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Cinnamomum zeylanicum Essential Oil Reduces Infestation by *Alphitobius diaperinus* in Poultry Litter

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ABSTRACT

***Background*:** Even though insecticides are managed and the period of sanitary emptiness in poultry is respected, the elimination of *Alphitobius diaperinus* may not be successful. The use of essential oils of plant origin presents as a good alternative in the substitution of insecticides with synthetic molecules, since they are easy to obtain, with rapid degradation and without risk of residues for non-target organisms. The main objective of the present study was to examine whether *Cinnamomum zeylanicum* oil reduces *Alphitobius diaperinus* (Panzer) (Coleoptera: Tenebrionidae) infestations under experimental conditions, without causing toxicity to broilers chicks exposed to treated litter.

***Materials, Methods & Results*:** The experimental design was completely randomized, with four replications per treatment. The treatments were as follows: solvent control using the diluent Dimethyl Sulfoxide 5% (oil diluent); chemical control using 5 g/m² cypermethrin; one spray of *C. zeylanicum* 5% oil; and two sprays of *C. zeylanicum* 5% oil. Each experimental unit was infested with 150 lesser mealworm adults. At 15 days of the broiler chick's life, blood was collected for biochemical analysis (alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, total protein, albumin, globulin, triglycerides and uric acid), and liver fragments were isolated for histopathological analysis. Using *TupeTrap* devices, we counted lesser mealworm 40 days after treatment. The treatments did not alter biochemical variables, and did not cause histopathological lesions in liver. The treatments with *C. zeylanicum* 5% oil with one and two sprays efficiently reduced lesser mealworm infestation compared with solvent control. Cypermethrin treatment had no effect.

***Discussion*:** Many of the commercial products present low effectiveness in the control of *A. diaperinus*, since the target organisms develop resistance to the product. In the present study, we used a higher cypermethrin dose than that recommended by the manufacturer, in order to increase efficacy in the face of possible resistance. Even so, cypermethrin did not efficiently control the organism. The effectiveness of the essential oil of *C. zeylanicum* tested can be attributed to the compounds found in greater quantity in the oil composition, such as cinnamaldehyde (41.27%), linalool (13.05%) and methyl eugenol (10.87%), characterized as responsible for the action of oil repellency. Monoterpenoid compounds found in essential oils extracted from plants have insecticidal action acting on the central nervous system of insects, which impairs their development, being characterized as neurotoxic compounds. The results found with the essential oil of *C. zeylanicum* are of great importance, since the control of *A. diaperinus* is not efficient because this organism has behavior that favors reinfestation in the poultry houses, such as shelter in cracks, in the draperies, below the feeders and in the soil. The biochemical analyzes of the blood can as important tools to assist in the monitoring of broilers health, in the diagnosis and treatment of diseases, and therefore the results presented are of great importance since they assist in the search for alternative methods for the control of *A. diaperinus*, where we can affirm that the essential oil of *C. zeylanicum* does not cause toxicity to broilers. Based on these results we can affirm that essential oil of *Cinnamomum zeylanicum*, 5%, is an effective substitute for existing commercially-available insecticides.

Keywords: alternative control, cinnamon oil, insecticide, lesser mealworm.

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INTRODUCTION

The lesser mealworm (*Alphitobius diaperinus*) is a major nuisance in poultry farming, disrupting the production chain, causing reductions in broilers weight gain, depreciating the value of facilities, and acting as disease vectors for pathogens such as fungi, viruses and bacteria [5]. Considered a difficult-to-manage pest, lesser mealworms are often treated with pyrethroid insecticides, such as cypermethrin, and organophosphates such as chlorpyrifos [19].

Currently, there is a great interest in the use of plant extracts to control natural pests, as alternatives to commercially-available insecticides. Among them, cinnamon essential oil (*Cinnamomum zeylanicum*) stands out for its biological effects in analgesics, antiseptics, astringents, homeostatics, antibacterials, antifungals, repellents, antiparasitics, and insecticides [2,4,27]. These attributes are due to their chemical constituents, including cinnamaldehyde [24].

Recently, studies have highlighted the efficiency of alternative treatments for pest and parasite control in animal production that have no effects on non-target organisms [14,27]. Based on these results, we carried out the present study to examine whether *C. zeylanicum* oil can reduce infestations by *A. diaperinus* under experimental conditions: that is, whether we could reduce lesser mealworm infestation in broilers chicks and poultry litter without causing toxicity to the animals.

MATERIALS AND METHODS

Cinnamomum zeylanicum *and cypermethrin*

The *Cinnamomum zeylanicum* oil used has a composition as previously described by Volpato *et al*. [27]. Cypermethrin was also purchased commercially in a flask containing 20 g of cypermethrin in 100 mL of the product. It was used as a chemical control.

Poultry litter and Alphitobius diaperinus

The poultry litter used was from an experimental shed, located on the western campus of the State University of Santa Catarina (UDESC), using pine wood (*Pinus elliottii*) as an absorbent substrate for broilers excreta. There were six lots, each used for up of 45 days. After collection, poultry litter was composted with the objective of simulating the type of management carried out on rural properties: the bed was wrapped in layers and covered by black tarpaulin for 7 days.

After this process, the litter was distributed to the boxes with dimensions of 0.035 m³ (experimental unit), simulating the poultry houses environment. One hundred fifty adult specimens of *Alphitobius diaperinus* were distributed in each experimental unit. The *A. diaperinus* were collected in a property located in the western region of Santa Catarina/ Brazil, and in the laboratory. They underwent a 30-day adaptation process under conditions that simulate their actual survival conditions (plastic containers with the same litter used in the experiment and later used in the experimental units).

Treatments

The design was a completely randomized, with four replications per treatment. Litters containing *A. diaperinus* were sprayed, and after a grace period of five days, the chicks were transferred to the litters. The treatments were as follow: A) solvent control (5% dimethyl sulfoxide); B) chemical control, with cypermethrin at 5 g/m² [9]; C) one spray of C. zeylanicum essential oil 5%; and D) two sprays of 5% *C. zeylanicum* essential oil. The 5% dilution was chosen based on previously-reported results by Volpato *et al*. [27]. In treatment D, the second spray occurred on the 15th day after the first spray.

Animals, diet and management

One hundred seventy eggs from Cobb laying hens were incubated, with the expectation of a 70% hatching ratio of males/females. After hatching the broilers were sexed and allocated to the experimental units. In each experimental unit, we allocated three males and two females, totaling 60 broilers chicks per day. The broilers received feed and water *ad libitum*, according to animal welfare regulations, with temperature and light controlled according to the requirements of the lineage. The experimental ration described in Table 1 was calculated to meet the broilers nutritional requirements. The litter was changed twice a day, water was changed whenever the drinking troughs were dirty, or when the temperature exceeded a level desirable for bird consumption. The feeders were filled whenever necessary.

Table 1. Composition of experimental diets provided to chicks.

Ingredient	kg
Corn	62.3856
Soybean meal 45%	32.8279
Dicalcium phosphate	1.7660
Soybean oil	0.8979
Limestone	0.8236
Salt	0.4331
Mineral and vitaminic supplement	0.4000
DL-Methionine	0.2241
L-Lysine·HCl	0.2013
L-Threonine	0.0405

Sample collection

On the 25th day of the experiment, corresponding to day of life 15, all broilers were anesthetized with inhaled isoflurane (in an anesthetic chamber), followed by blood collection for biochemical analysis. Blood was placed in tubes without anticoagulant and centrifuged at 5,600 *g* for 10 min. The serum was collected, stored in microtubes and frozen (-20ºC) until analysis. Broilers chicks were then sacrificed by cervical dislocation. Liver tissue samples were collected (left and right lobes) for histopathological analysis.

Fifteen days after broilers removal (corresponding to the 40th day after starting the experiment), two *TupeTrap* devices were installed in each experimental unit to verify the effectiveness of the treatments in reducing *A. diaperinus* infestation. The trap was adapted from Safrit & Axtell [20], using a PVC pipe (3.8 cm in diameter and 12 cm in length) with cardboard twisted inside the barrel. The traps were left for 15 days, after which time they were removed and placed in labeled plastic bags. They were frozen in order to kill the collected organisms and to facilitate counting.

Serum biochemistry

Alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (FA), as well as the levels of total proteins, albumin, uric acid and triglycerides were analyzed using commercial kits (Analisa®)[1], and were read on a semi-automatic equipment (Bioplus 2000®)[2]. Serum globulin levels were calculated as total proteins - albumin.

Liver histopathology

Liver histology was examined from in 3 mm sagittal sections fixed in 10% buffered formalin. Slides were stained with hematoxylin and eosin (H&E) for histopathological analysis. They were evaluated by a pathologist who was blinded to treatment arm.

Statistical analysis

First, the data were submitted to the normality test. Those that did not present normality (beetle count, alkaline phosphatase and triglycerides) were transformed to logarithmic form (log x). Subsequently, the analysis of variance was performed, followed by the Tukey test ($P < 0.05$).

RESULTS

The amount of *A. diaperinus* collected in the TupeTrap traps were significantly different among groups ($P < 0.05$), with the highest values in the control and cypermethrin treatments. There was a significant reduction of organisms in both treatments with *C. zeylanicum* oil (Figure 1).

Biochemical serum analysis of chicks exposed to cypermethrin and *C. zeylanicum* 5% oil are displayed in Table 2. ALT, AST, FA, total proteins, albumin, globulins, uric acid and triglycerides did not differ significantly ($P < 0.05$) among treatments. On histopathological analysis, no cell lesion was observed regardless of treatment; observed cells maintained normal architecture.

Table 2. Mean values and standard deviation of serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (AP), total proteins (TP), albumin, globulin, uric acid, and triglyceride of chicks.

Variable	Solvent Control	Cypermethrin	Cinnamon oil 5% (1x)	Cinnamon oil 5% (2x)	*P* values*
ALT (U/L)	4.12 ± 1.26	3.92 ± 1.49	3.69 ± 1.10	4.0 ± 1.25	0.547
AST (U/L)	218.0 ± 44.4	234.7 ±33.1	231.3 ± 41.8	204.2 ± 37.3	0.135
AP (U/L)	189.2 ± 122.3	231.3 ± 106.2	191.8 ± 129.6	231.6 ± 118.7	0.369
TP (mg/dL)	2.59 ± 0.29	2.51 ± 0.55	2.65 ± 0.43	2.51 ± 0.50	0.754
Albumin (mg/dL)	1.62 ± 0.10	1.70 ± 0.34	1.64 ± 0.19	1.65± 0.39	0.654
Globulin (mg/dL)	0.96 ± 0,25	0.81 ± 0.44	1.01 ± 0.36	0.85 ±0.40	0.425
Uric acid (mg/dL)	6.42 ± 1.61	6.31 ± 2.11	5.97 ± 2.50	4.93 ± 1.66	0.084
Triglyceride (mg/dL)	78.6 ± 30.9	84.4 ± 30.4	74.1 ± 39.5	58.3 ± 23.4	0.071

*Averages compared by the Tukey test ($P < 0.05$).

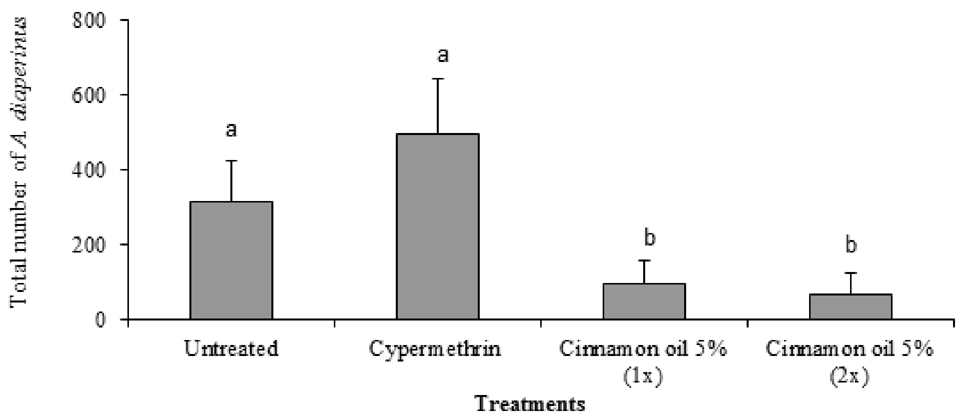

Figure 1. Number of live *Alphitobius diaperinus* (mean + SD) collected in TupeTrap trap in poultry litter contaminated with cypermethrin, C*innamomum zeylanicum* essential oil 5% one application and *C. zeylanicum* 5% essential oil two applications. Different letters in the graph show differences between groups in the Tukey test ($P < 0.05$).

DISCUSSION

Many commercial products have low effectiveness in the control of *A. diaperinus*, since the target organism develops resistance [25]. This phenomenon was also described by Chernaki-Leffer *et al*. [6], who found high resistance of *A. diaperinus* to cypermethrin. Resistance to organophosphate insecticides and pyrethroids by *A. diaperinus* has been reported frequently in the literature [6,9,12]. In the present study, we used a higher cypermethrin dose than that recommended by the manufacturer, in order to increase efficacy in the face of possible resistance. Even so, cypermethrin did not efficiently control the organism.

The mode and timing of insecticide application affects the action of the insecticide. For cypermethrin, cases of re-infestation are common. The use of higher doses of cypermethrin should be questioned, since in addition to its low effectiveness against *A. diaperinus*, cypermethrin causes ecotoxicological effects, negatively affecting biological markers in soil fauna [29]. Thus, it should be used with caution, in doses safe for the ecosystem. In addition to its environmental effects, cypermethrin causes toxic effects to broilers that may manifest as biochemical, histopathological [1] and/or clinico-hematological changes [23].

The insecticidal and larvicidal action of *C. zeylanicum* oil against *A. diaperinus* was first observed *in vitro* [27]. We confirmed this finding in an experiment simulating field conditions. The most active compounds found in the *C. zeylanicum* essential oil tested are cinnamaldehyde (41.27%), linalool (13.05%) and methyl eugenol (10.87%). These are credited as responsible for its repellent properties. Monoterpenoid compounds found in essential oils extracted from plants are insecticidal via their effects on the insect central nervous system, impairing their development [26]. As neurotoxic compounds [28], these agents interrupt the physiological functions of the GABAergic [26] and aminergic systems [10], as well as by inhibition of acetylcholinesterase [13].

Contrary to what was described in the literature [2], that *C. zeylanicum* oil is a repellent, in our study we observed insect mortality. This effect may have been potentiated by a combination of the 30 compounds found in *C. zeylanicum* oil combined with the organic matter present in the poultry litter. The insecticidal action of *C. zeylanicum* oil was also evaluated [8], who showed that the oil controlled *Sitophilus zeamais* (Coleoptera: Curculionidae), insects that are resistant to traditional insecticides and nevertheless use physiological and behavioral mechanisms to inhibit activity of the oil. The versatility of *C. zeylanicum* oil have been demonstrated in the literature, where it has been shown to work as an acaricide, and a fungicide, in addition to its insecticidal effect [4,17,18,21].

Our results with *C. zeylanicum* oil are important because control of *A. diaperinus* tends to be inefficient. The organism's behavior favors re-infestation in poultry farming: they shelter in cracks, in drapery, below the feeders, and in the soil [7]. Most control treatments involve the use of chemical insecticides with short residual periods. Their use leads to merely temporary population reduction [16].

Data on other essential oils used for the management of *A. diaperinus* can be found in the literature; *C. zeylanicum* oil is currently unavailable on the market. In an experiment using a mix of various essential oils (*Ocimum basilicum* (Lamiales: Lamiaceae), *Carum carvi* (Apiales: Apiaceae), *Laurus nobilis* (Laurales: Lauraceae), *Citrus limon* (Sapindales: Rutaceae), *Origanum vulgare* (Lamiales: Lamiaceae), *Salvia officinalis* (Lamiales: Lamiaceae), and *Thymus vulgaris* (Lamiales: Lamiaceae)) investigators found no significant difference in serum biochemical variables among the treatments evaluated [11]. This result was similar that observed in our study. Serum biochemical analysis is an important tool to assist in the monitoring of broilers health, in the diagnosis and treatment of diseases, and animal health maintenance in general [22]. Our results are important because they contribute to the search for alternative methods of *A. diaperinus* control. We can affirm that *C. zeylanicum* oil is not toxic to broilers chicks.

The diagnosis of hepatic diseases in broilers is not a very simple practice, as there is not always a correlation between alteration in liver enzyme levels and liver toxicity [15]. Variations in the serum levels the various enzymes in other studies may be related to the choice of commercial kit, the equipment used, the method of sampling, and methods of obtaining serum [3]. However, based on these data, we verified that none of the treatments were hepatotoxic.

CONCLUSIONS

We can conclude that essential oil of *Cinnamomum zeylanicum*, 5%, is an effective substitute for existing commercially-available insecticides. Its insecticidal properties were superior to those of cypermethrin in the control of *Alphitobius diaperinus*. It was not toxic to broilers chicks, even when used in poultry houses in which the broilers were housed.

MANUFACTURERS

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Ethical approval. This experiment was approved by the Animal Ethics Commission of the State University of Santa Catarina (protocol number 8941181116).

Declaration of interest. The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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