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ORIGINAL PAPER

Suceptibility of *Delia platura* to seven entomopathogenic nematode isolates from the Central Andes region of Colombia

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Abstract

The seed maggot, *Delia platura*, is a major pest of spinach crops in the savanna of Bogotá. In Colombia, chemical insecticides are used to manage the pest; however, because its management is not integrated, information about pest management in spinach is still undetermined. Here, we evaluated the susceptibility of *D. platura* to seven species of entomopathogenic nematodes from the central Andean region of Colombia. Additionally, under laboratory conditions, we produced and evaluated different doses of infective juveniles (IJs) of the most virulent species. In the laboratory, we used yellow potatoes (*Solanum phureja*) for breeding to obtain third instar larvae; we then exposed them to infective IJs 2500/species. Once we selected the most virulent species, we exposed *D. platura* to 500, 1000, 2000, 4000 and 8000 IJs/larvae. We obtained the best results with *Steinernema* sp.3 with mortality of 75-88% at doses of 4000-8000 IJs, and found that DL_{50} is 1314 JIs/larvae and DL_{95} is 15259 JIs/larva. We also evidenced the successful reproduction of *Steinernema* sp.3 in *D. platura*, with a mean production of 670±7.67 JIs/larvae for eighteen days. Thus, the seed fly is highly susceptible to *Steinernema* sp.3 making this species a potential controlling agent for this pest.

Keywords: *D. platura*, seed fly, biological control, Steinernematidae, Heterorhabditidae, Diptera.

Introduction

The seed fly *Delia platura*, Meigen (Diptera: Anthomyiidae), is a cosmopolitan pest that affects more than 40 species of plants such as spinach (*Spinacia oleracea L.*), artichoke (*Cynara scolymus L*), beet (*Beta vulgaris L*) and wild cabbage (Brassica oleracea L.) among others (Darvas and Szappanos 2003, Valenciano et al. 2004). *D. platura*, is widespread; it is a major pest in Europe, North and South America, and very common in northern Africa, Japan, India, Australia and New Zealand (Valenciano et al. 2004). This dipteran attacks during germination reducing emergence

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and causing economic losses affecting up to 30% of production (Valencia et al. 2004). The life cycle of the fly is synchronized with the sowing and harvesting stages. During sowing, the females, attracted to the organic matter, oviposit approximately 100 eggs in the soil near the stems (Capinera 2001, Gouinguene and Stadler 2006); then, during germination, the eggs hatch and the first instar larvae feed on seedlings. As the larvae erode the roots and stems, the plants rot, turn yellow and die (See et al. 1975). Larvae feed on plant bulbs and shoots during the harvest, causing great economic losses to producers (Valenciano et al. 2004, Morelock & Correll 2008).

In Colombia, the seed maggot mainly affects spinach crops; it reduces annual production between 5-30%, which represents a loss of between 85.5 and 513 ton/ha (Gil et al. 2007), and is normally controlled using broad-spectrum organophosphate insecticides such as Monitor, methamidophos 600 (not specific to *D. platura*). However, the inadequate use of pesticides and eventual resistance developed to them by the pest, their high cost (3% of production), and high toxicity and a lack of acquaintance with the natural enemies present in the Cota, Cundinamarca area have led to the exploration of new management plans. Among these plans is biological control using entomopathogenic nematodes given their seeking and specificity capabilities, and their wide host range and reproduction in the field (Valenciano et al. 2004, Gil et al. 2007, Delgado and Saenz 2012).

Nematodes *Steinernema* spp. Travassos (Rhabditida: Steinernematidae) and *Heterorhabditis* spp. Poinar (Rhabditida: Heterorhabditidae) are obligate pathogens of insects associated with Enterobacteriaceae bacteria of the genus *Xenorhabdus* Thomas and Poinar, and *Photorhabdus* Thomas and Poinar, respectively. Once the nematodes have entered the host's (mouth, anus, spiracles, cuticle), the bacterium spreads producing toxic substances, which cause septicemia and the subsequent death of the insect (Nielsen 2003, Sáenz 2005 and Estrada 2008). Nematodes develop within the cadaver feeding on the tissues metabolized by the bacteria. If the infection is successful, the body of the insect can support the propagation of new infective juveniles in amounts proportional to the size of the host (Sáenz 2005, Stuart et al. 2006 and Estrada 2008).

Recently, the use of entomopathogenic nematodes (EPNs) has been considered a potential alternative to control *Delia* species (Chen et al. 2003a, Chen and Moens 2003 and Nielsen 2003). Laboratory tests have demonstrated that different doses of *Steinernema* spp. and *Heterorhabditis* spp. are efficient between 40-60% in the control of *Delia radicum* (Linnaeus) (sister species of *D. platura*). However, the results are inconsistent in terms of the larval stage, nematode species, strain, dose and experimental conditions (Bracken 1990, Royer et al. 1996 and Willmott et al. 2002). Both the state of development of the host and the nematode species are factors that significantly influence the mortality of *Delia* spp. (Willmott et al. 2002, Nielsen 2003).

Research to find new controls in a laboratory setting is a standard procedure, essential to test different nematode species on a given host in order to maximize their impact in the field. In Colombia, no research has been conducted using EPNs to control the seed maggot; the most akin are studies conducted by Gil et al. (2007) to establish the different pests affecting spinach. In consideration of this, this laboratory study evaluates the susceptibility of third instar larvae of *D. platura* to seven species of nematodes from the Central Andean region of Colombia, six doses of EPNs and the production of infective juveniles of the most virulent type.

Materials and methods

***Delia platura* Breeding:** *Delia platura* adults were captured in Cota Cundinamarca, at an altitude of 2,547 m, and an average temperature of 13.7°C, in a vegetable producing farm named Alcala. We placed sixteen 1500 ml plastic bottles, on the soil, near the plant for 24 hours, and filled each bottle with 2ml of rice masato (fermented beverage) homogeneously coating the bottle walls (**Figure 1a**). Once captured, we placed 10 *Delia platura* adults, and 25g of yellow potato (*Solanum phureja*) supported on three 1cm x 1cm styrofoam squares

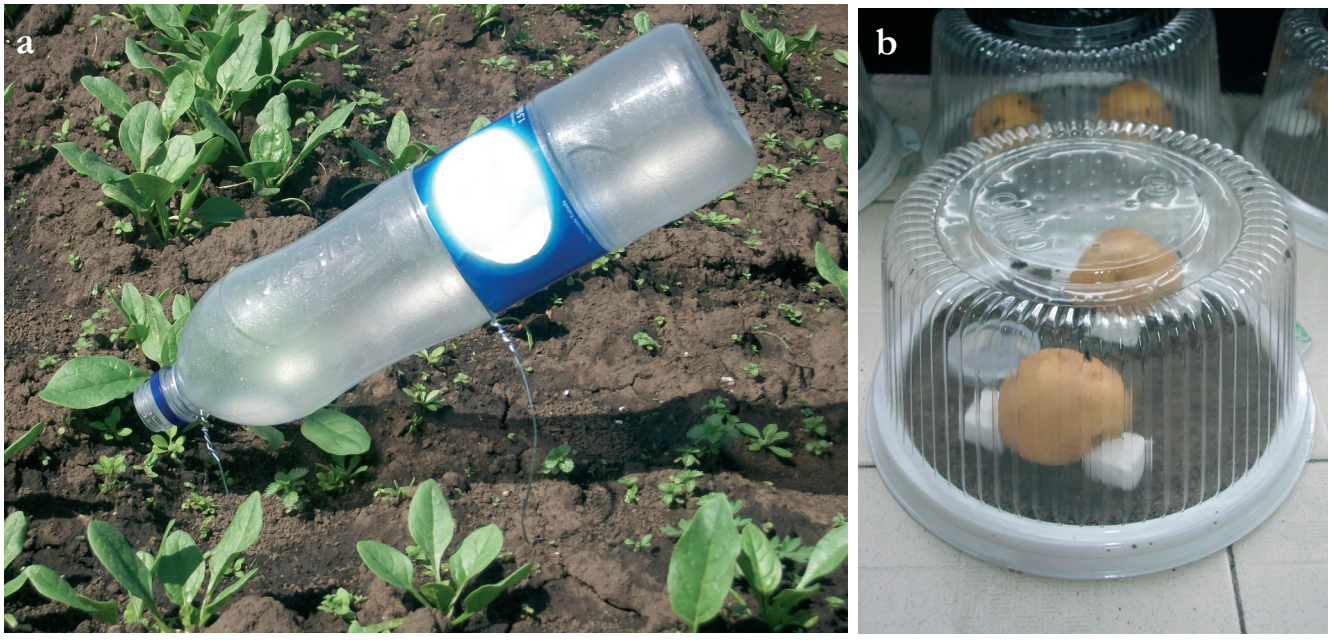

Fig. 1. *Delia platura* collection and seedstock. a. Trap for catching adults. b. Brood chambers.

and wet sand in sixteen 30x30x30 cm plastic breeding boxes. Additionally, and according to the methodology described by Jimenez et al. (2010) we also placed 1 ml of rice masato as a food source (**Figure 1b**). We stored the 16 breeding boxes for 10 days in the biological control laboratory of the Pontificia Universidad Javeriana under controlled conditions of 70% relative humidity, 12 hours light and 12 hours of darkness and average temperature of 14°C until 7mm (2mg) third instar larvae were obtained.

Obtaining entomopathogenic nematodes: We used seven isolates of nematodes from the Colombian Andean region (**Table 1**); six of these were obtained, through Convention No. 182 of

Table 1. Colombian soil nematode isolates evaluated on *Delia platura*. * Species isolated at the Pontificia Universidad Javeriana.

Species	Station	Origin	Strain
Steinernema websteri	Naranjal	Naranjal/Chinchiná/Caldas	JCL006
Steinernema sp.1	Paraguaycito	Buenavista/Quindío	JCL024
Steinernema sp.2	Naranjal	Chinchiná/Caldas	JCL007
Steinernema sp.3	Santa Bárbara	Sasaima/Cundinamarca	JCL027
Steinernema colombiense	Maracay	Quimbaya/Quindío	SNI0198
Heterorhabditis bacteriophora		Fresno/Tolima	HNI0100
Heterorhabditis sp.*		Alcalá/Valle del cauca	SL0708

2009, by the Pontificia Universidad Javeriana and Cenicafé (National Coffee Research Center). According to Lopez et al. (2007), the morphological characteristics of the strains are related to the species *Steinernema* sp.1 in the *feltiae* group, *Steinernema* sp.2 in the *bicornutum* group, and *Steinernema* sp.3 in the *carpocapsae* group. The *Heterorhabditis* sp. SL0708 strain was obtained from the Pontificia Universidad Javeriana. All the species were in 1'000.000 IJ foams, stored at 5-8°C. We used one day harvested, fresh nematodes for all the tests in this study.

Susceptibility of *D. platura* to entomopathogenic nematodes: The isolations in Table 1 were used in the bioassays as well as a control using water. For the experiment we used 120-14 cm^3 plastic cups containing filter paper moistened with 5µl of distilled water, 2500 IJs/larvae, 5g of yellow potato as food and a third instar larvae, in order to simulate field conditions, a total of 15 replicates per treatment, arranged in a completely randomized design. The cups were stored under controlled conditions at a temperature of 20±2°C, and 70% relative humidity. Following the methodology by Kaya and Stock (1997), Willmott et al. (2002), and Chen and Moens (2003), we evaluated mortality percent of the larvae in each treatment every 24 hours. The test was repeated three times.

Production of infective juveniles (IJs) in *D. platura* larvae: Because its mortality percentages were above 70%, we evaluated the production of IJs for the *Steinernema* sp.3 isolate. As recommended by Kaya and Stock (1997), 20 *D. platura* third instar larvae were infected with 2000 JIs/larvae, and incubated at 25°C. Upon completion of the eight-day nematode life cycle, the cadavers were transferred to modified white traps to recover IJs. The cadavers were counted directly using 10 aliquots per sample every 24 hours, until the depletion of the cadavers, following recommendations by Delgado and Sáenz (2012).

***Steinernema* sp.3 dose:** During the bioassay, to determine the recorded dosis presenting the highest mortality we used *Steinernema* sp.3 to evaluate the same doses used for *D. radicum* (Chen et al. 2003), that is, 0, 500, 1000, 2000, 4000, 8000 IJs/larvae. We used 14 cm^3 plastic cups containing filter paper moistened with 5µl of distilled water, 1µl nematode dose, 5g of yellow potato as food and a third instar larvae, for a total of 20 replicates and 5 repetitions per treatment. We stored the experimental units under controlled conditions at a temperature of 20±2°C, and a 70% relative humidity. The larval mortality for each treatment was evaluated every 24. Similary, we evaluated the LD_{50} and LD_{95} the test was repeated three times.

Statistical Analysis: Mortality was converted to a percentage and presented as an average ± the standard error: Unprocessed data met normality (Shapiro-Wilk and Kolmogorov-Smirnov), homogeneity (Levene) and independence (independence test) assumptions, they were therefore analyzed using a one-way ANOVA. To identify which species had the highest mortality and the most effective dose for control of *D. platura*, we analyzed the tests showing significant differences between treatments retrospectively using a Tukey test (parametric) at a significance level of $p \leq 0.05$, the differences are indicated in the figures with different letters on the bars. We calculated LD_{50}-LD_{95} values using the Probit analysis. The tests were performed using SPSS 19 software.

Results

***D. platura* susceptibility to entomopathogenic nematodes:** Third instar larvae were susceptible to *Steinernema* spp. and *Heterorhabditis* spp. with significant differences between treatments ($F_{(6,14)}$= 133.238, $p < 0.05$, **Figure 2**). *D. platura* larvae have increased susceptibility to *Steinernema* sp.3 (80±3%), *Steinernema* sp.2 (64±3%) and *Steinernema* sp.1 (57±3%) with significant differences ($p \leq 0.05$). Mortality does not exceed 50% in *H. bacteriophora* (47±3%), *Heterorhabditis* sp. SL0708 (37±3%), *S. websteri* (20±3%) and *S. colombiense* (17±3%) isolates. The highest mortality (≥50%) for all the isolates occurred from 48 to 72h post infection.

Production of IJs in *D. platura* third instar larvae: IJ production in larvae of *D. platura* was evaluated using *Steinernema* sp.3; this isolate presented the highest mortality (>70%). The production of IJs occurred from the third and

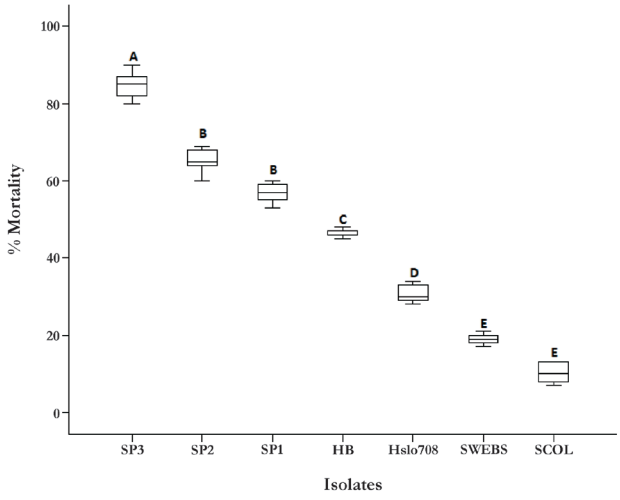

Fig 2. *D. platura* mortality percentage in seven isolates of entomopathogenic nematodes: *Steinernema* SP.3 = sp3. SP.2 = *Steinernema* sp.2. SP.1 = *Steinernema* sp.1. HB = H. *bacteriophora*. Hslo708 = *Heterorhabditis* sp. SL0708. SWEBS = S. *websteri*. SCOL = S. *colombiense*. The boxes represent the quartiles in which data is located. The horizontal bar indicates the data median. The vertical bar SE. The letters on the top of the bars express the differences at p <0.05 (Tukey).

fourth day, reaching 100% of the production from the ninth to the eleventh day (**Figure 3**). The average production of IJs of *Steinernema* sp.3 in *D. platura* third instar larvae was 670±7.67.

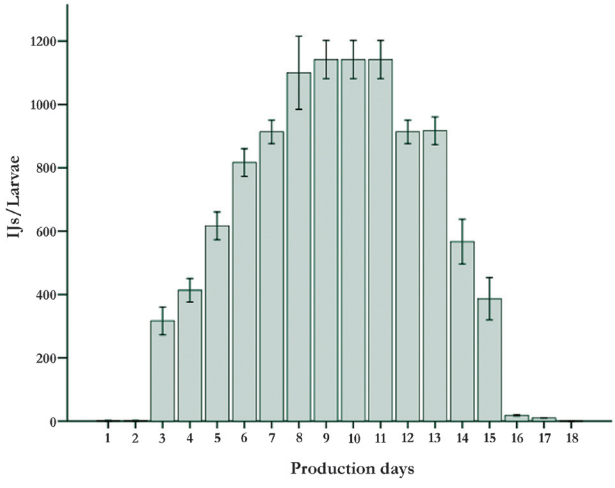

Fig 3. Production time (days) of IJs of *Steinernema* sp.3 in third instar larvae of *Delia platura*. The bar indicates the mean with its SE.

The maximum number of IJs possible in the larvaes was 1.125±10,550 and the minimum was 300±7.67 IJs/larvae, and only one generation of EPNs inside them.

***Steinernema* sp.3 dose:** *D. platura* third instar larvae were susceptible to the five doses applied, presenting a significant difference with the control ($F_{(5,24)}$=649.067, p <0.05, **Figure 4**). A higher mortality appeared (>60%) in doses from 4000 to 8000 JIs/larvae, presenting significant differences (p≤0.05), and gradually decreased (≤50%) using doses of 2000 JIs/larvae. For the seed fly, the LD_{50} is 1,314 IJs/larvae and the LD_{95} is 15, 259 IJs/larvae.

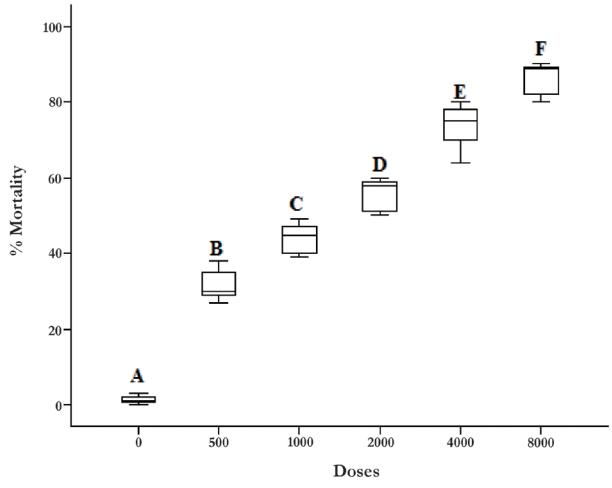

Fig 4. Percentage of mortality of third instar larvae of *D. platura* with six doses (IJs/larva) of *Steinernema* sp.3. The boxes represent the quartiles in which data is located. The horizontal bar indicates data median. The vertical bar SE. The letters on the top of the bars express the differences at p <0.05 (Tukey).

Discussion

There have been no previous studies in which these Colombian isolates have been tested on *D. platura*. We found that the seed fly was susceptible to the *Steinernema* and the *Heterorhabditis* isolates; four of the seven (*Steinernema* sp.1, *Steinernema* sp.2, *Steinernema* sp.3, *Heterorhabditis bacteriophora*) species produced 50-80% larval mortality with respect to the control; *Steinernema* sp.3 being the most virulent (80%) for

D. platura. Willmott et al. (2002) and Chen & Moes (2003) evaluated various species of larvae EPNs in *D. radicum*; their results showed that *Steinernema feltiae* (Filipjey) is more virulent (45%) than H. *bacteriophora* Poinar (0-16%), unlike the results from this study in which species of *Heterorhabditis* spp. only caused 40% mortality. This difference in virulence of EPN species may be explained by pathogenicity and the EPNs' ability to penetrate and avoid or to suppress the host's immune response (Lewis et al. 2006, Li et al. 2007).

The pathogenicity of nematodes is a complex state of attraction, penetration and multiplication (Lewis et al. 1992, Chen and Moes 2003). The IJs migrate to the host responding to stimuli produced by the host, such as CO_2, temperature, contact, cuticle, feces, these are in turn associated with nematode foraging strategies: ambushing, cruising. (Griffin et al. 2005; Lewis et al. 2006). Ambushers, such as *Steinernema* sp.3 are able to position themselves vertically erect, allowing them to affix to a substrate using the lowered surface tension (Lewis et al. 2006). In this position, IJs can actively scan the environment for volatile chemical signs such as CO_2 emitted by hosts; this facilitates finding mobile hosts (Chen et al. 2003b). In contrast, cruising foragers like *H. bacteriophora* and *S. colombiense* are able to identify signals associated with the cuticle and stool, allowing them to find sedentary (immobile) hosts, which explains their low mortality (≤50%) in *D. platura* larvae (Lewis et al., 2006, Delgado and Sáenz 2012). Like most dipterans, *D. platura* larvae continuously move within the substrate; therefore, the most effective way to colonize third instar seed fly larvae is using EPNs with an ambusher foraging strategy (Royer et al. 1996).

The number of IJs that can be produced within a host depends on the amount of IJs applied and the size of the host. Nielsen & Holger (2004) estimate the average production of IJs in a 1-2mg larvae in *D. radicum* is of 518IJs; these values are similar to those obtained in this study, the average production of IJs in *D. platura* larvae was 679 IJs/larvae for *Steinernema* sp.3. That is, third instar larvae can sustain the propagation of EPNs because of their large size and length (Nielsen 2003). Moreover, an increased production of IJs in *D. platura* larvae occurred from day 6 to day 12, otherwise to reports regarding *G. mellonella* Linnaeus larvae in which most of the production took place during the first three days and gradually decreasing over time, creating one and two short EPN cycles (Realpe et al. 2007). It can be inferred that only one long EPN cycle be can take place within *D. platura* larvae.

Susceptibility studies in populations of *Delia* spp, show a positive relationship between dose and larvae mortality, the higher the dose the higher the mortality (Willmott et al. 2002, Chen et al. 2003 a, Nielsen 2003). In this study, 4000-8000 IJs/larvae of *Steinernema* sp.3 produced the highest mortality (≥60%). We found that the medium lethal dose for *D. platura* larvae was 1,314 IJs/larvae and 1.4414 IJs/larvae was the maximum lethal dose. Chen & Moens (2003) reported the LD_{50} for larvae of *D. radicum* is 258 IJs/larvae and LD_{95} for *Steinernema arenarium* is 15259 IJs/larvae (Aetyukhovsky); this establishes that IJs doses may vary according to the species of EPNs used. The differences in the doses may be explained by the foraging strategy, recognition and penetration ability of the IJs in the host.

EPN species have different recognition capabilities, low or high, which affect their ability to penetrate a host. Chen et al. (2003b) found that *Steinernema carpocapsae* Weiser has weak recognition capabilities, inconsistent with its ambusher strategy, which prolong its penetration into the host, requiring of more individuals to generate the same mortality. Similarly, common to dipterans, *D. platura* has small, protected natural openings, which hinder the entry of IJs in larvae, affecting the IJs penetration ability (Capinera 2001). Therefore, the dose required of *Steinernema* sp.3 is 8000 IJs/larvae to generate 80% *D. platura* larval mortality.

Conclusion

D. platura larvae showed greater susceptibility to *Steinernema* sp.3 isolates using a dose of 8x103 IJs/larvae. These isolates also produced the highest number of IJs in third instar larvae of the seed fly. Accordingly, in the second phase of the study,

this dose will be evaluated in the field to determine its performance in the control of *D. platura* and its possible incorporation into integrated pest management programs in spinach crops in Cota, Cundinamarca.

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Conflicts of interest

The authors state that there are no interests other than scientific interests associated with the results of this research.

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Susceptibilidad de *Delia platura* a siete aislamientos de nematodos entomopatógenos de la zona Central Andina de Colombia

Resumen. La mosca de la semilla, *Delia platura*, es una de las principales plagas en los cultivos de espinaca en la sabana de Bogotá. En Colombia la plaga es controlada mediante la aplicación de insecticidas. Sin embargo, la información sobre el control específico en cultivos de espinaca es desconocida; dado que no se realiza manejo integrado. Con el fin de buscar alternativas para control de plagas en espinaca, se evaluó: 1. La susceptibilidad de *D. platura* a siete especies de nematodos entomopatógenos de la zona Central Andina de Colombia y 2. Diferentes dosis y producción de juveniles infectivos (JIs) de la especie más virulenta, bajo condiciones de laboratorio. Para obtener larvas de tercer instar en el laboratorio, se estableció la cría en papa amarilla (*Solanum phureja*), y se expusieron a 2500 JIs/especie. Seleccionada la especie más virulenta, se expuso *D. platura* a 500, 1000, 2000, 4000 y 8000 JIs/larva. Los resultados con mortalidad entre el 75 a 88% se encontraron con *Steinernema* sp.3 en las dosis de 4000 y 8000 JIs, encontrando que la DL_{50} es de 1314JIs/larva y 15259 JIs/larva para la DL_{95}. Así mismo, se estableció que *Steinernema* sp.3 se reproduce exitosamente en *D. platura*, encontrando un promedio de producción 670±7.67 JIs/larva durante un periodo de dieciocho días. La mosca de la semilla es altamente susceptible a *Steinernema* sp.3, el cual puede ser un agente potencial para el control de esta plaga.

Palabras clave: *D. platura*, control biológico, entomopatógenos, Steinernematidae, Heterorhabditidae, Diptera.

Suscetibilidade de *Delia platura* a sete nematoides entomopatogênicos isolados da zona Central Andina de Colômbia

Resumo. A mosca das sementes, *Delia platura*, é uma das principais pragas das culturas de espinafre na savana de Bogotá. Na Colômbia, a praga é controlada através da aplicação de inseticidas mas a informação sobre o controle específico é desconhecida. Assim, o objetivo deste estudo foi avaliar a suscetibilidade de *D. platura* a sete espécies de nematoides entomopatogênicos na Zona Central andina da Colômbia, além de avaliar as diferentes doses e produção dos juvenis infectantes (JIs) das espécies mais virulentas, em condições de laboratório. No laboratório foi estabelecida a reprodução do inseto em batata amarela (*Solanum phureja*) para larvas de terceiro instar, que foram expostos a 2500 JIs espécies. Foi selecionada a espécie mais virulenta e exposta a *D. platura* a 500, 1000, 2000, 4000 e 8000 JIs/larva. Os melhores resultados foram encontrados com *Steinernema* sp.3 com uma mortalidade de 75-88% nas doses de 4000 e 8000 JIs, encontrando que a DL_{50} é 1314 JIs /larva e a DL_{95} é 15259 JIs/larva. Além disso, foi estabelecido que *Steinernema* sp.3 reproduz-se com sucesso em *D. platura*, encontrando uma média de 670±7,67 de produção de JIs/larva por um período de 18 dias. A mosca da semente é altamente susceptível a *Steinernema* sp.3, e pode ser um potencial agente para o controle desta praga.

Palavras-Chave: *D. platura*, mosca da semente, controle biológico, Steinernematidae, Heterorhabditidae, Díptera.