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Effectiveness of flor-de-seda and pomegranate aqueous extracts on eggs of the *Heterakoidea* Superfamily isolated from naturally infected japanese quails

Eficácia dos extratos aquosos de Flor de Seda e Romã sobre ovos da Superfamília Heterakoidea isolados de codornas japonesas

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ABSTRACT

The present study aimed to evaluate the in vitro anthelmintic efficacy of *flor-de-seda* (*Calotropis procera S.W.*) and pomegranate (*Punica granatum L.*) extracts on gastrointestinal nematode eggs of Japanese quails. Stool samples were collected from a herd of 5,000 adult birds raised on the floor at a commercial farm in the state of Ceará, Brazil. The samples were packed in plastic bags, kept refrigerated and transported for laboratory analysis. The eggs were placed in 2.5 ml of the suspension and 2.5 ml of *flor-de-seda* and pomegranate extracts in concentrations of 50; 25; 12.5; 6.25 and 3.12%. Readings were subsequently taken at 24, 48 and 72 hours. The aqueous pomegranate extract at 25 and 50% was more efficient on the larvae hatching. The *flor-de-seda* aqueous extract in both concentrations and evaluation periods showed similar action to that of the positive control treatment (Albendazole 10%). The pomegranate and *flor-de-seda* aqueous extracts have anthelmintic activity on the development of larvae in eggs of the Heterakoidea Superfamily and are therefore presented as an alternative to replace the drugs commonly used to control helminths in Japanese quails.
Keywords: birds, gastrointestinal parasites, medicinal plants

RESUMO

O presente estudo teve por objetivo avaliar a eficácia anti-helmíntica "*in vitro*" dos extratos de flor de seda *(Calatropis procera S. W.)* e de romã *(Punica Granatum L.)*

sobre ovos de nematoides gastrintestinais de codornas japonesas. Foram colhidas amostras de fezes em um plantel de cinco mil aves adultas, criadas sobre o piso em uma granja comercial no estado do Ceará, Brasil. As amostras foram acondicionadas em sacos plásticos, mantidas refrigeradas e transportadas ao Laboratório de Doenças Parasitárias dos Animais Domésticos (LDPAD) do Centro de Saúde e Tecnologia Rural da UFCG. Os ovos foram colocados em 2,5 ml da suspensão e 2,5 ml dos extratos de flor de seda e romã, nas concentrações de 50; 25; 12,5; 6,25 e 3,12%. As leituras foram realizadas em 24, 48 e 72 horas. O extrato aquoso de romã a 25 e 50% apresentou uma melhor ação sobre a eclosão de larvas. Já o extrato aquoso de flor de seda em ambas as concentrações e períodos de avaliação apresentou ação semelhante a do tratamento controle positivo (Albendazole 10%). Os extratos aquosos de romã e flor de seda apresentam atividade anti-helmíntica sobre a eclosão de larvas de *Ascaridia* sp. e se apresentam como uma alternativa para substituição dos fármacos comumente utilizados no controle de helmintos em codornas japonesas.
Palavras-chave: aves, parasitoses gastrointestinais, plantas medicinais

INTRODUCTION

Quail farming is an activity which requires little space, low initial investment, has a short-term financial return and has shown a marked growth in the animal production scenario in recent years (Silva et al. 2018). Factors such as an increase in the consumption of eggs and quail meat by the population have led to an increase in this production sector.

Intestinal parasitosis is considered as one of the serious health problems in poultry, especially those raised on the floor, causing great economic losses due to growth retardation, reduced production rates such as egg production and increased susceptibility to diseases (Vitta et al. 2014).

Prophylactic treatment with synthetic anthelmintics is the first control measure in production systems. The incidence of anthelmintic resistance has sporadically been reported in certain populations of helminths. In the absence of highly effective drugs, the use of other less conventional methods can provide a solution to the problem (Tariq & Tantry, 2012; Vita et al. 2015). In addition, organic production, the growing concern with food security and the reduction of production costs justify the use of plants with proven antiparasitic action as an alternative to commonly used antiparasitic drugs.

The main gastrointestinal nematodes which affect birds raised on the floor are *Ascaridia galli* and *Heterakis gallinarum* due to direct contact with excreta, causing granulomas in the intestine, obstruction and death of animals (Abdelqader et al. 2012; Ruff, 1999).

Pomegranate is used in traditional medicine as an antimicrobial, anti-inflammatory and natural antioxidant with proven action on a wide class of bacteria and parasites, as it has polyphenols and tannins in its skin which make it impossible for helminths and microorganisms to develop (Dkhil, 2013; Abdel Monein, 2012). The *flor-de-seda* also has a proven action and 80 to 100% effectiveness in treating intestinal parasites in small ruminants (Cavalcante et al. 2016; Nery et al. 2009), however studies on the

evaluation of these plants in quails are still scarce.

Therefore, this study aimed to evaluate the anthelmintic activity of aqueous extracts from the flowers and fruit of *flor-de-seda* (*Calotropis procera*) and pomegranate skin (Punica granatum) on eggs of the *Heterakoidea* Superfamily isolated from naturally infected Japanese quails.

MATERIAL AND METHODS

The plants (*C. procera* and *P. granatum*) used were collected in the city of Patos, located in the Paraíba mesoregion, Brazil (07º 01' 28'' S / 37º 16' 48'' W). *P. granatum* peels (pomegranate) and the flowers and fruit of *C. procera* (*flor-de-seda*) were used to obtain the aqueous extracts.

The pomegranate and *flor-de-seda* samples were subjected to laboratory hygiene procedures under running water after collection and were placed in air drying for 48 hours, then taken to the forced ventilation oven at 60ºC for 24 hours. They were soon after weighed and ground in an industrial mill, according to the methodology described by Araújo et al. (2011).

Next, they were placed in sterilized glass containers to prepare the extracts using pomegranate and *flor-de-seda* bran separately in the proportion of 250g of plant material to 100g of distilled water, remaining submerged for a period of 24 hours. After this period they were filtered using filter paper. The filtered liquid (initial volume = v1) was transferred in small portions (100 ml) to amber containers, adding distilled water to obtain the desired concentrations (final volume = v2), and kept under refrigeration until the moment of carrying out the tests.

The animals' feces for in vitro tests were collected in the city of Lavras da Mangabeira, in the state of Ceará, Brazil, from a single Japanese quail (*Coturnix coturnix japonica*) breeding stock intended for commercial laying composed of five thousand adult birds raised on the floor with beaten sugarcane bagasse, all of them being of productive age. The excreta were collected using cardboard sheets placed on the bed in the morning and removed in the late afternoon, after which possible impurities were removed which would have adhered to the collected material and were later stored in plastic bags, refrigerated and immediately sent to the Laboratory of Parasitic Diseases of Domestic Animals (LDPAD) of the Federal University of Campina Grande, Campus de Patos-PB.

Parasite eggs were obtained using the technique by Girão & Ueno (1985). The material was crushed in a grail containing hypersaturated saline, then filtered through mesh sizes of 250 nm/nm (No. 60) and 180 nm/nm (No. 80). The obtained material was placed in 500 ml beakers and then three consecutive washes were carried out with distilled water. In the last wash, the sediment was maintained with a small volume of distilled water in order to compose a suspension with approximately 100 eggs/ml, followed by that applied by Hubert & Kerboeurf (1984). Petri dishes 10 cm in diameter were used with 2.5 ml of aqueous extract of pomegranate and *flor-de-seda* in concentrations of 50; 25; 12.5; 6.25; and 3.12% mg/ml^{-1} for every 250 eggs, meaning 2.5 ml of the filtrate. The test was carried out in triplicates. Next, 2.5

ml containing 250 eggs and 2.5 ml of distilled water were used for the negative control. The positive control was performed with Albendazole 10%.

The addition of extracts to the plates with recently collected eggs enabled evaluating their effect on the larvae development by the "in vitro" test, as performed by Coles et al. (1992). The plant extract action was evaluated during the incubation period of 24, 48 and 72 hours. The procedure was also repeated with the positive and negative controls. The readings were performed in optical microscopes at 100 times magnification. All the eggs present in the samples were evaluated, classifying them as viable and non-viable, and they were identified and counted in the petri dishes.

A completely randomized design with factorial arrangement (2x5+2) was used, and two plant extracts both with five concentrations (50; 25; 12.5; 6.25 and 3.12% of pomegranate and *flor-de-seda* extract), plus the positive and negative group, according to the methodology described by Hubert & Kerboeurf (1984).

The dilutions within each group (plant extract) were compared by analysis of variance (ANOVA) in a classification criterion, with multiple comparisons by the Tukey test. The comparison of several dilutions two by two between the different extracts was performed using the Student's t-test (Zar, 1999). The significance level adopted was 5% and the analyzes were performed using the MINITAB version 14.0 statistical program.

RESULTS AND DISCUSSION

The helminth eggs found in the study belong to the *Ascaridia* and *Heterakis* genera, belonging to the *Heterakoidea* Superfamily and the *Cyclophyllidea* order. Similar results were verified by Feitosa et al. (2013), Carneiro et al. (2011) and Sabri (2013) when searching for parasites in poultry feces.

The pomegranate aqueous extract activity calculated through the inhibition rate on the development of *Ascaridia* spp. and *Heterakis* spp. are shown in Table 1.

Table 1. Averages of the results of the activity of aqueous pomegranate extract calculated through the rate of inhibition of larvae development in eggs of the Heterakoidea Superfamily in different concentrations

Treatment	Concentrations (%)	Larval Development Inibition (%)		
		24 hours	48 hours	72 hours
Pomegranate	03.12	16.0 b	16.0 b	19.0 b
	06.25	22.0 b	15.0 b	20.0 b
	12.50	30.0 b	24.0 b	33.5 b
	25.00	63.0 a	77.5 a	78.0 a
	50.00	70.0 a	73.8 a	77.0 a
No treatment *	-	05.0 b	12.0 b	07.0 b
Albendazole **	10	20.0 b	15.0 b	21.0 b

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* Negative control (-)
** Positive control (+)
Means followed in different letters by the Tukey test (p<0,05)

Pomegranate extracts at concentrations of 25 and 50% showed the highest rates of hatch inhibition at each incubation time evaluated, and did not differ significantly between them. Similar results were verified by Fernandes et al. (2009) when working with ethanolic sugar-apple (*Annona squamosa*) extract in treating *A. galli* in poultry and observed similar effects to those found in this study, where it was possible to reduce the parasitic load of the animals by more than 60%. These authors dedicated the hypoglycemic action result of the plant on the parasite since it uses glucose as an energy reserve for maintenance and the plant used would probably not make glucose and other nutrients available to the parasites present in the intestinal lumen. Similar results to this study were also verified by Aziz et al. (2018) when evaluating the anthelmintic efficacy of pomegranate peels on birds naturally infected with *A. galli*. The results described by these authors demonstrated action on the parasites in the concentrations of 25 and 50 mg/ml of ethanolic extract of the pomegranate peel when compared with the drug commonly used for treating worms in birds, and are also very similar results to those found in this study.

According to Oliveira et al. (2010), the active compounds of vegetables are being studied to control intestinal parasites because they have several secondary compounds responsible for not providing nutrients for their development; among them the authors highlight the tannins, which are the most commonly found secondary metabolites in plants and present in large quantities in pomegranate.

The inhibition rates on the development of *Heterakoidea* superfamily larvae by *flor-de-seda* extract in different concentrations can be seen in Table 2.

Table 2. Averages of the results of the activity of the aqueous extract of silk flower, calculated through the rate of inhibition of the development of larvae in eggs of the Superfamily Heterakoidea in different concentrations

Treatment	Concentrations (%)	Larval Development Inibition (%)		
		24 hours	48 hours	72 hours
Flor de seda	03.12	10.5 a	16.0 a	14.0 a
	06.25	10.8 a	16.0 a	13.0 a
	12.50	10.5 a	15.5 a	18.0 a
	25.00	24.0 a	38.0 a	19.0 a
	50.00	30.5 a	31.0 a	21.5 a
No treatment *	-	05.0 b	12.0 b	07.0 b
Albendazole **	10	20.0 a	15.0 a	21.0 a

* Negative control (-)
** Positive control (+)
Means followed in different letters by the Tukey test (p<0,05)

There was a significant effect when comparing the different concentrations of *flor-de-seda* aqueous extract with the negative control treatment, and not differing in either concentration with the treatment using the drug (Albendazole 10%), although the concentrations of 25 and 50% in all analyzed periods (24 to 72 hours) showed superior larvae inhibition effect when compared with the positive control.

The most commonly identified active principles responsible for the anthelmintic activity of *C. procera* are: alkaloids, phenolic compounds and saponins; however, the action mechanisms involved have not been very well elucidated. The authors do describe the in vitro and in vivo spasmogenic effects of *flor-de-seda* latex on gastrointestinal smooth muscles (Kumar & Shivkar, 2004) and the in vitro spasmolytic effect of *C. procera* extract on smooth musculature (Iwalewa et al. 2005).

When analyzing the effect of the two aqueous extracts, the pomegranate extract showed up to 78% efficacy for the concentration of 25%, while the *flor-de-seda* extract in the concentration of up to 50% for the period of 72 hours showed similar activity to that of the drug used as a positive control (Albendazole 10%), demonstrating the potential of these herbal extracts to inhibit development of *Heterakoidea* superfamily larvae.

The results of this study corroborate other studies by Grzybek et al. (2016) and Cordeiro et al. (2010) who studied the use of herbal extracts in treating gastrointestinal helminths of farm animals and therefore recommended herbal extracts as a viable alternative for replacing antiparasitic agents used in the market.

The pomegranate (*P. granatum*) aqueous extract is effective in the "in vitro" treatment of gastrointestinal nematodes of Japanese quails (*C. c. Japonica*), with greater efficiency in concentrations at 25 and 50%. The *flor-de-seda* aqueous extract (*C. procera*) was less efficient when compared to that of pomegranate for inhibiting the development of gastrointestinal nematode larvae in quails. In vivo studies are needed to validate the use of alternative natural substances for the control of parasites in Japanese quail farming.

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