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Allamanda cathartica l. (Apocynacea) seeds induces changes on carbohydrates deposits of *Bradybaena similaris* (Férussac, 1821) (Mollusca, Bradybaenidae)

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Abstract. *Bradybaena similaris* (Mollusca, Gastropoda), is an intermediate host of parasites and a plague to agriculture. The World Health Organization (WHO) stimulate the research for natural moluscicides, once that they are usually the target of control of parasites. The powder of *A. cathartica* seeds and cold infusions made with this material, by 12 and 24 hrs, were used to analyze the effects of this plant on the carbohydrate deposits of *B. similaris*. Snails exposed to the infusion of seeds (E24) had higher mortality, but the exposition to the seed powder change significantly the content of carbohydrates in the different sites of the snails. The glycogen content in cephalopedal mass (CM) of snails exposed to the seed powder (3.554±0.307 mg of glucose/g tissue, wet weight) was significantly higher than that observed to the control group (0.073±0.038 mg of glucose/g tissue, wet weight) the same alterations were observed in the glycogen content in the digestive gland (DG). Similar response was observed to the galactogen content in the albumen gland (AG) of the snails exposed to the *A. cathartica* seeds powder (14.788±1.211 mg of galactose/g tissue, wet weight) when compared to unexposed snails (0.598±0.52 mg of galactose/g tissue, wet weight). But, the snails exposed to the 12h infusion the galactogen concentration was reduced (0.385±0.067 mg of galactose/g tissue, wet weight).

Key words: snails, glycogen, galactogen, comparative physiology, moluscicide.

Resumo. Sementes de *Allamanda cathartica l.* **(Apocynacea) induzem alterações nos depósitos de carboidratos de** *Bradybaena similaris* **(Férussac, 1821) (Mollusca, Bradybaenidae).** *Bradybaena similaris* (Mollusca, Gastropoda), é hospedeiro intermediário de parasitos e praga à agricultura. Recomendações da Organização Mundial da Saúde buscam por moluscicidas naturais, já que o controle de parasitos veiculados por moluscos consiste primariamente no controle destes hospedeiros. O pó das sementes de *Allamanda cathartica* e infusões a frio feitas com este pó, por 12h e 24h foram usados para testar sua atividade moluscicida. Moluscos expostos a infusão de 24h apresentaram maior letalidade, no entanto, a exposição ao pó da semente modificou severamente o metabolismo de carboidratos. A concentração de glicogênio na massa cefalopodal do grupo exposto ao pó (3,554 ±0,307 mg de glicose/g de tecido, peso fresco) foi significantemente maior que o grupo controle (0,073 ± 0,038 mg de glicose/ g de tecido, peso fresco), assim como, na glândula digestiva, relação semelhante foi observada na concentração de galactogênio na glândula de albúmen do grupo tratado com pó (14,788 ± 1, 211 mg de galactose/g de tecido, peso fresco) quando comparado com o grupo controle (0, 598 ± 0,52 mg de galactose/g de tecido, peso fresco), no entanto, o grupo submetido à infusão de 12h (0,385 ± 0,067 mg de galactose/g de tecido, peso fresco) apresentou menor concentração de galactogênio do que controle.

Palavras-chaves: moluscos, glicogênio, galactogênio, fisiologia comparada, moluscicida.

Introduction

The methodology to the snails control was directed to find chemical moluscicides and these substances had great acceptance. So, due the accumulation of these substances in the soil, contamination of water reservoirs, and by affect non target organisms, they caused several problems in the human health. In spite of this, still there are chemical moluscicides that are widely used, as niclosamide (Bayluscide® - Bayer), sodium pentachlorophenate (NaCCP), nicotinanilide and bromoacetamide, but these substances are very expensive (Yi *et al*., 2005).

In accordance with WHO recommendations, many studies had been made to know the toxic potential of plants aiming it use in the control of diseases, through the control of the vectors and hosts. The researches initially were focused in the control of *Biomphalaria glabrata* (Say, 1818), intermediate host of *Schistosoma mansoni* (Sambon, 1907). According to Mello-Silva *et al*.,(2006) some questions must be remembered to the selection of moluscicides, as toxicity, viability, annual growth, adaptation to different weather conditions and the localization of substances with moluscicide activity in parts easily regenerated by the plant, as leaves.

Allamanda cathartica L.(Apocynaceae), presented widely distributed in Brazilian territory (Souza, 2002), being commonly used as ornamental plant in the gardens. Its toxic power is recognized, being found toxic substances in every part of the plant (Apollo *et al*., 2006). Popularly, this plant is used as antibiotic, laxative and wound healing according Nayak *et al.* (2006). These authors isolated flavonoids and triterpenoids from this extracts. Extracts of *A. cathartica* were tested as antihelminthic, against nematodes (Alen *et al.*, 2000), insecticides and acaricide (Potenza *et al.,* 2004, 2005).

The moluscicidal activity may be observed through the physiological alterations because the interaction of snails with the active substances of plants cause metabolic changes that compromise the reproduction e survival of the molluscs.

The terrestrial snail *Bradybaena similaris* (Férussac, 1821) found throughout Brazil, being an agricultural plague and intermediate host to parasites as *Angiostrongylus costaricensis* (Morera & Céspedes, 1971) (Teixeira *et al.,* 1993) that causes the abdominal angiostrongiliasis to humans and *Eurytrema coelomatium* (Giard & Billet, 1892) Looss, 1907, digenetic trematode that parasitizes pancreatic ducts of ruminants (Pinheiro & Amato, 1995).

The glicidic reserves in snails are represented by two polysaccharides, glycogen and galactogen. The last one is a D- and L-galactose polymer identified in a great number of pulmonate snails, and its presence is restricted to the albumen gland and newly laid eggs, being related to the reproductive biology of these animals (Goudsmit & Ashwell, 1965; Gomot *et al.* 1989). The glycogen exhibits a wider distribution in the body of molluscs and is used as an energetic source to metabolism and its movements and why the carbohydrate metabolism is directly affected by the stress conditions.

The aim of this study was analyze the effects of the exposition of *B. similaris* to *A. cathartica* seeds on the carbohydrates contents in the CM, DG and AG as a parameter to evaluate the moluscicide activity of this plant.

Material and Methods

Collection and maintenance of snails

The snails were collected from gardens located at BR465, Seropédica, RJ, Brazil (latitude: 22°46'59" S, longitude: 43°40'45" W, height: 33m). The animals were put in plastic boxes and transferred to glass vivaria (15.5x24.5x12.0cm) with a layer of earth at the bottom, previously moistened with tap water. The snails were fed *ad libitum* with fresh lettuce leaves (*Lactuca sativa* L.) and the food was renewed in alternate days.

Collection of *Allamanda cathartica* seeds and exposition of snails

Seeds of plants located at the UFRRJ Campus, Seropédica, RJ, Brazil, were manually collected in Summer, washed and macerated. Five grams of the macerated seeds were powdered over the snails (EP group). A 5% suspension was obtained by mixing 2,5g of macerated seeds in distilled water. After 12 hrs, 25ml of this suspension were filtered and sprayed over the snails E12 group (12,5ml each/vivarium) and after 24 hrs the same procedure was followed to E24 group (12,5ml each/vivarium). So, another group unexposed was formed and considered as control group (C). The groups were formed by two vivaria with ten snails each one.

Biochemical analysis

After 24 and 48 hrs post exposure, the vivaria were observed to search for dead animals and eggs laid. The survivor snails were dissected and the cephalopedal mass (CM) and digestive gland (DG) were separated to the glycogen determination. The albumen gland (AG) were separated to the galactogen determination. The glycogen and galactogen were extracted according Pinheiro & Gomes (1994) and quantified by the 3,5 DNS technique Sumner (1924), being expressed as mg of glucose/g of tissue, wet weight, and mg of galactose/g of tissue, wet weight, respectively. The spectrofotometric analyses were made in triplicates.

Statistical analysis

The results were expressed as mean ± standard deviation. The Tukey test was used to compare mean values (α=5%) (v. 3.06, InStat, GraphPad Prism Inc.).

Results and Discussion

The exposition to the powder of *A. cathartica* seeds and cold infusions showed a lethal effect on *B. similaris* after 48 hrs of exposure, being the major number of dead snails observed in the E24 group. The higher duration of the period of infusion resulted in a higher toxicity to the snails, but in all groups mortality was observed, except in group C. Nascimento *et al.*, (2006) showed the moluscicidal effect of *A. cathartica* extract on juveniles *B. similaris*. So, in the methodology used by these authors the terrestrial snails were submerged in solutions, thus the mortality observed may be due the immersion period of the snails in the solutions, resulting in an anoxic condition to the animals.

The galactogen content in EP group (14.788 ± 1.21) was 14 times higher than observed in group C (0.598 ± 0.52), but significant differences also were observed in E24 group (7.105 ± 1.25) when compared to C group and the minor content was observed in E12 group (Fig. 1). So, no group presented ovipository activity. The powder of seed exposure and the infusion of 24hrs affected more the snails, raising a major deposit of this polysaccharide in the AG. This result may be related to an increase of the activity of galactogen synthesis or an interruption of the reproductive processes, probably as an escape mechanism involving a future compensatory process. Oliveira (2007) showed that there is a negative relation between the galactogen content in the AG of *A. fulica* and the period of exposition to LD_{50} of *E. splendens* var. *hislopii* latex.

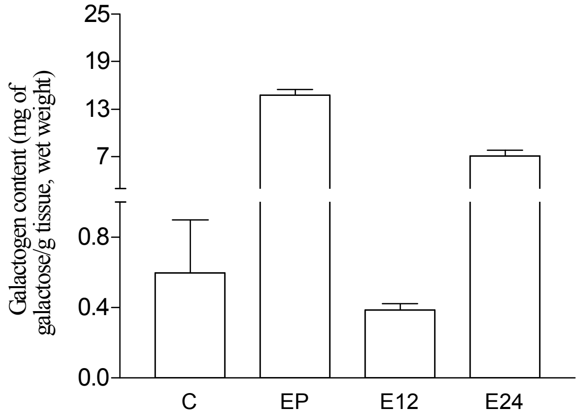

Figure 1. Relation between galactogen content, expressed in mg of galactose/g tissue, wet weight, in albumen gland of *Bradybaena similaris* exposed to seeds powder and infusions of 12hrs and 24 hrs of powder of *Allamanda cathartica* seeds.

The glycogen content in MC of C group was 0,073 ± 0,038 and only there was significant difference between this group and EP group, which had glycogen content 4862% higher. The E12 and E24 groups had glycogen content equal to 0.109 ± 0.22 and 0.040 ± 0.030, respectively, and were not significantly different of C group. The animals of EP group may have presented a dormancy behavior, reducing their mobility and, consequently, their metabolism. The seeds, as well the powder obtained from them, had a strong smell. Voss (2000), using olfactory stimuli, through neurophysiologic and behavioral methods, observed that Helix pomatia L. answered negatively to stimuli of different alcohol concentrations, presenting an escape behavior and, thus, a starvation state is established (Fig. 2).

The higher content of glycogen in DG was also observed in EP group (2.865 ± 0.168), which was 6095% higher than that observed to C group, it was followed by E24 group (1.068 ± 0.039), representing an increase of 2272% in comparison to C group. Between C and E12 groups there was not observed significant difference (Fig. 3). Differently Oliveira (2007), observed that the exposition of A. fulica to LD50 of E. splendens latex caused reduction in glycogen deposits in DG, as Mello-Silva et al. (2006) that observed a negative relation between E. splendens latex concentration and the deposits of glycogen in DG of B. glabrata (Tab.1).

In the present study, the activity of *A. cathartica* seeds on the carbohydrates metabolism of *B. similaris* was confirmed, pointing to its utilization as a moluscicidal plant. Alterations in the carbohydrate metabolism were observed, with the powder of the *A. cathartica* seeds presenting more significant effects on *B. similaris*, this fact may be related to a reduced metabolic activity in response to the strong smell of the seeds powder. So, the E24 group presented higher mortality, showing that this mode of application/ exposure presented the best moluscicidal effect when compared to the other here tested.

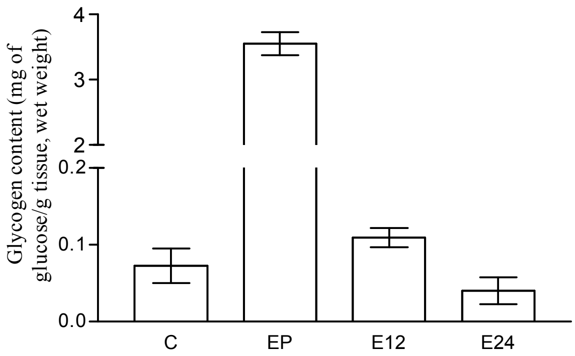

Figure 2. Relation between glycogen content, expressed in mg of glucose/g tissue, wet weight, in cephalopedal mass of *Bradybaena similaris* exposed to seeds powder and infusions of 12hrs and 24 hrs of powder of *Allamanda cathartica* seeds.

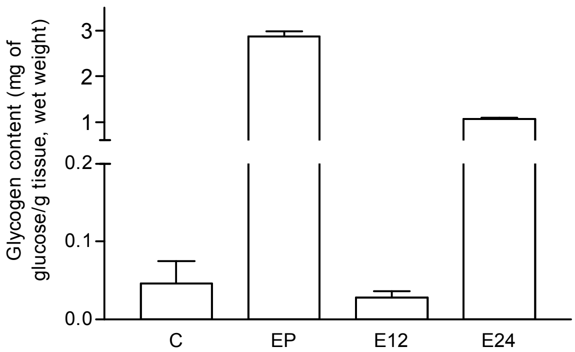

Figure 3. Relation between glycogen content, expressed in mg of glucose/g tissue, wet weight, in digestive gland of *Bradybaena similaris* exposed to seeds powder and infusions of 12hrs and 24 hrs of powder of *Allamanda cathartica* seeds.

Table 1. Glycogen content in cephalopedal mass (CM) and digestive gland (DG), expressed as mg of glucose/g tissue, wet weight; and galactogen content, expressed as mg galactose/g tissue, wet weight in *Bradybaena similaris* exposed to *Allamanda cathartica* powder seeds (EP) and 12 and 24 hours infusions (E12 and E24, respectively).

Groups	N	Glycogen content		Galactogen content
		Cephalopedal mass	Digestive gland	Albumen gland
		(mg of glucose/g tissue, wet weight)		(mg of galactose/g tissue, wet weight)
		X ± SD	X ± SD	X ± SD
C	20	0.073±0.039	0.047±0.049	0.593±0.524
EP	19	3.555±0.308	2.866±0.199	14.788±1.211
E12	17	0.109±0.022	0.028±0.014	0.385±0.067
E24	16	0.040±0.030	1.069±0.039	7.106±1.253

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