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Entomopathogenic nematodes (Rhabditida: Heterorhabditidae and Steinernematidae) screening for lesser mealworm *Alphitobius diaperinus* (Coleoptera: Tenebrionidae) control

Detección de nemátodos entomopatógenos (Rhabditida: Heterorhabditidae y Steinernematidae) para el control del escarabajo menor de la harina *Alphitobius diaperinus* (Coleoptera: Tenebrionidae)

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Abstract: *Alphitobius diaperinus* (Coleoptera: Tenebrionidae), an important poultry insect pest, is difficult because of the insect's behavior and biology and the restrictions for the used of synthetic insecticides. So, entomopathogenic nematodes are an alternative for *A. diaperinus* control. This study aimed to screen in laboratory entomopathogenic nematodes for *A. diaperinus* control. Fourteen strains were tested, and for the best LC_{50} and LC_{90} was estimated, as well as the control efficiency on adult insects at different temperatures (18, 22, 26, 30 and 35ºC). The poultry litter and sanitary products effect on nematodes were also evaluated. All strains were pathogenic with mortality values varying from 1 to 99%. *Steinernema arenarium* was the most effective causing 99% mortality. The estimated LC_{90} was 106 infective juveniles (IJ) per insect (approximately 25 IJ/cm^2). No nematodes activity was observed at temperatures above 30°C. The antagonistic action of poultry litter inhibit completely the effect on adult insects and a reduction on larvae control by 57%.

Key words: Biological control. Animal husbandry. Poultry farming. Isolate selection.

Resumen: *Alphitobius diaperinus* (Coleoptera: Tenebrionidae), es una importante plaga de aviarios de difícil control en función de sus hábitos y de la dificultad del uso de insecticidas sintéticos. Sin embargo, los nemátodos entomopatógenos tienen potencial como una alternativa para el control de éste insecto. El objetivo de este trabajo fue determinar el aislado de nemátodo entomopatógeno más eficiente para su utilización en el control de adultos de *A. diaperinus*. Fueron evaluados 14 aislamientos, y para aquel que presentó mejor virulencia fueran estimadas la DL_{50} y DL_{90}, como la eficiencia del nemátodo en diferentes temperaturas (18, 22, 26, 30 y 35ºC), sobre adultos del insecto. Se evaluó también para esto el efecto de la cama de aviário y de productos sanitarios sobre los nemátodos. Los 14 aislamientos evaluados fueron patogénicos con valores de mortalidad que variaron de 1 a 99%. *Steinernema arenarium* fue el más eficiente causando 99% de mortalidad. La LC_{90} estimada fue de 106 JI/insecto (aproximadamente 25JI/cm^2). Se observó que temperaturas superiores a 30ºC no fueron viables para los nemátodos y la cama de aviário presentó efecto antagonista a la acción del mismo, siendo su acción totalmente nula sobre adultos, y reduciendo 57% la eficiencia sobre las larvas.

Palabras clave: Control biológico. Producción animal. Avicultura. Selección de aislados.

Introduction

Alphitobius diaperinus (Panzer, 1797) (Coleoptera: Tenebrionidae), lesser mealworm, is one of the most and extremely harmful pests in poultry houses. It is mentioned as a vector of a role of diseases (Avian leukosis, Gumboro, Marek's, and Newcastle disease, smallpox, Avian influenza, coronavirus, and other bacterial diseases) (Watson *et al.* 2000; Chernaki-Leffer *et al.* 2002; Pinto *et al.* 2005; Vittori *et al.* 2007; Hazeleger *et al.* 2008; Chinivasagam *et al*. 2010; Abreu *et al*. 2011). Besides, the mealworm attracts poultry, they are fed by the birds, resulting in reduced feed intake and consequent weight loss and production decreasing (Matias 1992; Japp 2010). Due to the hardness of the adult elytra, the birds digestive tract may be injured, favoring disease contamination and hampering nutrient uptake (Despins & Axtell 1995).

The cryptic behavior and the environmental conditions, which are usually unfavorable for the establishment of natural enemies, make the control of lesser mealworm difficult. Chemical control is most commonly used, but not always with success, since the alkalinity of the litter (due to the decomposition of uric acid into ammonia) deactivated chemical active ingredients, reducing the residual period and the control efficiency. In addition, the risk of contamination of poultry products and the possibility of emergence of resistant populations oppose a widespread use of this control method (Matias 1992; Steelman 1996; Chernaki Leffer 2004).

However, some poultry management techniques can contribute to reduce the insect population, e.g., litter piling, covering the litter during lot intervals causing insect death by ammonia and CO_2 release; litter change in short time intervals and litter treatment at temperatures below 17ºC may reduce the insect population (Chernaki-Leffer *et al.* 2001).

Regarding biological control, there are evidences of the occurrence of fungi on lesser mealworm adults, both in Brazil and the USA (Steinkraus *et al.* 1991; Alves *et al.* 2004;

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Alves *et al.* 2005a). However, most studies were realized in the laboratory (Geden & Steinkraus 2003; Oliveira 2005; Rohde *et al.* 2006; Chernaki-Leffer 2007).

On the other hand, it has been demonstrated under laboratory conditions that there is a potential for use of entomopathogenic nematodes (EPN), of the genera *Steinernema* spp. and *Heterorhabditis* spp., to the control of *A. diaperinus* (Geden *et al.* 1985; Szałanski *et al* 2004; Alves *et al.* 2005b).

Studies data in the field are scarce, but the few existing studies indicate the possibility of a successful control of lesser mealworm by EPN strains (Geden *et al.* 1987; Rodrigueiro *et al.* 2008).

Entomopathogenic nematodes action on a wide range of insect pests with cryptic habits, as well as the tests already carried out with *A. diaperinus* indicate the potential success of its use in controlling this insect. The purpose of this paper was to select EPN isolates, determine optimal concentrations and the tolerance of the best isolate in environment abiotic factors changes that can influence their performance.

Materials and Methods

Isolate selection. The isolates were obtained from the collection of entomopathogens of the laboratory of insect pathology, Universidade Federal de Lavras (UFLA) and the Instituto Biológico de São Paulo.

Nematodes multiplication was performed *in vivo*, using last-instar larvae of *Galleria mellonella* L., as described by Poinar (1979). After the production, the infective juveniles (IJ) were collected and stored in Erlenmeyer flasks at 16°C in aqueous suspension for at the most one week before use in the experiments.

Fourteen isolates of EPN belonging to the genera *Heterorhabditis* and *Steinernema* (Table 1) were evaluated on *A. diaperinus* adults, using a methodology adapted by Rohde *et al.* (2006) and Alves *et al.* (2005b).

The insects were collected periodically from commercial poultry houses, and maintained at laboratory conditions in plastic containers with perforated cover, along with chicken litter and food, in 26 ± 1ºC, and natural photoperiod. Adults with good mobility were selected for the bioassays.

The isolates were screened in a Petri dish (diameter 9 cm), lined with double sterilized filter paper at the bottom, where the EPN was applied at a concentration of 100 IJ/cm^2, suspended in 2 ml of distilled sterilized water (the control treatment in water only). So, 15 adults were placed on each plate along with a small amount of sterilized chicken feed.

The experiment was performed under controlled conditions (25 ± 1 ºC, RH of 70 ± 10%, in the dark), and evaluated on the 5th day after installation. The dead insects were counted and dissected to confirm death by the nematode.

The experiment was conducted in a completely randomized block design, with four replications. Data were subjected to analysis of variance (ANOVA) and the means test of Scott-Knott ($P < 0.05$), using the software package SISVAR (Ferreira 2003).

Estimation of the LC_{50} and LC_{90}. After isolating the selection, the most virulent isolate *S. arenarium* was selected for subsequent tests and exposed to the test concentration to estimate LC_{50} and LC_{90} by the methodology described above for isolate selection.

The test was conducted with 15 adults in a Petri dish (9 cm diameter), and nine nematodes concentrations were evaluated: 1, 5, 10, 50, 100, 150, 200, 300, and 400 IJ/insect were evaluated. In the plates 2 ml of nematodes suspension in distilled water and to each concentration were applied in four plates. LC_{50} and LC_{90} were determined by the Probit analysis using the Polo-PC program, according to Haddad (1998). Furthermore, the data were submitted to polynomial regression analysis.

Effect of temperature and poultry house litter. The pathogenicity of *S. arenarium* on adult insects was evaluated at five temperatures: 18, 22, 26, 30 and 35ºC, according to the methodology described above for the isolated selection. Each

Table 1. Isolates and origin of the entomopathogenic nematodes used in the isolate selection tests.

Isolates	Origin
Steinernema (= *anomali*) *arenarium**	Voronezh / Russia
Heterorhabditis bacteriophora	New Jersey / USA
Heterorhabditis sp. (JPM4)	Lavras / MG / Brazil
Heterorhabditis sp. (JPM3)	Lavras / MG / Brazil
Heterorhabditis sp. (PI)	Teresina / PI / Brazil
Heterorhabditis sp. (RSC 01)	Benjamim Constant/ AM / Brazil
Heterorhabditis amazonensis	Benjamim Constant/ AM / Brazil
Steinernema carpocapsae (CB 02)	Florida / USA
Heterorhabditis sp. (CB 10)	Santa Fé do Sul / SP / Brazil
Heterorhabditis sp. (CB 13)	Pindorama / SP / Brazil
Heterorhabditis sp. (CB 24)	Piracicaba / SP / Brazil
Heterorhabditis sp. (CB 31)	Naviraí / MS / Brazil
Heterorhabditis sp. (CB 33)	Naviraí / MS / Brazil
Heterorhabditis sp. (CB 40)	Campinas / SP / Brazil

* Nomenclature of the species according to the list proposed by Nguyen (2002), available at [http://nematology.ifas.ufl.edu/nguyen/morph/steinsp].

treatment was repeated four times, in a completely randomized design. The data were subjected to analysis of variance and the mean test by Scott-Knott, using the statistical program SISVAR ($P \leq 5\%$) (Ferreira 2003). Data were also submitted to regression analysis to estimate the optimum temperature range for insect mortality on the 5th day caused by EPN action, to establish a curve of the nematode activity decrease with increasing (or decreasing) temperature.

To evaluate the litter effect, the experiment was performed using plastic containers of 20 × 15 × 15 cm (length × width × height) lined with a layer of hydrophilic cotton (1 cm) and immediately moistened with 30 ml of distilled water. A sheet of filter paper was placed on the bottom and then 200 g of sterile soil. The soil was inoculated with the nematode at a concentration of 100 IJ/cm^2 (the control with distilled, sterilized water only). Then 50 g poultry litter was added and the insects were released (50 insects/pot).

The influence of litter on the entomopathogenic activity of EPN on larvae and adults was evaluated, and a control treatment was conducted for each insect life stage. Each treatment was repeated four times, totaling 16 plots, arranged in a completely randomized design. The pots were covered with voile and maintained under controlled conditions (25 ± 1ºC, 12 h photophase). Every three days, 30 ml of water was added to the containers to keep the humidity. Seven days after application, the number of dead insects was counted and the data were subjected to analysis of variance and the means to the test of Scott-Knott, using the software package SISVAR (Ferreira 2003).

Results and Discussion

Isolates selection. All isolates were pathogenic, with variations in virulence, with mortality levels between 1.0 and 99.0%. *Steinernema arenarium* with 99.0% was the highest virulence and it was chosen to continue in the following experiments due to its high activity (Table 2).

In a study conducted by Alves *et al.* (2005b) the pathogenicity of *S. carpocapsae* and *S. glaseri* for this insect was evaluated by immersion in aqueous suspension containing the nematodes. However, the mortality values did not exceed 48%, emphasizing the importance of evaluating a large number of isolates, as in this study, where in spite of the non-significant mortality of some isolates, others were very efficient, reaching up to 99% adult mortality.

Geden *et al*. (1985) and Szalanski *et al.* (2004) evaluating the pathogenicity of different EPN isolates on *A. diaperinus* adults, also pointed out that the variation in mortality obtained was rather significant, highlighting the importance of selection.

Estimation of the LC_{50} and LC_{90}. The lowest concentration used (1IJ/insect) induced 17% mortality, a value that rose as the concentration was increased. The mean lethal concentration (LC_{50}) was estimated at 10.18 IJ/insect and a high lethal concentration (LC_{90}) at 106.2 IJ/insect, or approximately 25 IJ/cm^2 (Fig. 1). Similarly, in a study aiming to determine LC_{50} and LC_{90} of *Steinernema feltiae* and *Heterorhabditis heliothidis* isolates on *A. diaperinus* adults, Geden *et al.* (1985) obtained values of 42 and 724 IJ/insect, respectively, for LC_{50}, while for LC_{90} the values were 616 IJ/insect for *S. feltiae* and 10,516 IJ/insect for *H. heliothidis*. On the other hand, in this study, in spite of using similar conditions lower concentrations were found, suggesting greater virulence of the isolate evaluated here.

Table 2. Mortality percentage of *Alphitobius diaperinus* adults caused by different nematodes isolates of Steinernematidae and Heterorhabditidae, under laboratory conditions in incubator at 25 ± 1ºC 12 h photoperiod in an concentration 100 JIs/cm^2.

Nematodes	Mortality (%)
Steinernema arenarium	99.0 ± 0.45 A*
Heterorhabditis sp. (CB 40)	40.0 ± 2.73 B
Heterorhabditis bacteriophora	38.0 ± 2.50 B
Heterorhabditis sp. (CB 24)	22.0 ± 1.81 C
Steinernema carpocapsae (CB 02)	19.0 ± 4.49 C
Heterorhabditis sp. (CB 10)	17.0 ± 1.81 C
Heterorhabditis sp. (CB 31)	15.0 ± 1.00 C
Heterorhabditis sp. (CB 13)	15.0 ± 2.04 C
Heterorhabditis sp. (RSC 01)	10.0 ± 2.21 D
Heterorhabditis sp. (JPM 3)	9.0 ± 2.16 D
Heterorhabditis sp. (CB 33)	9.0 ± 1.30 D
Heterorhabditis amazonensis	4.0 ± 1.09 D
Heterorhabditis sp. (JPM 4)	2.0 ± 0.89 D
Heterorhabditis sp. (PI)	1.0 ± 0.45 D
Control	0.0 ± 0.54 D
Variation Coefficient = 46.56%	

* Means followed by the same capital letter do not differ statistically by the Scott-Knott test ($P \leq 0.05$).

In another bioassay with *S. carpocapsae* and *S. feltiae* isolates, to control *A. diaperinus* adults, LC_{50} was estimated between 1.5 and 77 IJ/insect for *S. carpocapsae* isolates, and 4.2 to 62 IJ/insect for *S. feltiae* isolates, indicating great variation between the different tested isolates (Szalanski *et al.* 2004).

Since the values found in this study are similar to data from the studies mentioned, the use of *S. arenarium* may be

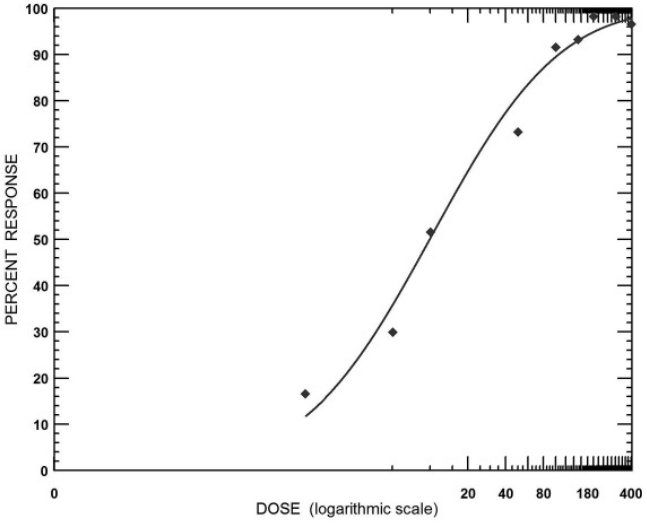

Figure 1. Adult mortality of *Alphitobius diaperinus* subjected to different concentrations of *Steinernema arenarium* (y = 5E-06x3 – 0.004x2 + 1.049x + 1.049x + 18.47).

Table 3. Infectivity of *Steinernema arenarium* on *Alphitobius diaperinus* in Petri dishes in incubator at 25 ± 1ºC 12 h photoperiod.

Concentrations*	0	1	5	10	50	100	150	200	300	400	LC_{50}	CL_{90}
Mortality %	0	17	30	40	73	80	93	100	100	100	10,184	106,201

X^2 Calculated = 2.7409; X^2 Tabled = 11.07; * (infective juveniles / insect).

viable for the control of *A. diaperinus*. It is noteworthy that in the present study assessments were performed with adults only, because in preliminary studies, larvae were always more susceptible than adults, so that the virulence found for adults will be repeated in tests with larvae (Geden *et al.* 1985; Szalanski *et al.* 2004; Alves *et al.* 2005b).

On the other hand, the concentration determined in the laboratory may not be suitable under field conditions where the number of uncontrolled factors is greater and at laboratory conditions, the insect is directly exposed to the pathogen, whereas under field conditions the nematode is exposed to a series of deleterious factors such as temperature and humidity, apart from having to search for the host (Alves *et al.* 2009).

Effect of temperature and poultry house litter. It was observed that at temperatures of 18.22 and 26ºC the percentage of mortality caused by the nematode was 72.71 and 68% respectively, with no significant differences. Besides, the temperatures of 30 and 34ºC interfered significantly with the results, so that there was no mortality in these two treatments (Fig. 2).

Similarly, Geden & Axtell (1988) observed that the efficiency of *S. feltiae* on *A. diaperinus* adults was reduced at higher temperatures and that the effect was accumulative, increasing during the evaluation period and the best results were obtained at 20 and 24ºC (mortality 84 and 100%, respectively) in the first two weeks of evaluation.

The negative effect of high temperature on the EPN action was confirmed in a study of Szczepanik (2000), with *S. feltiae* and *H. bacteriophora* isolates at 30ºC, who observed a significant reduction in the efficiency of both nematodes with maximum values of 5 and 50% mortality, respectively.

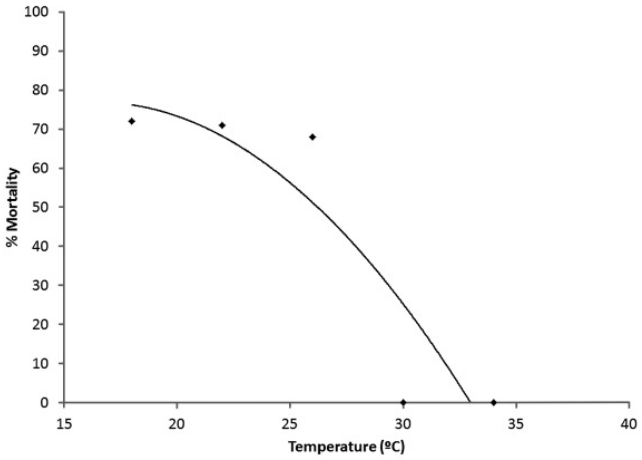

Figure 2. Mortality percentage of *Alphitobius diaperinus* adults induced by *Steinernema arenarium* at different temperatures conditions in incubator at 12 – h photoperiod in an concentration 100 JIs/cm^2 (y = -0.281 x2 + 9.25 x + 0.825).

No effect of poultry litter on adult mortality was observed, with no difference compared to control. For the treatment with larvae, the mortality was 43% (Table 3).

Geden *et al.* (1985) evaluated the effect of litter on the virulence of *S. feltiae* and *H. heliothidis* and observed a considerable increase of LC_{50} under the conditions described above (LC_{50} for adults was 42 IJ/insect in the laboratory and 971 IJ/insect in litter), corroborating the data in this study.

The variation of the physicochemical and microbial litter of poultry over the period of accommodation is quite evident. It is known that ammonia may have fungistatic effect and that it acts by inhibiting the growth of entomopathogenic fungi in this environment (Bacon 1986; Alves *et al.* 2008).

However, the effect of ammonia on EPN is unknown, but it is believed that it can influence the EPN metabolism, interfering with the efficiency of lesser mealworm control. This reinforces the need for integration of the nematode in a control strategy of the insect population, as mentioned above.

Although studies searching alternatives for the control of the lesser mealworm are still at an early stage, it can be said that factors such as environmental conditions and other techniques adopted in the poultry management can interfere with the nematode action. Knowledge about the possible interactions when using the nematode in poultry farming must therefore be acquired.

These results suggest that the use of EPN to control *A. diaperinus* in the field may be unfeasible, since temperatures above 30ºC are quite common, mainly in the summer. Therefore, the use of EPN has to be part of the population of lesser mealworm management. It could for example be applied when exchanging the poultry litter, onto the soil, to control the insects both in the larva and pupa, as well as adult stages. The nematode inoculation could thereby be increased, contributing to the insect control at every new cycle of poultry production.

It is possible to conclude that these agents can potentially control the insect pest, despite the need for field studies to compare poultry management strategies involving EPN.

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