INSECTICIDAL EFFICACY OF AQUEOUS EXTRACTS OF *Ricinus communis*, *Baccharis trimera* AND *Chenopodium ambrosioides* ON ADULTS OF *Alphitobius diaperinus*

EFICÁCIA INSETICIDA DE EXTRATOS AQUOSOS DO Ricinus communis, Baccharis trimera E Chenopodium ambrosioides EM ADULTOS DE Alphitobius diaperinus.

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SUMMARY

Seeking alternative strategies for health management to control mealworm in poultry barns, *in vitro* study evaluated the insecticide effect of *Ricinus communis*, *Baccharis trimera* and *Chenopodium ambrosioides* extracts against adults of *Alphitobius diaperinus*. The extracts were tested at concentrations of 0, 2, 4 and 8%. To study insecticidal effects was designed an experiment containing groups with 20 adults in 3 replicates for each treatment, evaluating the mortality percentage of 1, 3, 16 and 24 hours after treatment with counting of live and dead adults. Greater insecticidal effect was observed in treated mealworm with 8 % of *R. communis* aqueous extract (59.0%) compared to *C. ambrosioides* and *B. trimera* which had percentages of mortality of 44.6% and 0.0% at the same concentration, respectively. The aqueous extracts of *R. communis* and *C. ambrosioides* at 8% showed promising results for the control of adults mealworm. However, pharmacological studies must be designed to determine strategies and formulations for viable application of these extracts in poultry facilities, as well as to determinate the formulations stability, clinical and environmental safety treatments.

KEY-WORDS: Baccharises; Castorbean; Mealworm; Sanitary control; Wormseed

RESUMO

Na expectativa de buscar estratégias alternativas de manejo sanitário para controle do cascudinho em aviários, este estudo avaliou a eficácia inseticida *in vitro* dos extratos de *Ricinus communis*, *Baccharis trimera* e *Chenopodium ambrosioides* em adultos de *Alphitobius diaperinus.* Os extratos foram testados nas concentrações de 0, 2, 4 e 8%. Para avaliação inseticida foi delineado experimento contendo grupos de 20 adultos de cascudinhos em três repetições para cada tratamento, avaliando-se o percentual de mortalidade 1, 3, 16 e 24 h após o tratamento, com contagem dos adultos vivos e mortos. Foi possível observar maior efeito inseticida na formulação de extrato aquoso 8% do *R. communis* (59,0%), comparativamente ao *C. ambrosioides e B. trimera* que apresentaram percentuais de mortalidade de 44,6% e 0,0% na mesma concentração, respectivamente. Os extratos aquosos de *R. communis* e *C. ambrosioides* a 8% demonstraram resultados promissores para o controle de adultos de cascudinho. Contudo, estudos farmacológicos devem ser delineados visando determinar estratégias e formulações viáveis para aplicação destes extratos em aviários, assim como, defenir a estabilidade das formulações, segurança clínica e ambiental dos tratamentos.

PALAVRAS-CHAVE: Carqueja; Cascudinho; Controle Sanitário; Erva-de-Santa-Maria; Mamona

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INTRODUCTION

With the industrial poultry expansion, the darking beetle (*Alphitobius diaperinus*) can be considered a great obstacle in the production worldwide (DEL VALLE et al., 2016). The little beetles are also transmitters and reservoirs of disease-causing pathogens in poultries, such as bacterioses by *Escherichia coli*, *Enterobacter agglomerans*, *Citrobacter diversus*, *Klebsiella pneumoniae* (CHERNAKI-LEFFER et al., 2002) and *Clostridium perfringens* (VITTORI et al., 2007), as well as virus diseases such as Gumboro (MCALLISTER et al., 1995), Coronavirus (WATSON et al., 2000), Newcastle disease and Avian Influenza (SKOV, 2004).

In general, the use of chemical insecticides representes the main sanitary management to control the little beetles. Other studies have evaluated the strategic control of *A. diaperinus* in poultry farms using pyrethroids (HAMM et al., 2006; CHERNAKI-LEFFER et al., 2011), organophosphates (YEASMIN et al., 2014), imidacloprid (SINGH; JOHNSON, 2015), benzoylphenylurea (CHERNAKI-LEFFER et al., 2011) and spinosad (SOUZA et al., 2009; SINGH; JOHNSON, 2015).

The indiscriminate and incorrect use of these formulations in infested environments has been an unsuccessful strategy and may favor the selection of resistant insect strains (SOARES et al., 2009; BELO et al., 2012; SOUZA et al., 2017; CHARLIE-SILVA et al., 2018). The lack of drug efficacy monitoring in Brazil makes impossible a more accurate analysis on this question, probably it is one of the reasons for reports about the reduction in the activity of these compounds (CHERNAKI-LEFFER et al., 2011).

A number of strategies are employed to control the small beetles in the environment, such as the evaluation of biological control with entomopathogenic fungi (ALVES et al., 2015; SANTORO et al., 2015), microbial compounds (DEL VALLE et al., 2016), diatomaceous (SANTORO et al., 2010), hydrated lime, aluminum sulfate and gypsum (Wolfi et al., 2014).

The use of phytotherapics represents a viable strategy in the control of *A. diaperinus* (WANG et al., 2014). For these authors, phytotherapy is considered an important alternative in the control of insects, being able to reduce the economic and environmental impacts in relation to the use of synthetic pesticides, as well as to increase the viability of conventional insecticide compounds. Efficacy studies with essential oils of *Thuja occidentalis* and *Tanacetum vulgare* on *A. diaperinus* control did not demonstrate efficacy when compared to control insects (SZOLYGA et al., 2014). On the other hand, treatment with 5% *Cunila angustifolia* extract resulted in an insecticidal effect on larvae and adults of *A. diaperinus* (PRADO et al., 2013). Studies with neem essential oil (*Azadiractha indica*) demonstrated a 59% efficacy in the control of this beetle at 9% of concentration (MARQUES et al., 2013).

Currently, the research of plant extracts with activity on insects has grown considerably and presented promising results. Based on the importance of controlling this beetle in sanitary management of poultry farms and the need to establish effective strategies for application in the environment, the aim of this study was to evaluate the *in vitro* insecticidal activity of *Ricinus communis, Baccharis trimera* and *Chenopodium ambrosioides* extracts on coleoptera of *A. diaperinus* collected in naturally infested barns.

MATERIAL AND METHODS

Determination of extracts

Leaves of *R. communis, B. trimera* and *C. ambrosioides* were collected in the Descalvado - SP (21º54'14 "S - 47º37'12" W) and the extracts were prepared in the plant breeding laboratory of Brasil University. To obtain the aqueous extracts, the material was washed, dried and dehydrated in an oven with air circulation at 40ºC, for 48 h. Subsequently, the materials were finely ground in mill, obtaining powders of the vegetables that were stored in hermetically sealed containers and identified. Extract solutions were obtained by proportional addition of post-vegetables in 2, 4 and 8%, diluted in 50 mL of distilled water. The extracts were kept in sealed vials to extract the water soluble compounds and later were filtered on filter paper to obtain the aqueous extracts of each plant.

Collection of *A. diaperinus*

The little beetles were collected from poultry barns naturally infested belonging to commercial farma in the municipality of Itápolis, São Paulo State (21º30'55 "S - 48º46'40" W). The samples were transported to the laboratory in plastic pails covered with a screen and kept in the laboratory at ambient conditions (25 ± 1ºC and natural photoperiod) until the experiments were carried out.

Pharmacological evaluation of aqueous extracts

In order to evaluate the insecticidal effect of the extracts (*in vitro*), tests were done for each treatment and their respective concentrations. Initially, unsexed adult insects were randomly allocated to Petri dishes with a $4cm^2$ containing filter of paper. Each plate was set 20 adult specimens in three replicates for treatment. and 300 μL of extract were impregnated in the filter paper of each experimental unit. Two control groups were determinated, one treated with distilled water and another submitted to commercial drug formulations at a dose of 1.25 mL / L (Colosso ® Spraying, Ourofino Saúde Animal). Mortality observations were performed 1, 3, 16 and 24 hours post-treatment (HPT).

Data analysis

The data from dead beetle counts were submitted to the Shapiro-Wilk normality test and the analysis of variance, comparing the means by the Tukey test with 5% of significance ($p \leq 0.05$) in the R Core Team program, version 3.5 (2016). The percentages of extract effectiveness were calculated following the protocol established by the "Portaria No. 88, dated November 6, 2015", from Brazilian Ministry of Agriculture (MAPA, 2015).

$$Efficacy\ (\%) = \frac{(A - B)}{A} \times 100$$

A – Number of individuals living in the control group;
B – Number of living individuals in the treated group.

RESULTS

Observations of the extract pharmacological effects allowed to compile individualized data at the observational times proposed for the experiment and the results are expressed in the Table 1.

TABLE 1 - Mean values (± SD) and ANOVA[1] observed in the percentage of effectiveness of the efficacy percentage in the study of treatment with aqueous extracts of *Baccharis trimera, Chenopodium ambrosioides* and *Ricinus communis* on adults of *Alphitobius diaperinus.*

Treatment[2]		Mean efficacy of extracts			
		1 h	**3 h**	**16 h**	**24 h**
Negative Control		0,0 ± 0,0 c	0,0 ± 0,0 c	0,0 ± 0,0 c	0,0 ± 0,0 c
Positive Control		100,0 ± 0,0 a	100,0 ± 0,0 a	100,0 ± 0,0 a	100,0 ± 0,0 a
Baccharis trimera	2	0,0 ± 0,0 c	0,0 ± 0,0 c	0,0 ± 0,0 c	0,0 ± 0,0 c
	4	0,0 ± 0,0 c	0,0 ± 0,0 c	0,0 ± 0,0 c	0,0 ± 0,0 c
	8	0,0 ± 0,0 c	0,0 ± 0,0 c	0,0 ± 0,0 c	0,0 ± 0,0 c
Chenopodium ambrosioides	2	0,0 ± 0,0 c	0,0 ± 0,0 c	0,0 ± 0,0 c	0,0 ± 0,0 c
	4	0,0 ± 0,0 c	0,0 ± 0,0 c	0,0 ± 0,0 c	0,0 ± 0,0 c
	8	0,0 ± 0,0 c	30,0 ± 11,1 b	32,2 ± 18,2 b	44,6 ± 16,6 b
Ricinus communis	2	0,0 ± 0,0 c	0,0 ± 0,0 c	0,0 ± 0,0 c	0,0 ± 0,0 c
	4	0,0 ± 0,0 c	0,0 ± 0,0 c	0,0 ± 0,0 c	0,0 ± 0,0 c
	8	0,0 ± 0,0 c	36,3 ± 12,0 b	49,3 ± 7,0 b	59,0 ± 2,0 b

[1] Mean (n=3, totaling 60 adult coleoptera) followed by equal letters in the column do not differ from each other by the Tukey test with 5% significance (p≥ 0,05); [2] Negative control: treated with distilled water; Positive control: treated with Colosso® Spraying, Ourofino Animal Health Company, at the dose of 1.25mL/L; Concentration of plant extracts 2, 4 and 8%.

In the negative control (distilled water) and in the aqueous extract of B. trimera, 0.0% mortality was observed at all concentrations. The positive control group treated with the drug formulation (cypermethrin + chlorpyriphos + citronellal) presented 100% of mortality (Table 1).

The aqueous extract of *R. communis* leaves showed the highest pharmacological activity on adults of *A. diaperinus* at 8% of concentration, with 59.0% of the coleoptera died 24 HPT (Table 1).

The aqueous extract of Santa Maria herb (*C. ambrosioides*) presented a similar effect to those found for *R. communis*, but had a lower efficacy of 44.6% on *A. diaperinus* adults. In the statistical analysis, no significant difference was observed between the insecticidal activity of the extracts of *C. ambrosioides* and *R. communis* (P> 0.05) (Table 1).

DISCUSSION

The aqueous extract of *R. communis* leaves presented better insecticidal effects on adults of *A. diaperinus*. These findings may be related to the large amount of ricinoleic acid esters present in their leaves. Sampieri et al. (2013) confirmed the acaracide effect of these esters when added to the diet of rabbits infested with ticks *Rhipicephalus sanguineus*. Another study revealed repellent effects of ricinoleic esters on *Diaphania nitidalis* (Lepidoptera: Pyralidae) (LIMA et al., 2015). Studies with *R. communis* have demonstrated insecticidal effects on the control of *Callosobruchus maculatus* and *Spodoptera frugiperda* (ALMEIDA et al., 2006; ROSSI et al., 2010, respectively) and nematicide on *Meloidogyne javanica* (GARDIANO et al., 2009).

R. communis has developed a natural defense mechanism against insect attack throughout its evolution, including the synthesis and accumulation of substances that are used in its defense as the inhibitor of proteins called ricin, being the main responsible for toxicity in this plant (SANTORO et al., 2010). According to the United States germplasm bank, the concentration of ricin in *R. communis* leaves may vary between different genotypes, with levels of 1.5 to 9.7 mg / g being detected (PINKERTON et al., 1999). These results suggest that this inhibitory activity is attributed to a digestive enzyme that has a potential deleterious effect on insect development (ROSSI et al., 2010; DEL VALLE et al., 2016).

The insecticidal evaluation of the aqueous extract of Santa Maria herb (*C. ambrosioides*) showed 44.6% efficacy on adults of *A. diaperinus* at 8% concentration. The use of hexane and ethanolic extracts of *C. ambrosioides* resulted in efficacy of 32% and 8% in the control of this beetle, respectively (MARCOMINI et al., 2009). For these authors, the compounds with insecticidal activity present in *C. ambrosioides* extract are extracted in greater quantity of solvents with medium or low polarity.

The active principles, isolated from *C. ambrosioides* extracts are already known: they are (Z) - carboxol, (E) - ascaridol and carvacrol, whose toxic potential may become risky when the indiscriminate use of this herb extract for medicinal purposes. However, studies have attempted to evaluate the use of this herbal and found analgesic properties (Sousa et al., 2012), anti-inflammatory (DRAFT et al., 2015), anthelminthic (Alvarez et al., 2011), antileishmanial and schistosomicide (MACHADO et al., 2010).

In this investigation, the aqueous extracts of *R. communis* and *C. ambrosioides* at 8% demonstrated promising results for the control of *A. diaperinus* adults with 59.0 and 44.6% efficacy. However, pharmacological studies should be designed to determine viable strategies and formulations for the application of these extracts in aviaries, as well as to define formulation stability, clinical and environmental safety for the treatments.

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