Interação de um inseticida e de desinfetantes utilizados em aviários de corte com o nematoide entomopatogênico *Steinernema arenarium*

Interaction of insecticide and disinfectants used in a poultry house with the nematode entomopathogenic *Steinernema arenarium*

Interacción de uno insecticida y desinfectantes utilizados en un gallinero con el nematodo entomopatógeno *Steinernema arenarium*

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Resumo

Este trabalho teve como objetivo avaliar o efeito de um inseticida a base de cipermetrina e desinfetantes de diferentes grupos químicos, ambos utilizados no manejo de aviários de corte, sobre a viabilidade e a infectividade do nematóide *Steinernema arenarium*, bem como o efeito sobre a bactéria simbionte do nematoide. Para estudar o efeito sobre o nematoide, utilizou-se a metodologia de IOBC/WPRS, proposto por Vainio (1992), onde foi feita a mistura da solução do produto e suspensão de nematoide em água destilada (2000 Juvenis Infectantes (JIs)/mL). A testemunha constou apenas da suspensão do nematoide. Após 48 horas, avaliou-se a viabilidade e a infectividade dos nematoides sobre larvas de *Tenebrio molitor*. Para estudar o efeito sobre a bactéria simbionte, suspensões com 10^8 UFC/mL foram inoculadas em meio de cultura e adicionados três discos de papel (testemunha - água destilada, padrão de comparação - neomicina e um disco tratado com produto). Após 24 horas avaliou-se o

crescimento bacteriano. Todos os produtos reduziram a viabilidade de *S. arenarium*, porém apenas um desinfetante à base de cloro causou redução superior a 55%, e junto com o inseticida cipermil reduziram em 100% a infectividade, sendo considerados prejudiciais ao nematoide com base no E%. Todos os produtos afetaram o crescimento bacteriano.

Palavra-chave: Compatibilidade; Produção animal; Manejo de pragas.

Abstract

This study evaluated the effect of cypermethrin insecticide and disinfectants, both used in the management of poultry houses, on nematode *Steinernema arenarium*, as well as their effect on the bacterium symbiont of nematode. The IOBC/WPRS methodology proposed by Vainio (1992) was used to study the effect on the nematode. The solution of the product was mixed with 1ml of the nematode suspension in distilled water [2000 infective juveniles (IJs)/ml]. As control treatment, only the suspension of the nematode was used. After 48 hours, the viability and the infectivity of the nematode on the larvae of *Tenebrio molitor* were assayed. The effect of products on the symbiont bacterium was also evaluated. Suspension with 10^8 CFU (colony forming units)/mL were inoculated in culture medium and three paper discs added (control treatment – distilled water, comparison standard – neomycin, and a disc treated with each product) and the growth was evaluated after 24 hours. All the products reduced the viability of *S. arenarium*, although only the chlorine-based disinfectant caused reduction above 55%. For the infectivity, the chlorine-based product and the insecticide cipermil reduced in 100% this activity and were considered harmful based on E%. All the products affected the bacterial growing.

Keywords: Biological control agent; Compatibility; Selectivity.

Resumen

Este estudio tenía por objeto evaluar el efecto de un insecticida a base de cipermetrina y desinfectantes de diferentes grupos químicos, ambos utilizados en la gestión de los gallineros, en la viabilidad e infectividad del nematodo *Steinernema arenarium*, así como el efecto en la bacteria simbionte del nematodo. Para estudiar el efecto sobre el nematoide se utilizó la metodología de la IOBC/WPRS propuesta por Vainio (1992), en que la solución del producto se mezclaba con una suspensión de nematoide en agua destilada (2000 Infectious Juveniles (JIs)/mL). El testigo consistía sólo en la suspensión del nematodo en agua. Después de 48 horas, se evaluó la viabilidad e infectividad de los nematoides en las larvas de *Tenebrio molitor*. Para estudiar el efecto sobre la bacteria simbionte, se inocularon suspensiones con

108 UFC/ml en medio de cultivo y se añadieron tres discos de papel (testigo - agua destilada, estándar de comparación - neomicina y un disco tratado con el producto). Después de 24 horas se evaluó el crecimiento bacteriano. Todos los productos redujeron la viabilidad del *S. arenarium*, pero sólo un desinfectante a base de cloro causó una reducción de más del 55%, y junto con el insecticida cypermyl redujo la infectividad en un 100%, considerándose perjudicial para el nematoide a base de E%. Todos los productos afectaron el crecimiento bacteriano.

Palabras clave: Compatibilidad; Producción animal; Gestión de plagas.

1. Introduction

Brazil is currently the second largest producer of chicken meat in the world, and in the year 2019 reached a total production of approximately 13.2 million tons, of which 4.2 tons were destined for export (ABPA, 2020).

However, chicken production still faces many challenges and has not significantly grown in the last three years (*Ibid*, 2020). Among the limiting factors for production are the continuous need for investment in the sector, the adoption of adequate environmental practices, and the implementation of sanitary strategies that guarantee participation in the international market and consumer satisfaction (Raimundo, 2017).

Regarding health strategies, disease and pest control deserve special mention. The lesser mealworm (*Alphitobius diaperinus*) (Panzer) (Coleoptera: Tenebrionidae) is one of the most important pests of modern aviculture. Larvae and adults of this insect colonize the poultry litter and are ingested by the chickens instead of fodder, affecting the development of the poultry (Despins and Axtell, 1995). Moreover, they are potential vectors of bacteria pathogenic to poultry and humans (Hazeleger et al., 2008).

In addition, the uninterrupted presence of birds in the aviary and the insect's habit of sheltering in wall cracks, floor cracks, underneath feeders, drinking fountains, near the support pillars of the constructions, and below ground hinders control and favors re-infestations (Alves et al., 2005). The insects are usually controlled by applications of chemical insecticides on the poultry litter surface and soil (Dias et al., 2013). In addition to the insecticides against the lesser mealworm control, disinfectants for the control of bacteria and other microorganisms are frequently used in poultry sheds.

Despite their relative efficiency, the insecticides cause problems such as intoxication, contamination, and destruction of natural enemies (Japp et al., 2010). Thus, interest is

increasing in alternatives measures not only to control pests but also for the diseases which attack the poultry production.

Thus, several studies have examined the efficiency of alternative methods for control of *A. diaperinus*, such as the diatomaceous earth (Oliveira et al., 2017) and entomopathogenic fungi (Alves et al., 2015) and nematodes (Alves et al., 2005; Alves et al., 2012).

The entomopathogenic nematodes (EPNs) have some advantages over the other control agents due to their ability to search for their host. They are also generally compatible with other products (Kaya and Gaugler, 1993), persist for long periods in the natural environment, and do not affect humans or the other vertebrates (Ferraz, 1998).

Moreover, entomopathogenic nematodes kill their hosts quickly, due to symbiosis with entomopathogenic bacterium of the genus *Xenorhabdus* and *Photorhabdus* which associate with *Steinernema* and *Heterorhabditis*, respectively, and they cause their hosts to die by septicemia (Dowds and Peters, 2002).

Some laboratory studies showed the potential and possibility of using entomopathogenic nematodes to control *A. diaperinus* (Alves et al., 2005; Del Valle et al., 2015). A recent study on the *S. arenarium* obtained 99% of mortality of the lesser mealworm being (Alves et al., 2012), corroborating with previous studies (Rodrigueiro et al., 2008).

Hence, nematodes are a viable alternative to control *A. diaperinus* in poultry. In the near future they may become a control alternative with selected isolates and availability of a formulated product, which are not yet available in Brazil. However, studies under field conditions are still needed to demonstrate the best application and the appropriate conditions to perform them.

No information is available about the effect on entomopathogenic nematode of the insecticides and disinfectants commonly used in the poultry house. This is very important for the future use of nematodes to control *A. diaperinus*. Thus, this study was carried out with this objective.

2. Material and Methods

This study consisted of a laboratory research (Pereira et al., 2018), which was developed together with other experiments aiming at the control of the insect *A. diaperinus*, the lesser mealworm. It is a quantitative study, which sought to support other tests to validate the use of entomathogenic nematodes as an alternative control for this insect.

The *S. arenarium* (isolate SA) originated from Voronezh, Russia, and is part from the

collection of Federal University of Lavras, Lavras, MG, Brazil. This isolate was evaluated in Alves et al. (2012), which obtained 99% mortality of *A. diaperinus* in laboratory conditions, showing potential for its use in biological control programs.

The nematode was multiplied *in vivo* in larvae of *Tenebrio molitor* L. (Coleoptera: Tenebrionidae) from laboratory rearing (Molina & López, 2001).

We evaluated the insecticide Cipermil® (cypermethrin) and four disinfectant recommended for use in poultry house management: AVT-80® (quaternary ammonia), TH4+® (quaternary ammonium and glutaraldehyde), Glutaquat® (glutaraldehyde and benzalkonium chloride), and Aviclor® (sodium dichloroisocyanurate).

Based on IOBC/WPRS protocols, proposed by Vainio (1992), solutions of the products were prepared with double the concentration recommended by the manufacturer. Thus, 1 mL aliquots of each solution was transferred to glass tubes (capacity 30 ml) and 1 ml of suspension of the nematode in distilled water [2000 infective juveniles (IJs)/ml] was added. The control treatment contained only the nematode suspension (2000 IJs/ml) in 2 ml of distilled water. Tubes were kept at 22 ± 1 °C and 14 hr of photoperiod, for 48 hr, then the viability and infectivity assays were performed. The experiment was conducted in completely randomized design, with 5 tubes (repetition) for each treatment and control.

To assess viability, after tubes agitation, a 0.10 ml sample was transferred to an Elisa plate, and the number of IJs alive and dead were counted up to the total of 100 IJs, being considered dead those that did not reacted when touched with a probe.

To evaluate infectivity, 3 ml of distilled water was added to the tubes and left to decantation for 30 min at 10 °C. Supernatant was discharged and the procedure was repeated three times to eliminate the residues of the products from the nematodes. After that, 0.2 ml of the washed suspension of nematodes with about 200 IJs were placed in five Petri dishes (9 cm of diameter) with filter paper lining the bottom with 10 larvae of *T. molitor*. The dishes were kept in 26 ± 1 °C and 14 h of photophase for five days. The dead larvae were transferred to a dry chamber where they were kept for three more days. Dissection of cadavers were done under a stereoscopic microscope to observe the presence of nematodes and to confirm mortality. All experiments were repeated twice.

The data of viability and the infectivity (insect mortality) were submitted to analysis of variance, and data were analyzed by ANOVA (test F) and Turkey's test ($P \leq 0.05$), with Sisvar (Ferreira, 2019).

The values of nematode mortality were corrected by the formula:

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

Where: Mc% = Corrected mortality; Mo% = Observed mortality; and Mt% = Control mortality.

The infectivity reduction caused by the treatments was determined by the formula:

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

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

The Peters & Poullot (2004) modified formula was used to determine the effect of the treatments on entomopathogenic nematodes (E%), and the data of production reduction were not used, because some treatments had not infectivity and production.

E% = 100 – (100 – Mc% - Rinf%)

Based on E% value, insecticides were classified as: 1 – Innocuous (<30%); 2 – slightly harmful (30%–79%); moderately harmful (80%–99%), and 4 – harmful (>99%).

The symbiont bacteria were isolated as in Kaya and Stock (1997). *Galleria mellonella* L. (Lepidoptera: Pyralidae) larvae were infected with *S. arenarium* (isolate SA) as described by Molina and López (2001). After 24 h of the larvae infection, its hemolymph was collected by cutting the abdominal legs. Hemolymph was immediately inoculated on the surface of the culture medium nutrient agar (agar 20 g, meat extract 3 g, peptone 5 g) in Petri dishes. The material was incubated at 25 °C for 24 hr. The colonies obtained were multiplied in the same culture medium in assay tubes to obtain the inoculum which was kept at 10 °C.

The effect of the products was evaluated based on the technique by Ostrosky (2008). From the bacterial inoculums, a bacterial culture was prepared in Brain Heart Hinton (BHI) medium, incubated at 35 °C for a period of 24 hr. After that, the concentration was standardized by adding sterile saline solution (0.9%) until the concentration of 10^8 CFU/mL (colony forming units), based on the scale of 0.5 of MacFarland.

This solution was inoculated with a sterile *swab* with Mueller-Hinton agar culture in a Petri dish. Three paper discs (5 mm diameter) previously immersed in sterilized distilled water (control treatment), antibiotic neomycin 30 UCG (comparison standard), and one of the tested products were placed in the dishes, which were then incubated at 27 ± 1 °C for 24 hr in the dark. For each treatment, five Petri dishes were prepared, each considered a repetition.

The evaluation verified the presence or not of a halo of inhibition at the bacterial growing and its respective measurement, with two measurements perpendicular between them. All experiments were repeated twice.

The experiment was conducted in completely randomized design. The data were analyzed by ANOVA (test F) and Tukey´s test (P≤0.05), with Sisvar (Ferreira, 2019).

3. Results and Discussion

All tested products reduced the nematodes' viability (14 to 55% reduction) with Aviclor the most active against nematodes (55% reduction of nematodes viability) (Tab. 1). Aviclor and Cipermil reduced the infectivity 100%, which is the capacity of the IJs to cause mortality to the *T. molitor* larvae, and were considered harmful to the nematode. The other products have light effect, being classified as slightly harmful to *S. arenarium* (isolate SA) (Tab. 1).

Table 1. Viability and infectivity (± EP) of nematode *Steinernema arenarium* (isolate SA) on the larvae of *Tenebrio molitor,* after 48 hours exposure to an insecticide and disinfectant used in poultry houses and classification of compatibility.

Treatments	Viability (%)[a]	Infectivity (%)	Mc%	Rinf%	E%	IOBC Classification[b]
Control	100.0 ± 0.00 a	100.0 ± 0.00 a	0	0	0	Innocuous
AVT-80	86.0 ± 1.44 b	72.0 ± 18.27 a	14	28	42	Slightly harmful
Cipermil	84.8 ± 2.13 b	0.0 ± 0.00 b	15,2	100	115	Harmful
TH4+	83.2 ± 1.46 b	84.8 ± 7.71 a	16,7	15,2	31	Slightly harmful
Glutaquat	81.4 ± 1.40 b	81.4 ± 3.70 a	18,6	18,6	37	Slightly harmful
Aviclor	44.0 ± 4.98 c	0.0 ± 0.00 b	44	100	144	Harmful
C.V.	27.11	32.68				

[a] Means followed by the same letters in a column do not differ significantly by the Tukey test (p ≤ 0.05). [b] Toxicity classification of insecticides by IOBC: 1– Innocuous (<30%), 2– slightly harmful (30–79%), 3 - moderately harmful (80%–99%), and 4 – harmful (>99%). Mc% = Corrected mortality. Rinf% = Infectivity reduction. E% = Insecticide effect. Source: author.

Cipermil affected both the viability and infectivity of the nematodes. The elevated toxicity of the insecticide was already expected, due to the presence of chlorpyrifos, cypermethrin (pyrethroid), and citronella oil. Chlorpyrifos belongs to the organophosphate

group, which compatibility studies have found to be the most toxic for entomopathogenic nematodes, as its mode of action inhibits the enzyme acetylcholinesterase (Rovesti and Deseo, 1990; Monteiro et al.*,* 2014). Cypermethrin (pyrethroid) has lipophilic action and acts on the sodium nervous channels (Eells and Dubocovich, 1988), and citronella, which repels insects. Probably, the combination of these different active ingredients affected the behavior and infective capacity of the nematodes.

Other studies on the compatibility of chemical products and entomopathogenic nematodes also found that some fungicides and insecticides can affect the nematode's infectivity, although without harming their viability (Ishibashi and Takki, 1993; Bortoluzzi et al.*,* 2013).

According to Ishibashi and Takki (1993), some products can affect the nematodes moving, and these movements can be different from those considered normal for the entomopathogens (sinusoidal movement). Furthermore, Gaugler and Campbell (1991) observed greater locomotor activity of the nematode *S. carpocapsae* after exposition to the product oxamyl, although the authors concluded that the increase of the sinusoidal movement is not necessarily related to the improved searching of nematode for the host.

Besides that, according Andaló et al. (2010), some chemicals reduce the amount of lipids in nematodes, and this can affect the infectivity, because lipids are an important energy source for these organisms, and this factor may had affected the nematode.

The product Aviclor, when mixed with water, due to the presence of the free chloride in its formula, forms hypochlorous, dichloramines, hypochlorite ions, among others, depending on the composition of water. Furthermore, the small size of the molecules and the electric neutrality permits fast penetrability into the cell, allowing the oxidation of the cell components (Meyer, 1994). The mortality of the nematode (66%) when exposed to the product Aviclor can be due to this capacity of the product to cause cell oxidation.

All the products studied reduced the bacterial growth compared to the control treatment (Table 2).

Among the products tested, Aviclor presented the greatest halo of inhibition (2.8 cm), which was on the same level as that observed with antibiotic – comparison standard (2.7 cm), followed by TH4+, which caused the formation of a halo equal to 1.6 cm. For the other products, the inhibition halo varied from 1.1 and 1.3 cm. Jaenisch et al. (2010) also verified that products derived from active chloride caused the greatest inhibition in the growth in three different species of bacteria.

Bortoluzi et al. (2013) evaluated the interaction of an insecticide based on carbofuran

on the growth of the symbiont bacterium and observed no effect.

Table 2. Formation and size of inhibition halo (± EP) *in vitro* of symbiont bacteria of the nematode *Sterneinema arenarium* (isolate SA) with insecticide and disinfectants used in poultry houses.

Treatments	Halo (cm)[a]
Control	0.0 ±0.00 a
Antibiotic	2.7 ± 0.02 d
AVT-80	1.3 ± 0.06 b
Cipermil	1.1 ±0.08 b
TH4+	1.6 ± 0.05 c
Glutaquat	1.1 ± 0.03b
Aviclor	2.8 ± 0.09 d
C.V.	8,7

[a] Means followed by the same letters in a column do not differ significantly by the Tukey test ($p \leq 0.05$). Source: author.

Although the action of the tested products on the bacteria has been proven, it cannot be said that such products have the capacity to penetrate the nematode body via cuticle and affect the bacteria dwelling at the digestive system from nematodes. In addition, studies with disinfectants based on free chloride, hydrogen peroxide, acetic acid, citric acid, and lactic acid aiming to control bacteria (S*almonella* spp. and *Escherichia coli*) found that after their ingestion by the nematode *Caenorhabditis elegans* (Rhabditidae), the products were inefficient and did not affect both bacteria. The disinfectants can still effect the bacteria that stay on the surface of the nematode, without affecting those which are internalized into the digestive system (Bichai et al., 2009).

Thus, the reduction of the infectivity caused by the insecticide could be associated to the effect of the product on the nematode behavior. According to Cuthbertson *et al.* (2007), cypermethrin caused 100% mortality of *S. feltiae*, and is not viable to use in integrated pest management (IPM) strategies. Furthermore, Singh et al. (2012) found that products based on cypermethrin can act on the central nervous system, and cause a motor deficit, what may have caused the capacity of movement and the infection of the juvenile infectant.

However, the elevated toxicity of Aviclor on the nematode can be related to the capacity of the product to cause cell oxidation. The disinfectant could have also changed the

behavior of the nematode, harming its capacity to search and penetrate the juvenile infectant, because the viability was reduced by 66% and the infectivity was null.

4. Final Considerations

In this study, we observed that some products used in poultry house can influence the survival (infectivity and viability) of the nematode, and consequently interfere with control.

However, new studies are necessary to test the survival of the bacteria recovered from treated nematode, as well as the effect of the products classified as incompatible (Aviclor and Cipermil) on the moving and the search capacity of the entomopathogen.

Bear in mind that laboratory tests force the contact between the products and the nematode, and that these conditions are not found in the field, where direct contact between the products and the nematode can be avoided. With proper use of avian management techniques, and planned applications of both products and nematodes, we believe that the use of EPNs is a viable alternative to *A. diaperinus*.

Acknowledgements

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