Crigis RDN, Terra VR, Pani G, Satos JB, Silva AG,Cruz ZMA (2012) Determination of LD_{50} of the latex of *Euphorbia splenders* var. *hislopii* N.E.B (syn. *Euphorbia milii* Des Moul. var. *splendens* (Ursch & Leandri) against *Achatina fulica* (Bowdich, 1822). Natureza on line 10 (2): 77-80.

Natureza on line

Submetido em: 05/05/2012 Revisado em: 23/05/2012 Aceito em: 15/06/2012 ISSN 1806–7409

Determination of LD_{50} of the latex of *Euphorbia splenders* var. *hislopii* N.E.B (syn. *Euphorbia milii* Des Moul. var. *splendens* (Ursch & Leandri) against *Achatina fulica* (Bowdich, 1822)

Determinação da DL50 do látex de *Euphorbia splenders* var. *hislopii* N.E.B (syn. *Euphorbia milii* Des Moul. var. *splendens* (Ursch & Leandri) em *Achatina fulica* (Bowdich, 1822)

Renan DN Crignis[1], Vilma R Terra[2], Gisele Pani[3], Janaina B Santos[3], Ary G Silva[1,4], Zilma MA Cruz[1,4]*

1.Programa de Pós-graduação em Ecologia de Ecossistemas. Universidade Vila Velha - UVV. Rua Comissário José Dantas de Melo, 21, Boa Vista, Vila Velha, Espírito Santo, Brasil. CEP 29102-770. 2.Instituto Federal de Educação do Espírito Santo - IFES, Campus de Colatina. 3.Curso de Graduação em Ciências Biológicas. Universidade Vila Velha - UVV. Rua Comissário José Dantas de Melo, 21, Boa Vista, Vila Velha, Espírito Santo, Brasil. CEP 29102-770. 4. Bolsista de Produtividade em Pesquisa FUNADESP.
*Autor para correspondência: zilma.vix@terra.com.br

Resumo Diferentes moluscicidas têm sido utilizados no controle de numerosas espécies invasoras, tal como o caramujo do leste africano. O presente estudo avalia o efeito de látex *in natura* de *Euphorbia splendens* var. *hislopii* contra *Achatina fulica*. A toxicidade do látex foi avaliada por exposição de 120 caramujos a diferentes concentrações de latex (3,75-7,50 g / L) durante 96 h. A toxicidade do látex foi avaliada com base no comportamento dos caranujos após a exposição às concentrações utilizadas. Os resultados indicaram uma DL50 = 4,67 g / L, com doses mínimas e máximas num intervalo de confiança de 3,98 e 5,60 g / L, respectivamente. Os valores de confirmação foram $\chi^2 = 0,60$, df = 2 e $p \geq 0,05$. Os resultados obtidos revelam a eficácia do látex como molusquicida, embora também sugiram a necessidade de testes complementares de ecotoxicidade, utilizando *Achatina fulica* e outras espécies no mesmo ecossistema.

Palavras chaves: espécies invasoras, caramujo africano, DL_{50}, molusquicidas, *Euphorbia splendens*, *Achatina fulica*

Abstract Different molluscicides have been used in the control of numerous invader species,like the East African land snail. The present study evaluates the effect of *in natura* latex of *Euphorbia splendens* var. hislopii against *Achatina fulica*. Latex toxicity was evaluated by exposure of 120 snails to different latex concentrations (3.75 to 7.50 g/L) for 96 h. Latex toxicity was observed based on the behavior of snails after exposure to the concentrations used. The results indicated LD_{50} = 4.67 g/L, with minimum and maximum confidence doses of 3.98 and 5.60 g/L, respectively. The confirmation values were $\chi^2 = 0.60$, df = 2 and $p \geq 0.05$. The results obtained reveal the efficiency of latex as a molluscicide, though they suggest the need for supplementary ecotoxicity tests both using *Achatin afulica* and other species in the same ecosystem.

Keywords: Invader species, African land snail, $DL_{50,}$ molluscicide, *Euphorbia splendens*, *Achatina fulica*.

Introduction

Achatina fulica (Bowdich, 1822), known as the East African land snail, is an invader species responsible for infestations in urban environments, affecting human health (Chang 2002) ecosystems (Teles and Fontes 2002), and crops, with economic losses in Brazil (Araújo 1989).Evidence of public health risks posed by this snail species has been described by Carvalho *et al*. (2003) and related to the potential role the snail plays as a vector to abdominal angiostrongyliasis caused by *Angiostrongylus costaricensis* (Moreira and Céspedes 1971) and eosinophilic meningoencephalitis caused by *Angiostrongylus cantonensis* Chen, 1935. The possibility of increased disease transmission in areas infested with this mollusk is a major health concern, even when *A. fulica* specimens are not infected (Vasconcelos and Pile 2001).

Control measures using non-specific molluscicides have been described for over a century. However, more specific compounds are increasingly being used, in approaches that replace toxic chemicals with phytocontrol agents that are more efficient in controlling the mollusk and less detrimental in terms of residual effects on the environment (Vasconcelos et al. 2003b). The latex of *Euphorbia splendens* var. hislopii N.E.B. (syn *Euphorbia milii* Des Moul var. splendens Ursch & Leandri) (Carter 1994) has been shown to exert an important molluscicide effect against aquatic snails, like *Lymnaea columella* (Say, 1817) (Vasconcelos and Amorin 2003), the intermediate host of hepatic fasciola (*Fasciola hepatica* Linnaeus 1758), as well as in the control of *Biomphalarai glabrata* (Say, 1818),

78
ISSN 1806–7409 - http://www.naturezaonline.com.br

the intermediate host of *Schistosoma mansoni* (Sambom, 1907) (Baptista *et al*. 1994). Comparative studies between the latex of *Euphorbia splendens* and niclosamide (Bayluscide WP™) in control strategies against the snails of the family Planorbidae showed that the phytopesticide is almost as potent as the chemical molluscicide (Mello-Silva *et al*. 2006, Oliveira-Filho and Paumgartten 2000).

The present study determines the median lethal dose (LD_{50}) of *Euphorbia splendens* latex against *Achatina fulica*, and evaluates the effects of different latex concentrations on the species' behavior.

Methods

Euphorbia milli latex extraction

Samples were collected from plants cultivated in a garden, in Itacibá, municipality of Cariacica, State of Espírito Santo (ES), Brazil, in summer. The stem was cut near the plant apex and the latex was allowed to drain into a sandblasted glass vial kept protected from light and in a heatproof container with ice. The container was transported to the laboratory and the vial placed in a refrigerator upon use.

Acclimation of snails

Specimens of *Achatina fulica* were collected in Nova Itaparica, municipality of Vila Velha, ES, transported to the Laboratory of Environmental Contamination and Genotoxicity Biomarkers, Centro Universitário Vila Velha, ES, and placed in plastic boxes (40 x 30 x 25 cm, W x H x L) filled with a specific substrate composed of vermiculite 50%, clay 30%, dolomitic lime 10%, shell lime 10% according to Bessa and Araújo (1995). Specimens were kept in a room at 22 ± 4.4ºC and relative humidity of 52.8 ± 8.3%, under 12-h dark/light cycles. Snails were fed on pelleted commercial rabbit feed supplemented with lettuce leaves and carrot and banana slices. All feeds were replaced every 24 h.

Bioassay

After acclimation, feeding was discontinued for 24 h and *A. fulica* specimens were divided in five groups, based on mean size (5.6 ± 0.3 c) and body mass (30.0 ± 4 g). Five groups were formed and placed in plastic boxes as described above. Four groups were exposed to *E. splendens* latex concentrations, one group each, as 3.75 g/L, 5.0 g/L, 6.25 g/L and 7.5 g/L. Minimum latex concentration was determined in a preliminary experiment and chosen as the first concentration tested that killed 20% of snails.One control group was not exposed to any treatment.

Latex exposure was performed by aspersion using a sprayer. The sprayer nozzle was positioned 20 cm away from each snail. Spurts were directed along all the body length of the contracted snail. One latex application was conducted. Snails were left exposed to this latex treatment for 96 h, deprived of food but with *ad libitum* access to water. During this period, mucus secretion, display of cephalopod mass, and increasingly random mobility were the behavioral parameters measured. At the end of the 96-h exposure stage, the number of dead snails was recorded for each latex concentration.

Statistical analysis

Numbers of dead snails after exposure to different *E. splendens* latex concentrations were analyzed to calculate LD_{50} using the software Trimmed Spearman-Karber, version 1.5. The LD_{50} calculated was 4.67 g/L. Next, a new bioassay was performed by exposing another group of 30 snails to LD_{50} of *E. splendens* latex as described above, to confirm results. The results obtained were compared to the expected results using the chi-square test.

Results

Evaluation of *Achatina fulica* behavior

The effect of *E. splendens* latex on *A. fulica* behavior during the 96-h exposure period is shown in Table 1. During the collection and acclimation periods no change in behavior (changes in mucus secretion, display of cephalopod mass, and increasingly random mobility) was detected. During exposure, changes were compared to the behavior of the control group. The parameters were more extensively observed among snails with increased latex concentrations, and were recorded for 100% of specimens exposed to 7.50 g/L of latex.

Determination of LD_{50}

The molluscicide activity of *E. splendens* latex *in natura* is shown in Figure 1. Mortality of *A. fulica* correlated directly with increasing doses. LD_{50} was found to be 4.67 g/L, with minimum and maximum reliability values of 3.89 g/L and 5.60 g/L, respectively. The chi-square test revealed that calculated LD_{50} did not differ significantly from expected and observed values ($\chi^2 = 0.60$, df = 2, $p \geq 0.05$), confirming the LD_{50} observed in tests.

Discussion

The results obtained in the present study show that the response of snails to latex exposure was characterized by contraction

Table 1 Percentage behavioral changes in specimens of Achatina fulica caused by exposure to the latex of *Euphorbia splendens* var. *hislopii*.

Latex concentration (g/L)	Behavior		
	Mucus (%)	Display of cephalopode mass (%)	Random mobility (% s)
Control	0	0	0
3.75	80	70	40
5.00	100	100	90
6.25	100	100	100
7.50	100	100	80

ISSN 1806–7409 - http://www.naturezaonline.com.br

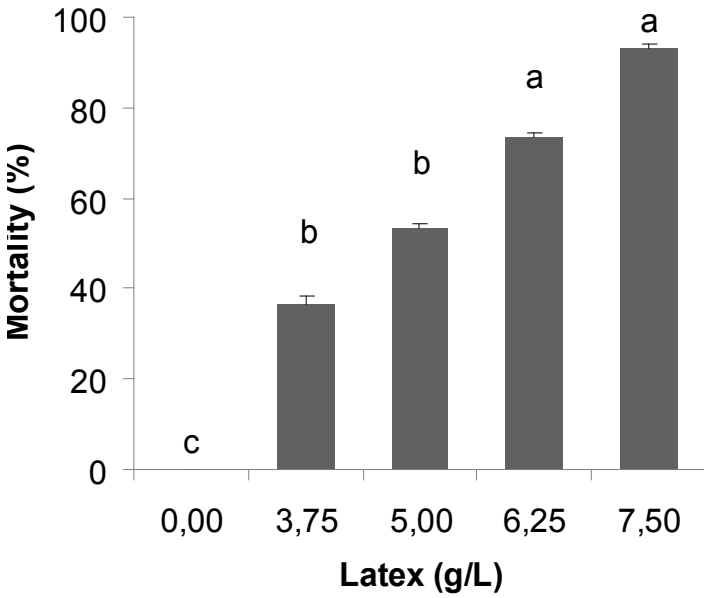

Figure 1 Determination of LD50 – mortality of Achatinafulica (%) after exposure to *Euphorbia milii* (var. *hislopii*) latex for 96 h. Controls were not exposed to latex. Different letters indicate significant differences in the Duncan test ($p \leq 0.05$). Values of confirmation observed were $\chi^2 = 0.60$, df = 2.

of the body immediately followed by stretched exposure of cephalopod mass. This contrasts with the body retraction reported by Giovanelli et al. (2001), in a study that evaluated exposure of *Biophalaria glabrata* to high latex concentrations.

The display of cephalopod mass by *A. fulica* was observed in 70% of specimens soon after spraying of the first latex solution. On the other hand, high mucus production, in all individuals, was similar to what has been reported for *B. glabrata* (Gioavanelli *et al*. 2001). The terrestrial habit of *A. fulica* may explain the intense mucus secretion as a protective physiological response to the molluscicide activity. It is likely that secretion was responsible for the high LD_{50} observed for *A. fulica*. Random movements were observed in 100% of specimens exposed to latex 6.25 g/L (Table 1), contrasting with the findings reported for *B. glabrata* (Giovanelli *et al*. 2001).

However, the latex concentration that killed 50% of the snails exposed (4.67 g/L) is higher than the values described for aquatic organisms, for which LD_{50} are of the order of mg/L. Giovanelli *et al*. (2001), for instance, described LD_{50} of 3.57 mg/L for *Melanoides tuberculata*(Muller 1774). On the other hand, Vasconcelos and Amorin (2003) reported that LD_{90} for *Lymnes columella* (Say, 1817) varied with season (1.51 mg/L, 0.55 mg/L, 0.74 mg/L and 0.93 mg/L in spring, summer, fall and winter, in that order). Activity of phytomolluscicides varies with concentration and target-organism. The results obtained for *A. fulica* in the present study revealed a median lethal dose well above the values obtained by Afonso-Neto *et al*. (2010). The authors described 100% lethal effect of several solutions (diluted up to 1:800) of *E. milli* var. splenders against *L. unilamellata*.

On the other hand, Oliveira-Filho and Paumgartten (2000) compared the effects of lyophilized latex and niclosamide, concluding that the former presents variable toxicity against organisms like oligochaeta and planktonic crustaceans, though it was non-toxic to bacteria and larvae of the mosquito *Aedes aegypti*, among others. Nevertheless, Mello-Silva *et al*. (2007) demonstrated that the LD_{50} of latex extract (1 mg/L) interfered in the reproductive process of *B. glabrata.*

In the experimental design of the present study, special care was taken to select snails 5.6 ± 0.3 cm in length and 30 ± 4.0 g in weight, so as to prevent the influence of size in final response against latex. However, a study by Oliveira-Filho and Paumgartten (1999) demonstrated that specimen size has little influence in lethal effect of latex and in LD_{50}.

The use of other molluscicides of plant origin has been described in tests using terrestrial snails, like the study by Ferreira *et al*. (2009) with *Subulina octona* (Brugüière, 1789). The authors obtained mortality of 47.5% when using a 5-g/L dose of caffeine, similar to the value of 4.67 g/L observed in the present study.

The high LD_{50} observed in the present study affords to suggest the need for further research using non-target organisms present in the ecosystem, although the report by Schall *et al*. (1991) proves the lack of acute toxicity or mutagenic effect in *Photobacterium phosphoreum*, exposed to concentrations above 445 μg/mL latex.

The results obtained in the present study demonstrate the efficiency of *E. milli* as molluscicide, though it poses the disadvantage of having a high LD_{50} for *A. fulica* (4.67 g/L). This value is higher than that observed for aquatic snails, which shows the need to assess biotoxicity, in agreement with the recommendation by the World Health Organization (WHO 1983), so as to prevent negative consequences to the ecosystem.

Acknowledgements

We are grateful to MsC Frederico Jacob Eutrópio for statistical analyses; to FUNADESP, for the researcher grat of Ary G Silva and Zilma MA Cruz, This research was supported by Universidade Vila Velha (UVV), FAPES (Grant #0021/2008) conceded to Zilma MA Cruz. ACR laboratory is supported by FUNADESP (Grant #07/10).

References

Afonso-Neto IS, Bessa EA, Soares GLG (2010) Assessment of moluscicidal activity of latex of three *Euphorbia* (Euphorbiaceae) species on *Leptinariaunilamellata*d'Obigny 1835 (Gastropoda – Subulinidae). **Revista Brasileira de Parasitologia Médic**a 12: 90-95.

Araujo JLB (1989) Moluscos de importância econômica no Brasil: I. Xanthonychidae: Bradybaenasimilaris (Férussac, 1821). (Mollusca, Gastropoda, Pulmonata, Stylommatophora). **Revista Brasileira de Zoologia** 6: 583-592.

Baptista DF, Vasconcelos MC, Lopes FE, Silva IP,Schall VT (1994)

ISSN 1806–7409 - http://www.naturezaonline.com.br

Perspectives of using *Euphorbia splendens* as molluscicide in Schistomiasis control programs. **Southeastern Asian Journal of Tropical Medicine and Public Health** 25:419-424.

Carter S (1994) A preliminary classification of *Euphorbia* subgenus *Euphorbia.* **Annals of the Missouri Botanical Garden** 81: 368-379.

Carvalho OS, Teles HMS, Mota EM, Mendonça CLGF, Lenzi HL (2003) Potentiality of *Achatina fulica* Bowdich, 1822 (Mollusca: Gastropoda) as intermediate host of the *Angiostrongylus costaricensis* Morera & Céspedes 1971. **Revista da Sociedade Brasileira de Medicina Tropical** 36: 743-745.

Chang CP (2002) *Bradybaena similaris* (Férussac) (Bradybaenidae) as a pest on grapevines of Taiwan. In: Barker GM (ed.) **Molluscs as crop pests.** Miaoli, CAB International, pp. 241- 244

Ferreira P, Soares GLG, D'avila S, Bessa EAC (2009) The influence of caffeine and thymol on the survival, growth and reproduction of *Subulina octona* (Brugüère,1789) (Molusca, Subulinidae). **Brazilian Archieves of Biology and Technology** 52: 945-952.

Giovanelli A, da Silva CLPAC, Medeiros L, Vasconcelos MC (2001) The molluscicidal activity of niclosamide (Bayluscide WP70®) on *Melanoides tuberculata* (Thiaridae), a snail associated with habitats of *Biophalaria glabrata* (Planorbidae). **Memórias do Instituto Oswaldo Cruz** 97: 743-745.

Mello-Silva CC, Vasconcelos MC, Pinheiro J, Rodrigues MLA (2006) Physiological changes in *Biofalaria glabrata* Say, 1818 (Pulmonata:Planorbidae) caused by sub-lethal concentrations of the latex of *Euphorbia splenders* var. *hislopii* N.B.E (Euphorbiaceae). **Memórias do Instituto Oswaldo Cruz** 101:3-8.

Mello-Silva CC, Vilar, MM, Bezerra JCB, Vasconcelos MC, Pinheiro J, Rodrigues ML (2007) Reprodutive activity alterations on the *Biomphalaria glabrata* exposed to *Euphorbia splenders* var. *hislopii* latex. **Memórias do Instituto Oswaldo Cruz** 102: 671-674.

Moreira P, Céspedes R (1971) *Angiostrongylus costaricensis* n sp. A new lungworm occurring in man in Costa Rica. **Revista de Biologia Tropical** 18:173-185.

Oliveira-Filho EC, De-Carvalho RR, Paumgartten FJR 1999. The influence of environmental factors on the molluscicidal activity of *Euphorbia milii* latex. Journal of Environmental Sciences and Health B 34: 289-303.

Oliveira Filho EC, Paumgartten FJR (2000) Toxicity of *Euphorbia milli* latex and niclosamide to snails and nontarget aquatic species. **Ecotoxicology and Environmental Safety** 46: 342-350.

Schall VT, Vasconcelos MC, Valent GU, Sato MIZ, Burlan EV, Sanchez PS (1991) Evaluation of the genotoxicity activity and acute toxicity of *Euphorbia splenders* Latex, a molluscicide for the control of schistosomiasis. **Brazilian Journal of Medical and Biological Research** 24: 573-582.

Teles HMS, Fontes LR (2002) Implicações da introdução e dispersão de *Achatina fulica* Bowdich, 1822 no Brasil. **Boletim do Instituto Adolfo Lutz** *12*: 3-5.

Vasconcelos MC, Pile E (2001) Ocorrência de *Achatina fulica* no Vale do Paraíba,Estado do Rio de Janeiro, Brasil. **Revista de Saúde Pública** 35: 582-584.

Vasconcelos MC, Amorim A (2003a) Molluscicidal action of the latex of *Euphorbia splenders* var. *hislopii* N.E.B. ("Christ's Crown") (Euphorbiaceae) against *Lymnes columela* (Say, 1817) (Pulmonata: Lymnaeidae), intermediate host of *Fasciola hepatica* Linnaeus, 1758 (Trematode:Fasciolidae). 1- Test in laboratory. **Memórias do Instituto Oswaldo Cruz** 98: 557-563.

Vasconcelos MC, Amorim A (2003b) Activity of *Euphorbia splenders* var. *hislopii* N.E.B. (Euphorbiaceae) latex against *Lymnes columella*(Say, 1817) (Pulmonata:Lymnaeidae), intermediate host of *Fasciola hepatica* Linnaeus, 1758 (Trematode:Fasciolidae). 2: limited field-testing. **Memórias do Instituto Oswaldo Cruz** 98: 981-985.