept at 25 ± 1º C and 14 hours photoperiod for seven days, when the dead individuals were counted. The mortality confirmation by nematodes was made through the observation of specific symptoms caused by infection with EPNs, accompanied by transfer of dead insects to a dry chamber for a period of 24h, followed by dissection to display EPNs within them.

Bioassays were performed in completely randomized experimental design, repeated six times, each one consisting of a group of five adult individuals of *C. sordidus* held in plastic chamber. Data were submitted to descriptive statistical analysis (Mean Standard Error MSE) and more efficient nematodes in insecticidal activity were selected to the next stage for comparison.

Assessment of nematodes production in vivo: Procedures for inoculation, incubation and sampling were the same as those used for the EPNs multiplication to evaluate the production; the methodology was based on studies performed by Barbosa (2005). To this end, seven days after inoculation 45 caterpillars that had symptoms characteristic of infection by EPNs were selected, for each isolate tested, which were divided into groups of five individuals, representing nine replicates. Each group was weighed and the individuals were transferred to White's traps and maintained at 25 ± 1º C and 14h photophase.

Daily, nematodes were collected and quantified from each replicate by up to 10 days. Thus, 20 mL aliquots were collected from the original suspension and placed on plates for ELISA tests. For each replication five counts were performed. Data were analyzed as described before.

Compatibility with the insecticide

Product effect on entomopathogenic nematodes: the carbofuran-based insecticide (2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcar-bamate) is registered and used in banana culture for borer control with commercial name Furadan 350FS (Agrofit, 2003). It was adopted the methodology of Negrisoli Júnior et al (2008a) adapted from Vainio (1992) at the concentration recommended by the manufacturer to pest control in banana crops (400 mL/100 L of water).

The mixture of insecticide + nematodes (2,000 IJ/mL) was prepared, while in the control was used distilled water to nematodes suspension at same IJ concentration, in glass tubes. The tubes were kept at 25 ± 1º C and 14h photophase for 48h, when it was assessed the viability and infectivity. Experiment was repeated five times.

To evaluate nematodes viability, tubes were closed with plastic film and vortexed for 1 minute. After that 0.1 mL samples were taken and transferred to count at test Elisa plates as described.

Table 1 – Isolates of Steinernematidae and Heterorhabditidae nematodes used in bioassays and their origin place.

Species	Isolate	Origin
Steinernematidae		
Steinernema carpocapsae	SC	North Carolina, USA
Steinernema carpocapsae	IBCBn02	Florida, USA
Steinernema anomali	SA	Voronezh, Russia
Heterorhabditidae		
Heterorhabditis indica	IBCBn05	Itapetininga, Brazil
Heterorhabditis sp.	IBCBn10	Santa Fe do Sul, Brazil
Heterorhabditis sp.	IBCBn13	Pindorama, SP, Brazil
Heterorhabditis sp.	IBCBn24	Piracicaba, Brazil
Heterorhabditis sp.	IBCBn33	Naviraí, MS, Brazil
Heterorhabditis sp.	IBCBn40	Solo, Tabapuã, SP, Brazil
Heterorhabditis sp.	IBCBn44	Santa Adelia, Brazil
Heterorhabditis sp.	JPM 3	Lavras, MG, Brazil
Heterorhabditis sp.	JPM 4	Lavras, MG, Brazil
Heterorhabditis sp.	RSC 01	Benjamin Constant, AM, Brazil
H. amazonensis	RSC 05	Benjamin Constant, AM, Brazil
Heterorhabditis sp.	PI	Teresina, PI, Brazil
H. bacteriophora	BAC	New Jersey, USA

For infectivity assessment distilled water was added in these tubes to complete 3 mL volume. Subsequently, they were kept at 9 ± 1º C for 30 minutes , in order to IJ decantation and the supernatant was rejected. This washing procedure was repeated three times and then 0.2 mL aliquots of suspension with approximately 200 IJ were collected from each tube and transferred to five Petri dishes (9 cm diameter) containing filter paper at bottom and 10 last instars larvae of *G. mellonella* were added, maintained at 25 ± 1º C and 14h photophase for five days. The dead larvae were transferred to a dry chamber where they remained for three days, then being subjected to dissection to observe the presence of nematodes and death cause confirmation.

Product effect symbiotic bacteria: Symbiotic bacteria *Photorhabdus* sp. were isolated according to Kaya; Stock (1997). Thus, *G. mellonella* larvae were inoculated with IBCBn40 strain and 24 h after infection, hemolymph samples were taken and inoculated on the surface of nutrient agar culture medium (agar 20 g, meat extract 3 g, peptone 5 g). After incubation at 25° C for 24h, colonies obtained were multiplied in the same medium in test tubes resulting in the inoculum that was maintained at 10º C in refrigerator for later use.

The product effect assessment was based on the technique of Ostrosky et al. (2008), modified from Barry; Thornsberry (1991), in which bacterial suspensions were prepared from the inoculums in Brain Heart Hinton Broth (BHI) and incubated at 35 °C for 24 hours. The standardization was performed in sterile saline solution (0.9%) for the concentration of 10^8 CFU/mL (colony forming units) using the 0.5 MacFarland scale.

Afterwards, this suspension was inoculated with a sterile *swab* on the surface of a Petri dish containing Mueller-Hinton Agar. Filter paper disks (5 mm of diameter) were prepared with the aid of a paper punch and previously autoclaved at 121º C for 15 minutes and then immersed for 1 minute in the treatments (insecticide – 400 mL/100 L and control - distilled water), running up the excess. In the case of antibiotic, the disk was impregnated with neomycin 30 SCU. These discs were applied on to the surface of the medium on Petri dish freshly inoculated with the microorganism to be tested. Each plate contained one disc of each treatment: sterile distilled water, neomycin and carbofuran solution at twice the recommended concentration. Experiment was repeated four times.

Plates were kept at 35° C for 24h in total darkness. The evaluation consisted of verifying the presence or absence of an inhibition halo of bacterial growth and its diameter (mm) measured in two perpendicular lines.

Data were subjected to analysis of variance (ANOVA) and differences among treatments were compared using Tukey mean separation test ($p < 0.05$) using the statistical program Sisvar (Ferreira, 2011).

RESULTS AND DISCUSSION

Isolates selection

Insecticidal Activity: Adults of *C. sordidus* were susceptible to almost all isolates tested, there being great variation in results with values between 0 and 36.7% of confirmed mortality (Table 2).

Table 2 – Average mortality of *Cosmopolites sordidus* adults by different isolates of entomopathogenic nematodes of the families Steinernematidae and Heterorhabditidae (after 7 days at 25 ± 1º C and 14h photophase on banana pseudostem with 100IJ/cm²)

Isolate	Mortality ± SE (%)
Steinernematidae	
SC	0.0 ± 0.00
IBCBn02	3.3 ± 3.33
AS	10.0 ± 4.47
Heterorhabditidae	
IBCBn05	3.3 ± 3.33
JPM3	6.7 ± 4.22
IBCBn44	10.0 ± 4.47
BAC	13.3 ± 8.43
IBCBn10	16.7 ± 6.15
RSC05	16.7 ± 8.03
IBCBn13	20.0 ± 5.16
RSC01	20.0 ± 10.33
IBCBn33	23.3 ± 9.55
JPM4	23.3 ± 6.15
PI	26.7 ± 6.67
IBCBn24	33.3 ± 6.67
IBCBn40	36.7 ± 12.02
Control	0.0 ± 0.00

It was observed that two isolates caused mortality above 30% and differed from the control: IBCBn40 (36.7%) and IBCBn24 (33.3%). The isolates IBCBn33, PI and JPM4 caused mortality greater than 20%, the others reached lower values, and the majority belongs to *Heterorhabditis* genus.

Mortality percentual here obtained were inferior than other studies such as Rosales; Suárez (1998) and Sepúlveda-Cano et al. (2008), in which nematodes were applied on filter paper in Petri dishes. We used a technique quite similar to the nematode field application (i.e., application on soil).

Also, insect behavior and other factors linked to nematodes, such as foraging strategies, recognition, penetration and infection in the host are involved in specific processes, which cause large variations in the efficiency of different species or isolates of nematodes on certain hosts (Lewis et al., 2006).

It was observed in all treatments that most of dead insects was closer to the surface where nematodes were applied and, even a few dead insects were found inside the galleries opposite to the application, it was verified that most of insects immediately moved to several directions within the container and randomly started their galleries in the pseudostem. Thus, the contact time between them and the EPNs may have been insufficient for penetration and infection of insects. With regard to foraging strategies, it is unclear exactly what the behavior of isolates tested is; however, some evidence suggest that those who perform best in this study might be more active in the environment, which would facilitate the meeting with borer pests. The fact of the isolates SC and IBCBn02, both belonging to the species *S. carpocapsae*, have little or no activity on the insect is strong evidence about this fact.

S. carpocasae is a species with ambusher behavior (Lewis, 2002; Campbell et al., 2003; Lewis et al., 2006), its IJ have no active search behavior for the host, instead, they are waiting for it in nictation state, awaiting the right moment to jump toward the entry channels in the insect, not being indicated therefore, to pests control in cryptic environments as it is the case of *C. sordidus*.

Based on the considerations presented and observations of Rasmann et al. (2005), Elliot et al. (2000) and Lewis et al. (2006), it can be assumed that in this study, isolates that caused highest mortality rates, both from the Heterorhabditidae family, probably have moved toward the host, possibly responding to chemical signals coming from the insects inside the galleries, or even signals from the pseudostem injured by the insect. Also, as reported by Treverrow et al. (1991) when evaluating the EPNs effectiveness in field applied directly to the banana pseudostem, it is discarded the negative effect of specific compounds from exudates from banana trees on nematodes.

Both isolates which brought about the highest rates of mortality in this experiment are from the family Heterorhabditidae, fact also observed in other studies performed with *C. sordidus* or other Curculionidae (Treverrow; Bedding, 1993; Dolinski et al., 2006; Sepúlveda-Cano et al., 2008; Giometti et al., 2011; Mwaitulo et al., 2011). All the cited authors deduce that the greatest susceptibility to *Heterorhabditis* is probably related to the greatest facility of these ones to enter into the insect´s body, in relation to the *Steinernema*, once they are comparatively smaller and they have a capacity to pierce the cuticle, and their entrance is not restricted to the natural openings of the insect, what is important in the cases in which it is focused the control of adults which are more resistant to the EPNs than larvae (Mwaitulo et al., 2011).

Concerning to the IJ concentration used in this study (100 IJ/cm^2), it probably has not been a limiting factor for the low mortality observed. This is because Sepúlveda-Cano et al. (2008) tested EPNs suspensions at 10, 100 and 1,000 IJ/insect and found that *H. bacteriophora* caused higher mortality rates of banana's borers in the intermediate concentration of 100 IJ/insect. Also, Treverrow; Bedding (1993) emphasize that within certain limits, increasing the IJs concentration does not necessarily imply an increase in infectivity. Although, there is not a defined rule, once that in other studies there was the opposite result for the majority of the isolates tested, including for the IBCBn24 on adults of the weevil of the sugar cane *Sphenophorus levis* (V.) (Curculionidae) (Giometti et al., 2011; Mwaitulo et al., 2011).

The same is applied to the exposure time of insects to EPNs adopted in this work. Accordingly, studies show that the period in which the highest mortality of *C. sordidus* exposed to EPNs is between 2 and 7 days. After this period, the mortality curve remains constant or with small increments (Treverrow; Bedding, 1993; Rosales; Suárez, 1998; Sepúlveda-Cano et al., 2008).

However, even if the evidence observed in the experiment tend to point to habit and dispersal of nematodes as determining factors in infectivity, it must be considered the action of enzymes and toxins produced by symbiotic bacteria or defense mechanisms of insects.

Nematodes production in vivo: There were obtained 2.2×10^6 and 2.3×10^6 IJ/g of larvae (equivalent to $4,4 \times 10^5$ and $4,6 \times 10^5$ IJ/larvae), respectively to IBCBn24 and IBCBn40 isolates, with no statistical difference between them (Table 3).

Table 3 – IJ Production of the nematode *Heterorhabditis* sp. isolates IBCBn24 and IBCBn40 in *Galleria mellonella* larvae (10 days, 25 ± 1º C and 14h photophase).

Isolate	Average IJ/g of larvae
IBCBn24	2.2×10^6 a
IBCBn40	2.3×10^6 a
CV (%)	26.38

Means followed by same letter in same column do not differ between each other by the Tukey test ($P \leq 0.05$)

The values here obtained were higher than those reported by other authors using the same production method, such as Barbosa (2005) who found 4.9

× 10^5 IJ/g for H. *bacteriophora* and Costa *et al*. (2007) that found on average 1.6 × 10^5 IJ/g for *H. riobravis*.

Gaugler; Han (2002) considered *G. mellonella* a host that promotes IJ production over 1.0 × 10^5 IJ/larvae, a figure considered high by the authors. It is noteworthy that the values obtained for strains IBCBn24 and IBCBn40 are in this magnitude and constitute a positive factor, especially if they are used in pest management programs, since the application in field requires the use of a significant number of IJ (Treverrow; Bedding, 1993).

Regarding the rhythm of emergency, both isolates showed a small number of IJ emerging until the second day, including presenting repetitions with no emergency (Fig. 1). The peak in the production of IBCBn40 isolate occurred on the fourth day and for the IBCBn24 it was between the fourth and fifth days. According to findings of Ehlers (2001), there is a positive correlation between emergence time of *H. bacteriophora* and amount of food available. Thus, based on the high number of IJ produced in this experiment and the no immediate emergence of them, probably the host larvae have provided plenty of nutrients, allowing greater permanence of IJ and their evolution into adult forms and consequent rise of new generations.

This finding is reinforced by the absence of sudden interruption in emergence of IJ in larvae, since after the decline of production peak, the emergence continued until the tenth day (Fig. 1).

Compatibility with the insecticide

Product effect on entomopathogenic nematodes: The presence of the insecticide did not significantly affect the viability of IJ, however it caused a reduction of infectivity in 72% by EPNs after is contact with the product (Table 4).

Table 4 – Viability and infectivity of *Heterorhabditis* sp. isolate IBCBn40 on larvae of *Galleria mellonella* in compatibility test with carbofuran insecticide (25 ± 1º C and 14h photophase).

Treatment	Viability[1]	Infectivity[2]
IBCBn40	98.40 ± 0.68 a	79.20 ± 3.36 a
IBCBn40 + Carbofuran	88.50 ± 6.95 a	7.20 ± 3.03 b
C.V. (%)	9.66	34.53

Means (SE) followed by same letter in same column do not differ between each other by the Tukey test (P ≤ 0.05).
[1]viability after 48 h in aqueous suspension.
[2]infectivity for more 8 days in larvae of *Galleria mellonella*.

Andaló et al. (2004) also found that the insecticide did not affect the viability and they observed 50% of reduction in infectivity, but only 7 days after application. Negrisoli Junior et al. (2008b) had similar results testing the effect of carbofuran on *H. bacteriophora*.

Although, Gonzales (2010) observed that carbofuran affected 100% the viability of the IJ, at the concentration of 50% and 100% of the recommended dose by the manufacturer, making the performing of the infectivity tests unviable.

It is noteworthy that the observations are usually conflicting, since Rovesti; Deseo (1991) considered carbofuran prejudicial to the viability but not to the infectivity of *H. heliothidis*.

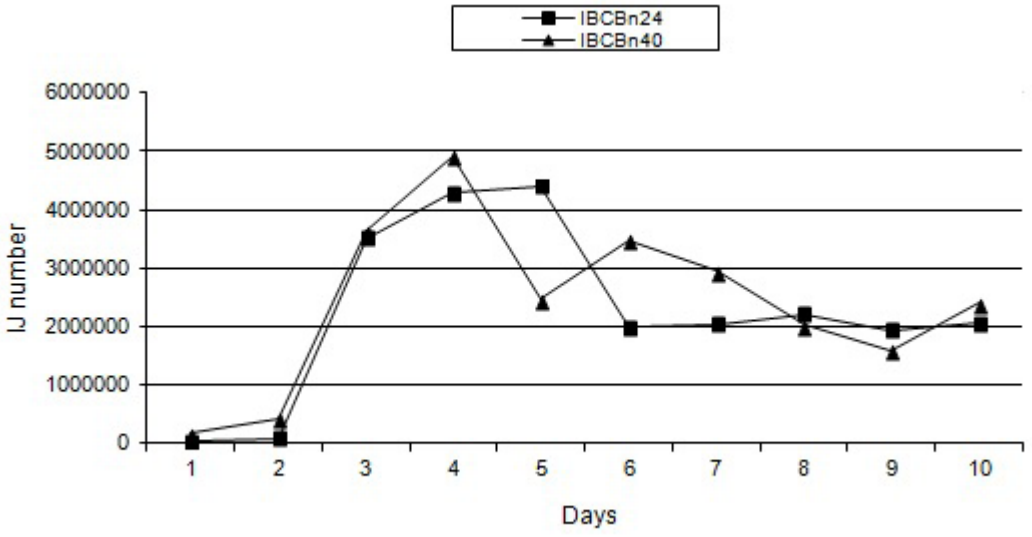

Fig. 1 - IJs daily emergency of *Heterorhabditis* sp. IBCBn24 and IBCBn40 isolates (10 days, 25 ± 1º C and 14h photophase).

Divergences are also found when the EPNs in question are from *Steinernema* genus (Gordon et al., 1996; Andaló et al., 2004; Negrisoli Júnior. et al., 2008b). Study carried out by Hara; Kaya (1983) showed that carbofuran caused alterations in the movements of *S. carpocapsae* and, consequently, in its virulence. Changes in the standard of movements of JIs from this species when exposed to other insecticides have also been reported (Ishibashi; Taki, 1993).

Assessing the information presented, it is observed that the interaction between EPNs and carbofuran does not follow a pattern. In the case of IBCBn40 strain, possibly this isolate was more sensitive to the inhibitory effect of carbamate to the acetylcholinesterase so that the mechanisms involved in the process of foraging and entering the host have been affected.

Similar data were found in other compatibility papers (Rovesti; Deseo, 1991; Ishibashi; Takii, 1993; Laznik et al., 2012), in which, according to the authors, the fact that there had been a meaningful effect on the infectivity and not on the viability it is because the product affects only the characteristics related to the behavior of the nematode, leading to the movement inhibition, dispersion and attraction by the host or even the capacity of reproduction and development, in such a way that although the nematode is alive, it is not able to cause mortality.

Effect on symbiotic bacteria: it was observed the absence of inhibition halo of bacterial growth around disks treated with carbofuran as well as in the case of disks previously emerged in distilled water, contrasting with the antibiotic treated disk in which led to the halo formation with 18.3 mm average diameter (Table 5).

Table 5 – In vitro growth of symbiont bacteria of *Heterorhabditis* sp. isolate IBCBn40 in Mueller-Hinton agar (35º C for 24h in total darkness).

Treatment	Halo (mm)
Bacteria + sterile distilled water	0.0 ± 0.00 b
Bacteria + neomycin	18.3 ± 0.16 a
Bacteria + carbofuran	0 ± 0.00 b
C.V. (%)	2.73

Means followed by same letter in same column do not differ between each other by the Tukey test (P ≤ 0.05).

There are many studies showing the capacity of some bacteria from different families (Gram positives or Gram negative) to degrade and metabolize carbofuran, growing on culture media with carbofuran, as *Arthrobacter* sp., *Pseudomonas* sp., *Sphingomonas* sp. and *Flavobacterium* (Chaudhry; Ali, 1988; Ramanand et al., 1988; Kim et al., 2004; Bano; Musarrat, 2004). But, no one study showed the ability of *P. luminescens* ability to degrade this product. So, we can say that *P. luminescens* grow on carbofuran metabolizing it or not causing injury on bacteria

Once the symbiotic bacteria of nematodes are within the host, they multiply and produce a series of proteins, enzymes, toxins and antibiotics (Poinar Junior, 1990). Thus, the lack of effect on the bacteria shows compatibility between the product and the bacteria opposing to the infectivity reduction. Consequently, the lack of effect on the viability of nematodes as well as the development of the symbiotic bacteria isolated from IBCBn40, it is likely that the infectivity reduction is due to some sublethal effect of the product on nematodes, indicating that the joint applications of this isolate and carbofuran should be avoided.

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Received on 21/9/11
Accepted on 12/4/12