RESEARCH NOTE

In vitro evaluation of the activity of Cinnamaldehyde as an inhibitor of the biological cycle of *Ctenocephalides felis felis*

Avaliação *in vitro* da atividade do Cinamaldeído como inibidor do ciclo biológico de *Ctenocephalides felis felis*

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Abstract

The objective of this work was to evaluate the *in vitro* activity of cinnamaldehyde as an inhibitor of the biological cycle of *Ctenocephalides felis felis*. Tests were carried out with six repetitions containing 10 eggs of *C. felis felis* together with 0.5g of diet and filter paper discs with area of 23.76 cm^2 impregnated with different concentrations of cinnamaldehyde (6; 8; 10; 12; 14; 16; 18; 20; 24 and 28 $\mu g.cm^{-2}$) in Petri dishes and incubated in a climate-controlled chamber at 27 ± 1 °C and RH 75 ± 10%, for 30 days. The tests included a placebo group containing the diluent (acetone) and a negative control group, without treatment. At the end of 30 days, the percentage of inhibition of adult emergence/mortality was calculated, as well as the LC_{50} and LC_{90}. Inhibition of adult emergence greater than 50% was observed from a concentration of 10 $\mu g.cm^{-2}$ and 100% inhibition from a concentration of 14 $\mu g.cm^{-2}$, obtaining LC_{50} and LC_{90} results of 8.75 and 13.57 $\mu g.cm^{-2}$, respectively. We concluded that the volatile compound cinnamaldehyde is effective *in vitro* as an inhibitor of the biological cycle of *C. felis felis*.

Keywords: biocontrol, cat flea, natural products.

Resumo.

O objetivo deste trabalho foi avaliar a atividade *in vitro* do cinamaldeído como inibidor do ciclo biológico de *Ctenocephalides felis felis*. Para realização do teste, seis repetições contendo 10 ovos de *C. felis felis* juntamente 0,5g de dieta e papel filtro de área 23,76 cm^2 impregnado com diferentes concentrações de cinamaldeído (6; 8; 10; 12; 14; 16; 18; 20; 24 e 28 $\mu g/cm^2$) foram armazenados em placas de petri e incubados em câmara climatizada à 27 ± 1°C e UR 75 ± 10%, por 30 dias. Além disso, o teste contou com um grupo placebo contendo o diluente (acetona) e um grupo controle negativo, sem tratamento. Ao final dos 30 dias, com os dados coletados foram calculados o percentual de inibição de emergência de adultos/mortalidade, como também a CL_{50} e CL_{90}. Com os resultados, foi observado uma inibição de emergência de adultos superior a 50% a partir da concentração de 10 $\mu g.cm^{-2}$ e uma inibição de 100% a partir da concentração 14 $\mu g.cm^{-2}$ obtendo como resultados de CL_{50} e CL_{90} de 8,75 e 13,57 $\mu g.cm^{-2}$, respectivamente. Conclui-se que o composto volátil cinamaldeído tem eficácia *in vitro* como inibidor do ciclo biológico de *C. felis felis*.

Palavras-chave: biocontrole, pulga do gato, produtos naturais.

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The flea subspecies *Ctenocephalides felis felis* (Siphonaptera: Pullicidae) is the most important parasitic insect of pets worldwide. Adults have hematophagous feeding habit, causing blood loss and allergic dermatitis from bites on its hosts, in addition to transmission of pathogens to animals and humans (Fular et al., 2020).

Currently, the main way to control infestations in dogs and cats is through application of synthetic chemical insecticides on animals or in the environment (Rust, 2020). However, the misuse of these compounds has caused toxicity to the environment and humans (Nerio et al., 2010). In addition, research has shown the emergence of strains of *C. felis felis* resistant to certain groups of ectoparasiticides, such as carbamates, organophosphates and fipronil (Coles & Dryden, 2014).

Therefore, researchers have been looking for other alternatives to control this ectoparasite, such as essential oils. These compounds are a mixture of plant metabolites with biological activity (Ellse & Wall, 2014). Aromatic plants, in particular spices, are the main sources (Narayanankutty et al., 2021). Cinnamon, *Cinnamomum* sp., is a spice with well-known medicinal uses, which can be attributed to its active component, cinnamaldehyde, which has efficient antimicrobial and insecticidal activity, combined with low toxicity to humans (Figueiredo et al., 2018). Therefore, the objective of this work was to evaluate *in vitro* the activity of cinnamaldehyde as an inhibitor of the biological cycle of *C. felis felis*.

C. felis felis eggs were used from a laboratory colony maintained under conditions approved by the Animal Use Ethics Committee under protocol number 4313110419. Cinnamaldehyde with purity ≥ 95% was purchased from Sigma-Aldrich® (W228613), packaged in amber glass bottles, protected with a stopper and screw cap, and stored in a refrigerator at 4 ºC.

The *in vitro* test initially consisted of diluting cinnamaldehyde in pure acetone to 99.5%, obtaining concentrations of 300; 400; 500; 600; 700; 800; 900; 1000; 1200 and 1400 $\mu g.mL^{-1}$. After this process, for each concentration, 0.470 mL of the solution was impregnated on filter paper discs (80 g) with area of 23.76 cm^2, obtaining corresponding concentrations of 6; 8; 10; 12; 14; 16; 18; 20; 24 and 28 $\mu g.cm^{-2}$. The negative control was not impregnated to evaluate the viability of the eggs used, and the placebo group was impregnated with the diluent.

Following impregnation, the material was left at room temperature for 30 minutes to completely evaporate the diluent. After this interval, the discs were placed in plastic Petri dishes, together with 0.5 g of flea diet, to meet the nutritional needs of the larvae, in a 1:1:5 ratio of wheat bran, dehydrated bovine blood and washed sand, as described by Correia et al. (2003), followed by addition of 10 eggs. The test had six repetitions.

The dishes were closed, with a lid and elastic band, and placed in a climate-controlled chamber with temperature of 27 ± 1 °C and relative humidity of 75 ± 10%, where they remained for 30 days. The percentage of inhibition of adult emergence was calculated as the number of adult fleas emerging from the puparium after the incubation period, with any egg that was not capable of generating an adult flea being considered dead. The data obtained were tabulated and the percentage of inhibition of emergence was calculated for each concentration, according to the formula described below: **Percentage inhibition of adult emergence (%) = (number of adult fleas not emerging in the treated group – number of adult fleas not emerged in the control group) x 100 / (100 – number of adult fleas not emerged in the control group).** Furthermore, the Lethal Concentration 50 (LC_{50}) and Lethal Concentration 90 (LC_{90}) were calculated through Probit analysis using the program RStudio (2020, RStudio: Integrated Development Environment for R., PBC, Boston, MA, USA) with 95% confidence interval ($p < 0.05$).

The inhibition percentages of adult emergence are presented in Table 1. The result of the Probit analysis revealed an LC_{50} value of 8.75 $\mu g.cm^{-2}$ (6.75 - 9.58 $\mu g.cm^{-2}$) and LC_{90} of 13.57 $\mu g.cm^{-2}$ (11.84 - 17. 25 $\mu g.cm^{-2}$), with a slope of 5.3, $R^2 = 0.999$ and $X^2 = 6.45$ ($p = 0.833$).

Subsequent to the analysis of the data, mortality of up to 10% in the negative control and placebo groups was observed, validating the biological material used, due to the emergence rate of adults in the biological cycle of *C. felis felis*, which varied from 70 to 100% (Rust & Dryden, 1997), due to losses from eggs not hatching, death of some larvae or death of adults while still in the puparium.

Comparison of our lethal concentration values with those obtained by Conceição et al. (2020) with the essential oil (EO) of *Cinnamomum cassia* on *C. felis felis* eggs (LC_{50} of 2.3 $\mu g.cm^{-2}$ and LC_{90} 11.4 $\mu g.cm^{-2}$) showed better pulicidal action of the EO against *C. felis felis* than its isolated major component, cinnamaldehyde.

Table 1. Rate of inhibition of adult emergence of *Ctenocephalides felis felis* from eggs exposed to different concentrations of cinnamaldehyde.

Concentration (μg.cm^{-2})	Total Eggs Incubated R1 - R6	No of emerged adults	Total Inhibition (%)	Inhibition Corrected (%)
6	60	40	33.33	27.3
8	60	32	46.67	41.8
10	60	20	66.67	63.6
12	60	12	80.00	78.2
14	60	0	100.00	100.00
16	60	0	100.00	100.00
18	60	0	100.00	100.00
20	60	0	100.00	100.00
24	60	0	100.00	100.00
28	60	0	100.00	100.00
Placebo	60	55	8.33	---
Control (-)	60	54	10.00	---

Although many researchers attribute the action of essential oils to their majority components (Ellse & Wall, 2014), in relation to cinnamaldehyde it is possible to suggest strong synergistic action with lesser compounds, due to our finding of higher lethal concentration values of essential oil. However, the different insecticidal activity values of compounds may occur due to factors intrinsic to the parasite, environmental factors, exposure time and mortality assessment technique, which can influence the results of the tested compounds (Blagburn & Dryden, 2009).

We can conclude that cinnamaldehyde is effective *in vitro* as an inhibitor of the biological cycle of *C. felis felis*, and is thus a promising bioproduct for the control of this ectoparasite and an alternative to chemical compounds that induce antiparasitic resistance.

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Ethics statement

The flea colony has been approved at the Animal Use Ethics Committee of the Veterinary Institute of the Federal Rural University of Rio de Janeiro with protocol number 4313110419.

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Conflict of interests

CLC, EOM, YHS, LOS, BGG, TMS, MECS, DRC, FBS, KC - No conflict of interest.

Authors' contributions

CLC - Development of methodology; preparation and writing the initial draft. EOM, YHS - Writing, Review and Editing manuscript. LOS, BGG, TMS, MECS - Development of methodology. DRC - Application of statistical study data, Review and Editing manuscript. FBS, KC - Acquisition of the financial support for the project leading to this publication

Availability of complementary results

We dont have complementary results, but suggest consulting the authors, in case of doubt, at the following emails: esterom17@outlook.com, ygorhenrique97@hotmail.com or diefrey8@gmail.com

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