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INSECTICIDAL EFFECT OF SYNTHETIC PRODUCTS AND PLANT EXTRACTS ON *ALPHITOBIUS DIAPERINUS*

Efeito inseticida de produtos sintéticos e extratos vegetais sobre Alphitobius diaperinus

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Abstract

Insecticidal effect of synthetic products and plant extracts on Alphitobius diaperinus. Popularly known as lesser mealworm or litter beetle, *Alphitobius diaperinus* (Panzer, 1797) has been causing damage to both the production and the health of poultry in confinement, becoming a major pest in the poultry industry. This study evaluated the insecticidal potential of plant extracts and synthetic products on adults of *A. diaperinus*. The experiments were conducted in laboratory conditions, under controlled temperature and relative humidity. The treatments consisted of extracts of *Cinnamomum verum* L., *Allamanda cathartica* L., *Ateleia glazioveana* Baill, *Cymbopogon* sp., *Chrysantemum* sp., a commercial product based on Neem (*Azadirachta indica* A. Juss.), insecticides lambda-cyhalothrin, dichlorvos and the control (distilled water). Survival evaluation was performed every 24 hours for 72 hours. The analysis of variance (ANOVA) was applied to compare the treatments. The most efficient treatments were dichlorvos and Neem, with 100% efficiency. There was no insecticidal activity against *A. diaperinus* for the commercially recommended insecticide (lambda-cyhalothrin) and for none of the plant extracts tested in the study. Finally, it is pointed out the advances obtained, as well as perspectives for effective use of products in the population control of the lesser mealworm.

KEYWORDS: Lesser mealworm, Commercial insecticides, Plant extracts

Resumo

Efeito inseticida de produtos sintéticos e extratos vegetais sobre Alphitobius diaperinus. Popularmente conhecido como cascudinho de aviário, *Alphitobius diaperinus* (Panzer, 1797) vem provocando danos tanto para a produção quanto para a saúde das aves criadas em confinamento, tornando-se importante praga no setor da avicultura. Este trabalho avaliou o potencial inseticida de extratos vegetais e produtos sintéticos sobre adultos de *A. diaperinus*. Os experimentos foram conduzidos em condições de laboratório, sob temperatura e umidade relativa do ar controladas. Os tratamentos consistiram em extratos de *Cinnamomum verum* L., *Allamanda cathartica* L., *Ateleia glazioveana* Baill, *Cymbopogon* sp., *Chrysantemum* sp., produto comercial à base de Neem (*Azadirachta indica* A. Juss.), os inseticidas lambda-cialotrina, diclorvós e o controle (água destilada). As avaliações de sobrevivência dos indivíduos foram realizadas a cada 24 horas, durante 72 horas. Foi utilizada a análise de variância (ANOVA) para comparar os tratamentos. Os tratamentos mais eficientes foram diclorvós e Neem, com 100% de eficiência. Não foi identificada a atividade inseticida para *A. diaperinus* no inseticida comercialmente recomendado (lambda-cialotrina) e para nenhum dos extratos vegetais testados no estudo. Por fim, são apontados os avanços obtidos, bem como perspectivas para uso efetivo de produtos no controle populacional do cascudinho de aviário.

PALAVRAS-CHAVE: Cascudinho de aviário, Inseticidas comerciais, Extratos vegetais.

1- Introduction

The growth of poultry production in Brazil has led to an increase in confinement poultry production (Santos *et al*. 2009). In addition to this process, there has been a proliferation of *Alphitobius diaperinus* (Panzer, 1797) (Coleoptera: Tenebrionidae) that has caused losses in production, making this insect an important pest in the poultry sector (Pinto *et al*. 2010). *Alphitobius diaperinus*, popularly known as lesser mealworm, can be found in poultry farms, mainly in poultry litter, under feeders and drinking fountains where food and moisture are found, favorable conditions for its development (Japp *et al*. 2010).

The proliferation of *A. diaperinus* in aviaries occurs rapidly. In the larval stage, this insect inhabits the poultry litter seeking the soil for its development (Moscarelli 2010). This behavior allows the survival during the sanitary break, allowing the population of the insect to grow again when a new flock of birds is placed in the aviary (Wolf *et al*. 2014).

The control of *A. diaperinus* is commonly performed using synthetic products such as pyrethroids and organophosphates (Japp *et al*. 2010). The products may be used alone or in admixture in commercial formulations. The reduced number of insecticides registered for the control of *A. diaperinus* allows this species to become resistant (Hickmann *et al*. 2018). Without much understanding of the insect's resistance pattern to insecticides, the products continue to be used even though they are not effective in controlling, which leads to an even greater selection of resistant individuals (Narinderpal & Johnson 2015). Despite the recommendation of management practices through the control using synthetic products, the incorrect and indiscriminate use of these products may cause damages to human health and the environment, causing risks to biodiversity (Krinski *et al*. 2014).

Another negative factor is the damage to the poultry health, since they use the larvae and adults of *A. diaperinus* as alternative food, replacing the balanced feed. This behavior causes a decrease in the average body weight of the birds (Marques *et al*. 2013). In addition, when they ingest adult individuals, they may suffer damage to the digestive tract caused by the elytra, making them vulnerable to pathogens (Alves *et al*. 2011).

The control of the pest population through insecticides from plant extracts has been promoted due to their lower impact on environment and human health when compared to potential synthetic insecticides (Busato *et al*. 2015). The growing concern with environmental sustainability is evident, and due to this there is an increase in investments and studies related to organic production that aims to minimize the impacts of the use of synthetic products and to seek alternative methods (Isidro *et al*. 2018).

Studies related to the insecticidal potential of plants have been used a long time and offer safer protection to the environment and non-target species. It is known that plants have represented a viable resource for alternative pest control (Correa & Salgado 2011). Due to the risks that synthetic insecticides can cause when misused, the search for alternative pest control products is relevant.

Allamanda cathartica (L.) belongs to the family Apocynaceae and is popularly known as golden trumpet, native to the Brazilian Atlantic Forest (Almeida 2016) and can be found all over the country. This plant contains toxalbumins as active principle, which are present in the latex from the leaves, which makes it toxic to humans. In cases of accidental ingestion, it may cause gastrointestinal disturbances (Lima & Santos 2016).

Chrysanthemum cinerariifolium (Trevir.) belongs to the family Asteraceae, is a millenarian plant native to Asian countries. It is popularly known as chrysanthemum. In Brazil, the plant is grown mainly in the South region. A study investigating the potential of chrysanthemum in the control of *Tribolium castaneum* (Coleoptera: Tenebrionidae), popularly known as a red flour beetle, demonstrated a 20% efficiency in the mortality of adults (Silva *et al*. 2015).

Cinnamomum verum (L.) belongs to the Lauraceae family, is native to Sri Lanka and South India. In Brazil, it is grown mainly in the states of São Paulo and Bahia. This plant contains components such as eugenol, cinnamaldehyde (cinnamic aldehyde) and tannins that are responsible for the antioxidant, antimicrobial and antidiabetic activity of the plant (Wickenberg *et al*. 2014).

Cymbopogon nardus (L.) belongs to the family Poaceae, is native to tropical Asia and popularly known as citronella. It is one of the best-known botanical insect repellents. There is a growing number of candles and incense that are made from extracts of this plant due to its repellent potential (Bueno & Andrade 2010). It can be cultivated in different regions of Brazil, mainly in the North of Minas Gerais (Rocha *et al*. 2012). Previous studies have demonstrated the insecticidal potential of *C. nardus* in the control of *A. diaperinus*, indicating the mortality of adult individuals after 24hours of exposure to the essential oil of the plant (Marques *et al*. 2013).

Ateleia glazioveana (Baill) is a tree of the family Fabaceae, popularly known as timbó. It can be found in the States of Rio Grande do Sul and Santa Catarina (Oldenburg *et al*. 2015). It was previously known for its insecticidal potential and showed an efficiency greater than 80% in the control of *Diabrotica speciosa* (Coleoptera: Chrysomelidae) (Migliorini *et al*. 2010).

Azadirachta indica (A. Juss) is a plant of the family Meliaceae, popularly known as Neem (Brasil 2013). It is a tree native to India, grown in several countries for medicinal use. Azadirachtin, the active principle of the plant is commonly used for the purpose of controlling agricultural pests. There is a record of insecticide potential against more than 400 species (Alves *et al*. 2012).

Thymus vulgaris (L.) is an aromatic shrub that belongs to the family Lamiaceae, popularly known as thyme (Ferreira *et al*. 2016). The plant is widely used for its medicinal properties, such as anti-inflammatory, antioxidant and antiseptic (Borges *et al*. 2012). Studies on the insecticidal activity of the plant have already been evidenced, and it is important to consider the study of El-Akhal (2016) testing the larvicidal effect of *T. vulgaris* against the malaria vector *Anopheles labranchiae* (Diptera: Culidae), where 100% larval mortality was found after 24 hours at 600 µg/mL concentration.

The mortality of 100% *A. diaperinus* individuals was observed by applying 2.5 mL ethyl alcohol (P.A) diluted in water (1: 1) for every 100 g poultry litter (Fogaça *et al*. 2018). Using a similar method, Alves *et al*. (2012) tested a commercial product based on Neem cake and it was verified that the insects minimized oviposition, reduced feeding, and disintegrated the insect population, but with a reduced insecticidal effect.

It is important to develop studies so that new products with insecticide potential are prospected. Prospecting aiming at absence of residues in food, reduction of aggression to non-target species, non-development of resistance in species with potential to become pests and reduction of environmental impacts (Migliorini *et al*. 2010).

Based on the economic importance, as well as on the damages caused by *A. diaperinus*, the present study was conducted with the objectives of a) evaluating the insecticidal activity of plant extracts against *A. diaperinus* in laboratory conditions, b) comparing the insecticidal effect of plant extracts with the commercially recommended products; c) evaluating the efficiency of the plant extracts obtained under different extraction methods on *A. diaperinus*.

2- Material and methods

The study was carried out in the city of Chapecó (27º 05' 47" S; 52º 37' 06" W), State of Santa Catarina, at the Laboratories of Pharmacognosy and Entomology, Unochapecó. The specimens of *A. diaperinus* were obtained from a poultry farm in the locality of Linha Feliz, municipality of Guatambú. The plants were collected on private properties in the same municipality.

The extracts were produced in the Laboratory of Pharmacognosy, and the experiments performed in the Laboratory of Entomology of the Community University of the Chapecó Region (Unochapecó), under temperature of 25ºC ± 3ºC and relative humidity of 70% ± 10%.

Synthetic products (Pyrethroids and Organophosphates) were evaluated at the dosages recommended by the manufacturer for the control of *A. diaperinus*: The active principal lambda-cyhalothrin belongs to the chemical group of Pyrethroids, it is recommended for the control of *A. diaperinus* in the concentration of 100 mL of the product diluted in 10 liters of water, applied evenly by spraying. This concentration covers a perimeter of 200 m² in places where there is occurrence of the pest. The active ingredient Dichlorvos belongs to the chemical group of Organophosphates and is indicated a dilution of 80 mL of the product in 10 liters of water. This formulation covers 200 m² surface area.

Fresh leaf samples were obtained of five native or cultivated botanical species. From the collection, branches were taken to the Herbarium of Unochapecó and exsiccates were registered with the following numbers: *Cinnamomum verum* (3955), *Allamanda cathartica* (3956), *Ateleia glazioveana* (3957), *Cymbopogon* sp. (3958), *Chrysantemum* sp. (3959). The rest of the material was used to produce aqueous and hydroalcoholic extracts.

Aqueous extracts were obtained by turbolysis (3 sets of 5 min) using plant material (leaves, 50 g) and distilled water (100 mL). The extracts were then filtered through cotton wool in a glass funnel (3 times). To obtain the hydroalcoholic extract, the same proportion of plant material was used, adding a hydroalcoholic solution (100 mL) in the proportion of 90:10 v/v (ethanol-H_2O), repeating the process of turbolysis extraction and filtration through cotton wool in a glass funnel. The extracts were identified, lyophilized, and stored at -20°C until testing.

A commercial Neem-based product was used as recommended: 1 mL and 10 mL, diluted in 100 mL water, and a pure concentration of the product, that is, without dilution in water, containing 0.12% the active ingredient (Azadirachtin) of the plant. The essential oil of thyme (*Thymus vulgaris*) was also added to the tests, which was purchased as ready for the test, with 1,000 mg essential oil diluted in 1 liter of water. The preparation of the concentrations of all treatments is presented in **Table 1**.

Sampling of *A. diaperinus* (adult individuals) was performed manually in a poultry farm. The specimens were packed and transported in plastic pots covered with porous nylon to the laboratory for testing.

A sample of 10 specimens of *A. diaperinus* per replicate were exposed to the synthetic products and the different concentrations of the plant extracts. Each treatment consisted of three replicates. Sterile filter paper disks embedded in the treatments of plant extract or dilutions of the synthetic products were used for the tests. The concentrations used for the plant extracts were the baseline 500 µg, one greater of 1000 µg and one lower of 100 µg, all diluted in 100 mL distilled water. The synthetic products and Neem were initially tested in their concentration recommended by the manufacturer. After the first test, treatments that showed some activity at the recommended concentrations, were repeated with smaller concentrations, and for the non-efficient, larger concentrations were tested (**Table 1**).

The specimens were packed in sterile 250 mL plastic pots (microcosms). The microcosm, in addition to the insects, contained food (protein and poultry feed) and water under the embedded filter paper. As a control treatment, only food and water were used.

The assessment was based on the number of insects that survived the exposure. The count of the surviving insects was performed every 24 hours for a period of three days (24, 48 and 72 hours) and the dead specimens were removed from the microcosm at each count.

One-way analysis of variance (ANOVA) was applied on the number of live insects to compare treatments. Only those treatments that showed some insecticidal activities were included in the analysis. The values were previously transformed into Log (x + 1). Tukey's post-test was used to check the difference between the treatments. The efficiency of the treatments was calculated by the formula of Abbott (1925).

3- Results and discussion

The exposure of *A. diaperinus* to aqueous and hydroalcoholic plant extracts and to essential oil of *Thymus vulgaris* (thyme) at the end of 72 hours did not result in mortality of *A. diaperinus* (**Table** 2). From this result, regardless of the concentrations tested or extraction methods (aqueous and hydroalcoholic), the extracts or essential oil tested showed no insecticidal activity. These results do not preclude the possibility of additional studies with extracts and essential oils on this insect. Pegorini (2016) observed an insecticidal effect for *Eugenia uniflora* essential oil in the larval phase of *A. diaperinus* (70.83% mortality) and Baretta (2018) detected effectiveness in the control of *A. diaperinus* using a phytotherapeutic preparation based on essential oil from *Cinnamomum zeylanicum* J. Presl., without revealing toxic effect on birds.

The insecticidal efficacy of essential oils extracted from plants appears to be related to the chemical constituents inherent in each plant. On the other hand, in the study by Volpato et al. (2018), which evaluated eight essential oils extracted from *Cymbopogon citratus* (DC.) Stapf., *Cedrus atlantica* (Endl.), *Zingiber officinale* (Rosc.), *Pelargonium graveolens* (L.), *Juniperus communis* (L.), *Cymbopogon martinii* (Roxb.) Wats, *Thymus vulgaris* (L.) and *Vetiveria zizanioides* (L.), in different concentrations, none of them showed a significant insecticidal effect.

These results for the aqueous and hydroalcoholic extracts as well as for the essential oils may be partially because volatile compounds known to have complex composition vary their chemical constituents among species and parts of the same plant. In addition, the same botanical species can be affected by the cultivation site, conditions of collection, stabilization, and storage, as well as the soil-climatic factors (Solórzano-Santos *et al*. 2012; Miranda *et al*. 2016).

The concentrations of 1mL and 3mL lambda-cyhalothrin as well as Neem at 1 mL and 10 mL showed similar insecticidal effect, were not efficient ($p > 0.05$). However, pure commercial Neem (0.12% azadirachtin) and the three concentrations of dichlorvos had a strong insecticidal effect compared to the control group ($p < 0.05$).

The active principal Lambda-cyhalothrin at the recommended concentration (1mL) and 3mL presented efficiency percentage of 23% and 7%, respectively (**Table** 3). Neem presented 10% efficiency in the concentration of 10mL diluted in 100mL water. Also, 100% efficiency was observed when the insects were exposed to Neem in its pure commercial formulation, that is, in the concentration of 0.12% of the active principle (Azadirachtin). This result corroborates Silva *et al*. (2017), who tested the Neem extract from the leaves in powder form for the control of *Sitophilus zeamais* (Motsch). The treatment using 0.5 grams of *A. indica* on 10 grams of corn caused mortality of 90% of the individuals, being also toxic to the adult individuals. Other studies related to the use of the plant also show promising results for the insecticidal effect, such as control of: *Brevicoryne brassicae* (L.), *Liriomyza sativae* (Blanchard), *Spodoptera frugiperda* (JE Smith), among others (Vieira & Peres 2017; Silva *et al*. 2016; Melo *et al*. 2018).

The continuous and improper use of a product contributes to the selection of individuals resistant to this type of insecticide, making it increasingly necessary to search for new active ingredients (Santos *et al*. 2009). Resistance, according to the World Health Organization (WHO), is defined as the ability of a population of organisms to tolerate a dose of insecticide that under normal conditions would cause their death.

The insecticide Dichlorvos, tested at commercial concentration of 0.8%, and lower concentrations (0.6% and 0.5%) showed 100% efficiency in *A. diaperinus* mortality (**Table** 3). Mortality was evidenced in the first 24 hours of exposure. Incorrect and indiscriminate use of organophosphate (Dichlorvos) concentrations is often associated with human contamination (Cavalcanti *et al*. 2016). Thus, additional studies in field conditions may point to the possibility of use at concentrations lower than those recommended by the manufacturer and thus reduce costs and damage to the environment.

The control of *A. diaperinus* is considered a challenge, considering the habits of sheltering in crevices of the aviary, avoiding contact with the insecticide during the application. The larval phase represents another challenge, since it can shelter in the deeper layers of the poultry litter or even in the ground, becoming inaccessible during the application of insecticides. Given the losses to poultry production, the control of *A. diaperinus* population remains as a scientific and technological challenge to be overcome (Pegorini 2016; Baretta 2018).

5- Conclusion

1. Plant extracts and the Thymol and Neen oil showed no insecticidal activity in the control of *Alphitobius diaperinus*.
2. The different concentrations or extraction methods of the natural extracts had no influence on the efficiency results, being necessary investigations with doses higher than those tested.
3. Dichlorvos may be used under field conditions in concentrations lower than those recommended by the manufacturer.
4. It was possible to show the no effectiveness of the commercially recommended insecticide (Lambda-cyhalothrin) and all the plant extracts for the control of *A. diaperinus*.
5. The essential oil of neem also presented insecticidal activity; however, additional studies are necessary to define the most suitable concentrations for application.

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8- Attachments

Table 1. Treatments tested on *Alphitobius diaperinus*, under laboratory conditions (temperature of 25ºC ± 3ºC, relative humidity of 70% ± 10%), 2018.

Treatmentos/ popular names	Solution/formulation	Concentrations
Allamanda cathartica (allamanda)	Aqueous / Hydroalcoholic	100; 500; 1000 (µg/mL)
Chrysanthemum cinerariifolium (chrysanthemum)	Aqueous / Hydroalcoholic	100; 500; 1000 (µg/mL)
Cinnamomum verum (cinnamom)	Aqueous / Hydroalcoholic	100; 500; 1000 (µg/mL)
Cymbopogon nardus (citronella)	Aqueous / Hydroalcoholic	100; 500; 1000 (µg/mL)
Ateleia glazioveana (timbó)	Hydroalcoholic	100; 500; 1000 (µg/mL)
Azadirachta indica (neem)	Commercial	1 mL; 3 mL; 5 mL; 10 mL; pure (0.12%)
Thymus vulgaris (thyme)	Aqueous	1000 mg/L
Lambda-cyhalothrin	Commercial	1mL; 2 mL; 3 mL
Dichlorvos	Commercial	0.4 mL; 0.6 mL; 0.8 mL

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Table 2. Analysis of variance (ANOVA) on the survival of adult *Alphitobius diaperinus* to treatments with synthetic insecticides, under laboratory conditions (temperature of 25ºC ± 3ºC, relative humidity of 70% ± 10%), 2018.

Causes of variation	**SQ**	**F**	**p-value**
Between groups	436.5	58.76	< 0.05
Within groups	17.3		
Total	453.8		

Treatments		**Average survival**	**p-value**
Control	ICON 1mL	8.33	= 0.55
	ICON 3mL	9.33	= 0.99
	Devetion 0.8	0.00	< 0,05
	Devetion 0.6	0.00	< 0,05
	Devetion 0.4	0.00	< 0,05
	Neem10mL	9.00	= 0.92

Table 3. Mean survival of *Alphitobius diaperinus* exposed to different concentrations of synthetic insecticides, Neem and control treatment, under laboratory conditions (temperature of 25ºC ± 3ºC, relative humidity of 70% ± 10%), 2018.

Group	Mean 24hs	Mean 48hs	Mean 72hs	Efficiency 72hs (%)	Significance (p)
Control (distilled water)	10 ± 0.0	10 ± 0.0	10 ± 0.0	0	0
Lambda-cyhalothrin 3mL	9.3 ± 1.2	9.3 ± 1.2	9.3 ± 1.2	7.0	0.99
Azadirachta indica 10mL	9.3 ± 1.2	9.0 ± 1.7	9.0 ± 1.7	10.0	0.95
Lambda-cyhalothrin 1mL	8.0 ± 2.6	8.0 ± 2.6	7.7 ± 2.3	23.0	0.55
Azadirachta indica commercial (0.12%)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	100.0	0.00
Dichlorvos 0.4mL	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	100.0	0.00
Dichlorvos 0.6mL	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	100.0	0.00
Dichlorvos 0.8mL	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	100.0	0.00