

Original Article

## Natural products as a control measure of the *Achatina fulica* (Gastropoda: Achatinidae)

Produtos naturais como medida de controle do molusco *Achatina fulica* (Gastropoda: Achatinidae)

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### Abstract

*Achatina fulica* is a terrestrial mollusk known as the giant African snail that is related to environmental, economic, urban, and public health problems. As control measures for this mollusk, cooking salt (NaCl) and calcium oxide (CaO) are used, and baits are composed of metaldehyde. However, these measures have environmental toxicity and impact the soil. In this way, natural products have been tested on this mollusk to discover and develop a substance to combat this urban and agricultural pest. This article aims to evaluate studies involving natural products to control the population of *Achatina fulica*. Articles and works published in books were included in the present work. A total of 1,103 works were found during the search. Of these, 14 works met the objective of these review and were included in this article. The tests do not possess methodological standardization, do not have a maximum concentration to be considered active, or a maximum exposure time. A lack of standardization in the methodology of tests on *A. fulica* was observed. The performance of tests on other life stages of the mollusk, as well as tests that analyze other parameters, are essential. Only one article analyzed presented phytochemical analysis. No ecotoxicity tests were reported either. Some extracts showed promising results, highlighting the aqueous extract of *Capsicum frutescens*. More studies investigating the molluscicidal activity of natural products on *A. fulica* are needed. It is very relevant that the new studies present a phytochemical analysis of the tested extracts, as well as ecotoxicity studies.

**Keywords:** public health, molluscicides, giant African snail, agricultural pest.

### Resumo

*Achatina fulica* é um molusco terrestre conhecido como caramujo gigante africano que está relacionado a problemas ambientais, econômicos, urbanos e de saúde pública. Como medidas de controle para esse molusco, são utilizados sal de cozinha (NaCl) e óxido de cálcio (CaO), e as iscas são compostas de metaldeído. No entanto, essas medidas têm toxicidade ambiental e impactam o solo. Desta forma, produtos naturais foram testados neste molusco para descobrir e desenvolver uma substância para combater esta praga urbana e agrícola. Este artigo tem como objetivo avaliar estudos envolvendo produtos naturais para controle da população de *Achatina fulica*. Artigos e trabalhos publicados em livros foram incluídos no presente trabalho. Um total de 1.103 trabalhos foram encontrados durante a pesquisa. Destes, 14 trabalhos atendiam ao objetivo desta revisão e foram incluídos neste artigo. Os testes não possuem padronização metodológica, não possuem concentração máxima para serem considerados ativos ou tempo máximo de exposição. Observou-se uma falta de padronização na metodologia de testes em *A. fulica*. A realização de testes em outras fases da vida do molusco, bem como testes que analisem outros parâmetros, são essenciais. Apenas um artigo analisado apresentou análise fitoquímica. Também não foram relatados testes de ecotoxicidade. Alguns extratos apresentaram resultados promissores, com destaque para o extrato aquoso de *Capsicum frutescens*. Mais estudos investigando a atividade moluscicida de produtos naturais sobre *A. fulica* são necessários. É muito relevante que os novos estudos apresentem uma análise fitoquímica dos extratos testados, bem como estudos de ecotoxicidade.

**Palavras-chave:** saúde pública, moluscicidas, caramujo gigante africano, praga agrícola.

## 1. Introduction

The mollusk *Achatina fulica* (Bowdich, 1822) belongs to the class Gastropoda and subclass Pulmonata, including slugs and other terrestrial mollusks (Brusca and Brusca, 2013). *Achatina fulica* is popularly known as “the giant

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African snail” or just “African snail” due to its large size and origin in the East-Northeast region of the African continent. Initially, the distribution was observed in the Kwazulu-Natal region (South Africa) to the northern region of Somalia (Sarma et al., 2015). Due to their easy adaptation to adverse environments, these snails can be found in different world regions, such as China, Thailand, the Pacific Islands, Australia, Japan, and the American continent (Thiengo and Fernandez, 2010). Such snails represent one of the worst invasive species in the world (Gołdyn et al., 2016).

*Achatina fulica* is related to environmental, economic, urban, and public health problems. The environmental impact is because these mollusks have clustering behavior, without dietary requirements, competing directly with native mollusks (Almeida et al., 2016). Direct competition for space and food can have negative impacts and can cause the extinction of native species. In addition, the absence of dietary requirements leads to an economic impact due to the rapid and voracious destruction of crops and gardens (Barros, 2011). Another essential factor is that *A. fulica* is an intermediate host of the parasitic worms *Angiostrongylus cantonensis* (Chen, 1935) and *Angiostrongylus costaricensis* (Morera and Cespedes, 1971) (Eammsobhana, 2014), which are etiological agents of eosinophilic meningitis and abdominal angiostrongyliasis, respectively (Zanol et al., 2010).

In recent years, mollusk control has been performed using cooking salt (NaCl) and calcium oxide (CaO). However, despite being effective, these substances are not selective, affecting natural species, and still impact the soil. These substances may change the soil properties, causing problems for planting (Singh et al., 2012; Moreau et al., 2015). In addition, baits composed of metaldehyde promote damage to the environment and toxicity to human health and other animals since these products do not have selectivity for *A. fulica* (Afonso-Neto et al., 2010; Ferreira et al., 2011; Moreau et al., 2015). For these reasons, some authors have been conducting studies with natural products as control measures for these mollusks to obtain an effective molluscicide with low environmental toxicity.

In this context, we propose to survey the current scenario of studies that seek molluscicidal activity with natural products against *A. fulica*. This article aims to evaluate studies involving natural products to control the population of *Achatina fulica*.

## 2. Materials and Methods

### 2.1. Quali-quantitative analysis

To carry out this study, we conducted searches on the following academic search platforms: National Library of Medicine (PubMed), Scientific Electronic Library Online (SciELO), Biblioteca Virtual em Saúde (BVS), Science Direct, Portal de Periódicos Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES periódicos) and Google Scholar, between January 2000 and March 2022. The associations between the descriptor “*Achatina fulica*” with the descriptors “molluscicidal activity”; “control”;

“natural products”; “plant extract” were used. Due to the low number of articles that met the objective of the work, and given the importance of the subject, in addition to the articles, works published in books were included in the present work. A total of 1.103 works were found on the search platforms in all: 49 works from PubMed, 30 works from SciELO, 61 works from VHL, 266 works from CAPES periodicals, 579 works from Science Direct, and 118 works from Google Scholar (Figure 1).

### 2.2. Criteria used

After the search on the platforms, the title and abstract of the works were read, and works that were not within the subject or that were duplicated on different platforms were excluded. Then, the other works were read through the full reading of the works, where the works that did not meet the objective of the present work were excluded.

Exclusion criteria were: a) studies that did not involve natural products b) duplicate articles; c) studies that did not meet the research aim; c) studies that were not published within the search period.

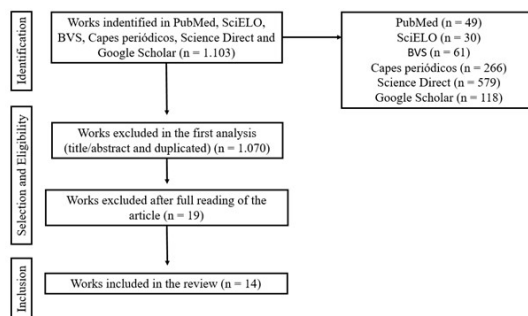
## 3. Results and Discussion

### 3.1. Tested molluscicides and their activities

During the search, a total of 1.103 articles were found and only 14 chosen: 12 articles and 2 works reported in books fit within the objective proposed by this review article, reporting natural products with molluscicidal activity on *A. fulica* (Table 1). Only two papers were published in a same journal – Toxicology.

Given the importance of the issue, which has economic and public health importance, we found a significantly low number of publications, especially when compared with studies involving the control of aquatic mollusks.

Rao and Singh (2000) tested against *Achatina fulica*, the molluscicides derived from *Azadirachta indica* (*A. Juss*) (neem) oil; *Cedrus deodara* (Roxb. ex. D. Don) (Himalaya cedar) oil; *Allium sativum* L. (garlic) bulb powder; and *Nerium indicum* L. (kaner) bark powder. In addition, the authors tested binary combinations between the *A. sativum* bulb powder and *C. deodara* oil and between *A. indica* and



**Figure 1.** Diagram of research and selection of articles in the databases for inclusion in the review.

**Table 1.** Activity of plants used experimentally to control *Achatina fulica*.

Species	Plant Parts	Observed Activity	Reference
<i>Azadirachta indica</i> (Neem, nimtree or Indian lilac)	<i>Azadirachta indica</i> oil; Binary combinations with <i>Cedrus deodara</i>	Mortality, fertility and ovicidal activity	Rao and Singh, 2000
<i>Cedrus deodara</i> (cedar; deodar; deodar cedar; Himalaya cedar)	<i>Cedrus deodara</i> oil; Binary combinations with <i>Allium sativum</i> bulb powder and <i>Azadirachta indica</i> oil		
<i>Allium sativum</i> (Garlic)	<i>Allium sativum</i> bulb powder; Binary combinations with <i>Cedrus deodara</i> oil		
<i>Nerium indicum</i> (Kaner)	<i>Nerium indicum</i> bark powder		
<i>Azadirachta indica</i> (Neem, nimtree or Indian lilac)	<i>Azadirachta indica</i> oil; Binary combinations with <i>Allium sativum</i> bulb powder and <i>Cedrus deodara</i> oil	Mortality	Rao and Singh, 2002
<i>Cedrus deodara</i> (cedar; deodar; deodar cedar; Himalaya cedar)	<i>Cedrus deodara</i> oil; Binary combinations with <i>Allium sativum</i> bulb powder and <i>Azadirachta indica</i> oil		
<i>Allium sativum</i> (Garlic)	<i>Allium sativum</i> bulb powder; Binary combinations with <i>Azadirachta indica</i> oil; <i>Cedrus deodara</i> oil; and NaCl		
<i>Nerium indicum</i> (Kaner)	<i>Nerium indicum</i> bark powder		
<i>Euphorbia splendens</i> var. <i>hislopii</i> (Crown of thorns; Christ thorn)	Latex	Mortality, mucus secretion, cephalopedous mass contraction, and mobility.	Crignis et al., 2012
<i>Morus rubra</i> (Blackberry; Red mulberry)	Leaves	Mortality, ovicidal activity and growth	Miranda et al., 2012
<i>Syzygium aromaticum</i> (Clove)	Flower buds	Mortality, ovicidal activity and growth	Gusman et al., 2014
<i>Ruta graveolens</i> (Rue)	Leaves	Mortality	Moraes et al., 2014
<i>Arnica chamissonis</i> (Arnica)			
<i>Baccharis dracunculifolia</i> (Rosemary)			
Semi-natural product	Rice husk ash silica (RHA) coated with biopesticides obtained from leaves of <i>Azadirachta indica</i> ; <i>Pongamia pinnata</i> ; <i>Nicotina tabacum</i> ; e <i>Calotropis procera</i>	Mortality, inactivation and loss of fluids	Selvi et al., 2015
<i>Baccharis dracunculifolia</i> (Rosemary)	Leaves	Mortality, ovicidal activity and growth	Vieira et al., 2016
<i>Morus rubra</i> (Blackberry; Red mulberry)	Leaves		
<i>Cyperus rotundus</i> (Nutgrass; Nutsedge)	Leaves		
<i>Euphorbia heterophylla</i> (Wild poinsettia)	Leaves		
<i>Syzygium aromaticum</i> (Clove)	Flower buds		
<i>Syzygium aromaticum</i> (Clove)	Essential oil	Mortality	Parvate and Thayil, 2017

Table 1. Continued...

Species	Plant Parts	Observed Activity	Reference
<i>Manihot esculenta</i> (Bitter cassava; manioc; tapioca; yuca)	Stems and leaves	Mortality, fertility, ovicidal activity and growth	Agostini et al., 2018
<i>Carica papaya</i> (Papaya)	Stems and leaves		
<i>Dieffenbachia seguine</i> (Dumb cane)	Stems and leaves		
<i>Anthurium</i> (Flamingo flower)	Stems and leaves		
<i>Schefflera arboricola</i> (Umbrella Plant; dwarf schefflera)	Stems and leaves		
<i>Philodendron</i> (Philodendron)	Stems and leaves		
<i>Psidium guajava</i> (Guava)	Stem ash		
<i>Syzygium aromaticum</i> (Clove)	Flower buds		
<i>Capsicum frutescens</i> (Chilli pepper)	Ripe fruits	Mortality	Silva Júnior et al., 2018
<i>Tabebuia rosea</i> (Pink poui)	Leaves	Mortality	Patiño-Montoya and Giraldo, 2018
<i>Gliricidia sepium</i> (Gliricidia, forest lilac, mexican lilac)	Leaves		
<i>Cuminum cyminum</i> (Cumin)	Commercial product		
<i>Capsicum frutescens</i> (Chilli pepper)	Fresh fruit	Mortality	Santos et al., 2018
<i>Strongylodon macrobotrys</i> (Jade vin; emerald vin; turquoise jade vin)	Flowers	Mortality and ovicidal activity	Pimenta et al., 2020
<i>Bidens pilosa</i> (Blackjack; beggar's Tick; farmer's friend)	Aerial parts		

*C. deodara* oil. In the tests, *A. fulica* individuals collected in the field with a shell diameter of 8 to 10 cm and weighing 75 to 100 g were subjected to spraying the sublethal concentrations of the molluscicidal plants (20% and 60% of the  $LC_{50}$  in 24 h), being 1 mL of 14.400 ppm and 43.400 ppm of bulb powder of *A. sativum*; 6.600 ppm and 19.900 ppm of *C. deodara* oil; 20.000 ppm and 62.000 ppm of the *N. indicum*; 34.000 ppm bark powder and 104.101 ppm of *A. indica* oil; 8.300 ppm and 25.000 ppm of *A. sativum* bulb powder + *C. deodara* oil; and 15.000 ppm and 45.000 ppm of *A. indica* oil + *C. deodara* oil. Two mollusks were used for each concentration. Three experiments evaluated the hatching of the eggs and the survival rate of the surviving snails until 72 hours after hatching and biochemical changes in the ovotestis, including estimates of free proteins and amino acids, nucleic acids, phospholipids, and lipid peroxidation (Rao and Singh, 2000). The results showed that all molluscicides tested in all experiments significantly reduced mollusk fertility. The best result was observed with the binary combination of *A. sativum*

bulb powder + *C. deodara* oil at a concentration of 60% of the  $LC_{50}$ , which obtained an average of 63.3 eggs per snail *A. fulica* in the third experiment. The most negligible reduction was for the *A. sativum* bulb powder, which had 222.3 eggs per snail. The egg viability analysis showed that all molluscicides tested in the second and third experiments diminished the viable eggs at all concentrations. In the first experiment, only the *N. indicum* bark powder and the binary combination of *A. sativum* bulb powder + *C. deodara* oil, both at a concentration of 60% of the  $LC_{50}$ , showed reduced viability eggs. The best result was 84.5% viable eggs for the binary combination of *A. sativum* bulb powder + *C. deodara* oil at a concentration of 60% of the  $LC_{50}$  (Rao and Singh, 2000). Therefore, this combination showed the best results in reducing *A. fulica* egg fertility and viability. Young snail survival also showed a significant reduction, obtaining a maximum decrease in young snail survival of 27.65% after 72 h for the binary combination of *C. deodara* oil + *A. indica* oil (Rao and Singh, 2000).

The study demonstrated by Rao and Singh (2002) presented a methodology for spraying mollusks that were 8 to 10 cm in size. The tested substances were *C. deodara* oil, *A. indica* oil, *A. sativum* bulb powder, *N. indicum* bark powder, and binary combinations of *A. indica* oil + *A. sativum* bulb powder, *A. sativum* bulb powder + NaCl, *C. deodara* oil + *A. sativum* bulb powder, and *C. deodara* oil + *A. indica* oil. The authors used concentrations ranging from 3.000 ppm to 120.000 ppm. The spraying was done uniquely in a volume of 1 mL. The tests were performed in a glass aquarium that aimed to mimic natural conditions. The mortality of *A. fulica* was evaluated every 24 h during the 96 h period.  $LC_{50}$  values were calculated for 24 h, 48 h, 72 h, and 96 h. The results showed by Rao and Singh (2002) demonstrated that in the final test period (96 h),  $LC_{50}$  of 14.700 ppm was calculated for *A. sativum* bulb powder, 12.200 ppm for *C. deodara* oil, 17.900 ppm for *N. indicum* bark powder, 4.300 ppm for the combination between *C. deodara* oil + *A. sativum* bulb powder, and 13.300 ppm for the combination of *C. deodara* oil + *A. indica* oil.

Crignis et al. (2012) evaluated the latex taken from *Euphorbia splendens* (Bojer. ex. Hooke) plant species (Christ thorn), which belongs to the family Euphorbiaceae, on the snail *A. fulica*. Snails approximately 5.6 cm in size were placed in contact with the latex by spraying at concentrations of 3.750 ppm, 5.000 ppm, 6.250 ppm, and 7.500 ppm. The authors reported snail separation into four groups but did not say how many snails were exposed by concentration. The control used is not specified, as only a mollusk group has not been exposed to any treatment. Another factor observed was the spraying description over the entire mollusk body length at a distance of 20 cm from each individual. However, the author does not make precise the latex sprayed amount on each mollusk. The test lasted 96 hours, and mortality was evaluated through mucus secretion, cephalopod mass extravasation, and mobility. The calculated  $LD_{50}$  was 4.670 ppm. For behavioral effects, an increasing behavioral difference was demonstrated according to the concentration of latex.

Miranda et al. (2012) tested aqueous *Morus rubra* L. leaf extracts (blackberry). The extracts were made at concentrations of 100.000 ppm, 300.000 ppm, 400.000 ppm, 500.000 ppm, 600.000 ppm, 700.000 ppm, and 1.000.000 ppm. Distilled water was used as a negative control. The tests were realized using 20 sprays on the mollusks for 15 days. The mollusks were used two days after hatching and ovicidal tests. At the end of this period, the extract, at the maximum concentration, did not influence the hatching reduction or the mollusk survival rate. However, the treatment caused interference in mollusk growth.

Gusman et al. (2014) used dried flower buds of *Syzygium aromaticum* L. (Merr. and L. M. Perry) (clove). The test was realized on *A. fulica* after two days of hatching, with 15 sprays on the mollusks, for 24 days. Additionally, the extract was used on the ovicidal test. The extract was diluted to 100.000 ppm, 300.000 ppm, 500.000 ppm, and 1.000.000 ppm, and distilled water was used as a negative control. Ten mollusks after two days of hatching and 10 eggs were used in the experiments. The tests were performed in 3 repetitions. The extract did not significantly affect

the eggs. However, at the end of the 24-day experiment, the authors report a mollusk survival reduction rate of up to 50%.

In work developed by Moraes et al. (2014), *Ruta graveolens* L. (rue) aqueous extracts from family Rutaceae as well as *Arnica chamissonis* (Less.) (arnica), and *Baccharis dracunculifolia* DC. (rosemary), both of the family Asteraceae, were tested at concentrations of 100.000 ppm and 200.000 ppm on adult *A. fulica*. The mollusks were exposed to the extracts through daily applications in 15 days, with the mollusks being divided into 3 blocks for concentration in 12 adult mollusks. The extracts showed low mortality of mollusks. *R. graveolens* extract showed mortality of 8.33% at 200.000 ppm and 25% at 100.000 ppm; *B. dracunculifolia* extract presented mortality of 8.33% at 200.000 ppm and 16.66% at 100.000 ppm. *A. chamissonis* extract obtained a mortality of 8.33% at 100.000 ppm, with no snail mortality at 200.000 ppm. The authors also observed that during *B. dracunculifolia* extract application, the mollusks showed more significant movement and fed better.

Selvi et al. (2015) tested the molluscicidal activity of biogenic silica obtained from rice husk ash, a seminatural product. Part of Microsilica was applied in powder and paste forms, and another part was coated with biopesticides obtained from aqueous leaf extracts of *A. indica*, *Pongamia pinnata* L. (Pierre), *Nicotiana tabacum* L., and *Calotropis procera* (W.T. Aiton). Microsilica applications were made of the powder and paste of rice husk ash on *A. fulica*. Powder and paste forms of silica were applied at concentrations of 5,000 ppm, 10,000 ppm, 15,000 ppm, 20,000 ppm, and 25,000 ppm. The microsilicas coated with biopesticides were applied at a concentration of 20,000 ppm. As a positive control, common salt at a concentration of 20.000 ppm was used. During the test, at 24-hour intervals, mortality, inactivation, and body fluid loss from snails were analyzed. As a result, silica in powder and paste forms reduced the mortality by 100% at 25.000 ppm after 26 minutes of testing for both forms. There was also 100% mollusk mortality at the lowest concentrations, however, after a longer period (50 to 56 minutes). For silica coated with biopesticides, snail mortality occurred after a longer period, with uncoated silica showing mortality after 71 minutes and silica-coated with *A. indica* obtaining mortality after 73.5 minutes.

Vieira et al. (2016) studied the molluscicidal activity of *B. dracunculifolia* (rosemary), *M. rubra* (blackberry), *Euphorbia heterophylla* L. (wild poinsettia), and *S. aromaticum* (clove) extracts. All extracts were evaluated at concentrations of 100.000 ppm, 300.000 ppm, 500.000 ppm, 700.000 ppm and 1.000.000 ppm using distilled water as a control. The authors used mollusks aged 10 days after hatching, and the tests were performed during the 30 days, using sprinkles interspersed every 2 days. The extracts were also applied to *A. fulica* eggs. The *E. heterophylla* and *S. aromaticum* extracts showed high mortality at 500.000 ppm, with  $LC_{50}$  and  $LC_{90}$  values not being presented. The other extracts had a low mortality rate and ovicidal action. Another aspect of toxicity studied in *A. fulica* was its physiological action on mollusks. All extracts reduced the *A. fulica* mass to 500.000 ppm, 700.000 ppm, and 1.000.000 ppm.

Parvate and Thayil (2017) tested the essential oil of *S. aromaticum* (Clove) on *A. fulica* adults. The mollusks weighed  $65 \text{ g} \pm 20 \text{ g}$ . The essential oil was tested at concentrations of 30,000 ppm, 60,000 ppm, 90,000 ppm, 150,000 ppm, 210,000 ppm, and 270,000 ppm. The essential oil was applied topically to snails, but the authors do not detail the method used. The mollusks were divided into groups of 10. The total period of the experiments was 96 h, with mortality observed every 24 h. An  $\text{LD}_{50}$  value of 39,160 ppm was obtained in the final experimental period (96 h).

In the study presented by Agostini et al. (2018), *Manihot esculenta* (Crantz) (bitter cassava), *Carica papaya* L. (papaya), *Dieffenbachia seguine* (Jacq.) Schott. (dumb cane), *Anthurium* (Schott) (flamingo flower), *Schefflera arboricola* (Hayata) Merr. (umbrella plant), *Philodendron* (Schott) (Philodendron) stem and leaves, *Psidium guajava* L. (guava) stem ash, and *S. aromaticum* (clove) flower buds were tested. The extracts were tested at 1.000.000 ppm. *A. fulica* presented oviposition, but the eggs did not present embryos inside after exposure to flower buds the extract of *S. aromaticum*. For the other extracts, no changes were observed. Although the authors reported the result involving the species *S. aromaticum* as promising, the results do not present statistical comparisons with the control, which does not assess whether the extract has a significant result. There is no report on the size of the mollusks used or the method of applying the extracts to snails.

In the work of Silva Júnior et al. (2018), the *Capsicum frutescens* L. (chili pepper) aqueous extract was evaluated on *A. fulica* at concentrations of 2.000 ppm, 3.000 ppm, 4.000 ppm, 5.000 ppm, 6.000 ppm, 7.000 ppm, 8.000 ppm, 9.000 ppm, and 10.000 ppm. The mollusks were 30 and 120 days old. The test was performed during 30 days. Drinking water was used as a negative control. The 30-day-old and 120-day-old individuals had sizes of 1.6 cm and 3.0 cm, respectively. The extract was applied directly on *A. fulica*. The application in concentrated groups of 5 snails, where the specimens remained in direct contact with 20 mL of the extract solutions for 72 h. The individuals presented 100% mortality for 30-day-old snails for all concentrations. In 120-day-old individuals, mortality of 100% of the mollusks was observed at concentrations of 7.000 ppm, 8.000 ppm, 9.000 ppm, and 10.000 ppm.

Patiño-Montoya and Giraldo (2018) used extracts of *Tabebuia rosea* (Bertol.) Bertero ex A.DC (pink poui), *Gliricidia sepium* (Jacq.) Kunth ex Walp. (gliricidia), and *Cuminum cyminum* L. (cumin). The extracts of the first two plants were tested at 600.000 ppm. Cumin was prepared from 55.000 mg of commercial cumin in 0.1 L of distilled water, 4% alcohol commercial beer, and a commercial molluscicide (4% metaldehyde) was also used. Distilled water was used as control. Mollusks were used between young and adult ages with varying sizes, and the shell length was measured, a fact that demonstrates a lack of size standardization for mollusk analysis. The tests were realized through 60 direct sprays on the terrarium, where the mollusks were placed after collection. Mortality was observed only for cumin extract and commercial products. In addition to the observed mortality, the surviving mollusks exposed to cumin showed physiological stress signals, such

as decreased mucus secretion, starvation, or inactivity, compared to commercial molluscicide. There is a lack of essential data throughout the article, such as mollusks size used, which is not clearly described. The article only reports that the mollusks were measured, but the size used was not informed. In addition, another factor to be observed is that cumin commercial was used without a test to check the presence of other substances (which is not well specified).

Santos et al. (2018) and Silva Júnior et al. (2018) tested the extract of *C. frutescens* (chili pepper) against *A. fulica*. The difference is that in the study of Santos et al. (2018), tests were performed with ethanolic extracts, and in the study by Silva Júnior et al. (2018), aqueous extracts were used. The authors tested the extract on young (2 cm) and adult (20 cm) mollusks. Mortality was assessed at 24 h and 48 h. Additionally, the authors performed an ovicidal activity test on 200 eggs of *A. fulica*, with the extract spraying manually. The concentrations of the extract tested were 50.000 ppm, 100.000 ppm, 150.000 ppm, and 200.000 ppm. In this study, a field test was carried out. Ninety adult mollusks of *A. fulica* were distributed in a field with 207 feet of lettuce, 10 snails per line, and 23 feet of lettuce per line. The mollusks were placed in cages fixed to the plants. The extracts were applied by hand spraying at concentrations of 50.000 ppm and 100.000 ppm. Mortality was verified after 48 hours. The extracts did not interfere with the hatching of the eggs. However, the extracts were applied directly on the mollusks using the spray 10 times and for 3 repetitions for 48 h. The authors reported a significant difference in mollusk mortality of 84% and 40% at concentrations of 10 and 5%, respectively, in field tests (Santos et al., 2018).

Pimenta et al. (2020) tested extracts of *Bidens pilosa* L. (blackjack) and *Strongylodon macrobotrys* (A.Gray) (jade vine) on young *A. fulica* snails ( $40 \pm 2 \text{ mm}$  in shell size). The extracts obtained were separated into hexane, methanol, and dichloromethane fractions at 500 ppm, 1000 ppm, and 1500 ppm. The mollusks were separated into groups of 10 and exposed to 30 ml of the aqueous solutions of the fractions by direct dermal contact. The animals were exposed for 72 h for those treated with *B. pilosa* and 96 hours for those treated with *S. macrobotrys*, with mortality verified every 24 h. The difference in test time for the two species was not justified. The best results obtained were a mortality of 53.3% for the crude extract of *S. macrobotrys* at a 1.500 ppm concentration and a mortality of 73.3% for the hydromethanolic fraction of *S. macrobotrys* at a 1.000 ppm concentration. *B. pilosa* had no effect on *A. fulica*.

Cantanhede et al. (2010) described vegetable molluscicide use as an effective and low environmental impact option for mollusk control. However, more studies are needed to correlate terrestrial mollusk control, considering that the vast majority of studies focus on aquatic mollusks. The few studies involving terrestrial mollusk control have been realized comparatively with previously used methodologies and are not entirely effective (Fischer and Costa, 2010). The articles found and used in the present study showed differences in the methodologies represented (Table 2), causing a lack of standardization for

**Table 2.** Technical characteristics of activity tests, used on *Achatina fulica*.

Reference	Mollusk sizes	Application method	Testing period
Rao and Singh, 2000	8 to 10 cm	Spray (1 mL)	0 to 60 days (fertility and ovicidal activity); 72h (mortality of surviving mollusks)
Rao and Singh, 2002	8 to 10 cm	Spray (1 mL)	96 h
Crignis et al., 2012	About 5.6 cm	Spraying the entire length of the mollusk body at a distance of 20 cm from each mollusk	96 h
Miranda et al., 2012	Two-day mollusks after hatching	15 sprays (5 mL every 2 days)	15 days
Gusman et al., 2014	Two-day mollusks after hatching	15 sprays	24 days
Moraes et al., 2014	Adult mollusks	Daily applications.	15 days
Selvi et al., 2015	Adult mollusks	An applied dose - Does not specify how it was applied	24 h intervals
Vieira et al., 2016	Mollusks after 10 days of hatching	Sprays interspersed every 2 days	30 days
Parvate and Thayil, 2017	Not reported. Only informs the weight and that adult individuals were used	Topical administration, but without further details	96 h
Agostini et al., 2018	Not reported	Not reported	30 days
Silva Júnior et al., 2018	Mollusks of 30 days (1.6 cm) and 120 days (3.0 cm) of age	Direct contact (20 mL)	30 days
Patiño-Montoya and Giraldo, 2018	Young and adult mollusks - Sizes varied	60 sprays	7 days
Santos et al., 2018	Newly hatched young mollusks (40 mm)	Direct contact	24 and 96 h
Pimenta et al., 2020	Young mollusks (2 cm) and adults (20 cm)	Spray	48 h

tests with *A. fulica*. We observed the necessity of developing controls for *A. fulica* mollusks and test standardization to improve future studies on the species.

The *C. frutescens* species (chili pepper), studied by Silva Júnior et al. (2018), demonstrated a high molluscicidal activity eliminating 100% of the mollusks in 30 days at a concentration of 2.000 ppm after 24 h. This compound exhibited the highest mortality and the lowest concentration with action on the mollusks among the articles found. However, in direct contact with the tested substance, the methodology used, adapted from Ferreira et al. (2010), makes it challenging to reproduce the product in the field. The methodology does not consider external factors to hinder product application in the field, such as climate change, mollusk escape, soil interference, or where the compound is applied, and is a more appropriate methodology for testing in laboratories. On the other hand, the methodology does not exclude the possibility of the plant being used as a molluscicide in the future. Valverde (2011) described that the *C. frutescens* species has capsaicin, ascorbic acid, and carotenoids, which are suggested to be molluscicidal molecules. However, the methodology applied by Silva Júnior et al. (2018) must be

adapted to determine whether the objective is to develop a product to control *A. fulica*.

Moraes et al. (2014) observed that *A. fulica* showed resistance to all extracts with lower mortality levels than those found in the study by Vieira et al. (2016). The higher mortality observed in the study by Moraes et al. (2014) was 16.66% at 100.000 ppm, thus reducing the possibility of these extracts acting on the control of terrestrial mollusks. Patiño-Montoya and Giraldo (2018) verified the presence of mollusks in a state of starvation. However, the product used was commercial cumin, which is different from other studies. It cannot be sure what caused the effect, as the commercial content can be either a natural or industrialized seasoning, which leaves a gap in the effectiveness of the test. Gusman et al. (2014) also did not verify significant action. However, the perceived activity can lead to future studies of *S. aromaticum* (clove) flower buds, since other studies, such as the one by Gusman et al. (2014) and Agostini et al. (2018), also found some activity using the same product. Parvate and Thayil (2017) also analyzed *S. aromaticum* (clove) and tested its essential oil. The authors obtained an LD<sub>50</sub> of 39.160 ppm in 96 h. However, when compared to the concentration obtained in the promising result reported by Silva Júnior et al. (2018), of 2,000 ppm,

it has a high concentration. The binary combination of *A. sativum* bulb powder + *C. deodara* oil studied by Rao and Singh (2000) showed a significant reduction in oviposition and viable egg production. However, the concentration used was 25.000 ppm, which when compared with the result obtained by Silva Júnior et al. (2018), this treatment also reveals a high concentration. Rao and Singh (2002) described that the best LC<sub>50</sub> obtained after 96 h of testing for the binary combination of *C. deodara* oil + *A. sativum* bulb powder was 4.300 ppm, with an even higher concentration compared with Silva Júnior et al. (2018). However, with a lower concentration compared to other studies. Crignis et al. (2012) also obtained a promising result, with an LD<sub>50</sub> of 4.670 ppm, a slightly higher concentration than Rao and Singh (2002). An exciting factor, both extracts with promising results reported above (Rao and Singh, 2000; Rao and Singh, 2002; Crignis et al., 2012; Silva Júnior et al., 2018) and for the other extracts showed some activity on *A. fulica*, even at higher concentrations, may be the formulation of baits with lower promising extract concentrations. For some time now, commercial baits have been used to control terrestrial mollusks, and in general, these baits are tablets made of metaldehyde, carbamates, and phosphate ions (Barker and Watts, 2002). They serve as a means of transporting the molluscicide and have attractions, increasing the substance palatability for mollusks (Edwards et al., 2009; Cardoso et al., 2015), acting as stomach poisons for mollusks (Salvio et al., 2008), with damage to gastric epithelium being the primary molluscicide bait mechanism of action (Ebenso, 2004; González-Cruz and Martín, 2013; Smith et al., 2013). Baits can increase the effectiveness of molluscicidal compounds because they can accumulate at higher concentrations in the animal intestine and cause an increase in damage.

Another factor for the increase in the effectiveness of the baits is that the contact area of the gastric epithelium is greater than the area of the cephalopedic surface, thus favoring a greater absorption of the molluscicide. Molluscicide ingestion by the mollusks that ingest the baits can still lead to easier molluscicide dispersion to other mollusk vital organs, such as liver tissues (Mandefro et al., 2018). Some studies have tested baits with synthetic molluscicides on terrestrial mollusk species, such as *A. fulica* (Smith et al., 2013), *Deroceras reticulatum* (Salvio et al., 2008), and *Eobania vermiculata* (Essawy et al., 2009). There are also reports of baits with plant extracts in terrestrial mollusks, such as *Archachatina marginata* and *Limicolaria aurora* (Ebenso, 2004). Therefore, bait formulations containing natural product extracts prove to be an up-and-coming technique that can be used for *A. fulica* mollusks.

### 3.2. Tests at different mollusk life stages and other parameters

In addition to testing the molluscicidal activity for adult individuals of *A. fulica*, Rao and Singh (2000) evaluated other parameters, such as the effects on fecundity of *A. fulica*. The authors also performed biochemical tests to analyze the mechanisms by which the tested extracts would act on the mollusks. Gusman et al. (2014) evaluated

the effect of the tested extract on adult individuals and the hatching of *A. fulica* eggs. They also evaluated whether the extracts altered mollusk growth by analyzing their final mass (g). Santos et al. (2018) tested the extract on eggs and on young and adult individuals, in addition to conducting a field trial with adult individuals of *A. fulica*. Agostini et al. (2018) analyzed changes made by the extracts on egg laying. The authors did not report the effects on adult individuals. Miranda et al. (2012) and Vieira et al. (2016) performed tests to evaluate the hatching of eggs, the survival rate, and mollusk growth. Parvate and Thayil (2017) performed histological tests to assess the toxic effect of the tested essential oil on different snail tissues. Patino-Montoya and Giraldo (2018) tested their extracts in young and adult individuals of *A. fulica*. The authors also assessed physiological aspects of snails during the trials, such as inactivity, starvation, and mucus secretion. Silva Júnior et al. (2018) also tested their extracts on individuals of different ages. Moraes et al. (2014) evaluated their extracts only on adult individuals of *A. fulica*. However, changes in movement and mollusk feeding were observed during the test. Selvi et al. (2015) observed the mortality, movement/inactivation, and loss of mucus from adult *A. fulica*.

Tests aimed at evaluating the extracts on other snail life stages are essential, given that the substance may not act on adult individuals. However, the extracts may have some effect on juvenile life stages. Given mollusk population control, the observation of changes in fertility is also essential. Other parameters, such as movement/inactivation, loss of mucus, feeding, and mollusk growth, are relevant to represent, as the extracts may not have a lethal effect and may change the behavior or physiology of a population.

### 3.3. Phytochemical analysis

Some studies involving natural products have demonstrated the molluscicidal activity of extracts, or isolated substances have indicated that molluscicidal activity may be linked to the presence of secondary metabolites, such as tannins and saponins terpenoids, steroids, and flavonoids (Cantanhede et al., 2010). Silva Júnior et al. (2018) performed a phytochemical test of the extracts. Thus, this test proves to be very interesting and necessary to identify and quantify the substances present in the extract, which may be the possible molluscicidal activity causes. In this study, phenolic compounds, tannins, flavonoids, and alkaloids were found in the *C. frutescens* aqueous extract. Saponins and anthraquinones were not found in the extract (Silva Júnior et al., 2018). In the other articles, no data from phytochemical analyses were found.

### 3.4. Ecotoxicity

The development of new molluscicides necessitates assessing the risk of causing ecotoxicity, limiting molluscicide use (Nunes et al., 2006). In the studies reported in this article, there are no reports of ecotoxicity tests. Models commonly used and recommended to assess ecotoxicity are the crustacean species *Artemia salina* (Linnaeus, 1758) (Nunes et al., 2006) and the fish of the species *Danio rerio* (Hamilton-Buchanan, 1822) (Bambino



and Chu, 2017). However, these organisms do not serve as a parameter for this review since the snail *A. fulica* is a terrestrial mollusk. Ecotoxicity tests must be realized with organisms that occupy the same habitat as these mollusks.

For comparison purposes, in studies that analyze possible molluscicides from natural products to combat aquatic mollusks, such as the genus *Biomphalaria*, it is possible to observe the concomitant performance of ecotoxicity tests in many of them. Tests involving the species *A. salina* (Rocha-Filho et al., 2015; Martins et al., 2017; Silva et al., 2018; Araújo et al., 2018), *Daphnia similis* (crustacean) (Rapado et al., 2013), *D. rerio* (Rapado et al., 2013; He et al., 2017; Pereira et al., 2017; Jia et al., 2019; Paula-Andrade et al., 2019), *Coturnix japonica* (bird) (Jia et al., 2019), and *Macrobrachium nipponense* (freshwater prawn) were performed (Jia et al., 2019). Therefore, we consider it relevant to perform ecotoxicity tests concomitantly with the tests for assessing molluscicidal activity for *A. fulica*. These tests would also facilitate extract identification at specific concentrations with action on *A. fulica* and do not induce toxicity to other nontarget organisms.

#### 4. Conclusions

*C. frutescens* (chili pepper) aqueous extract presents the most promising extract in the search for a naturally occurring molluscicidal compound. Other interesting extracts are the binary combination of *C. deodara* oil (Himalayan cedar) + *A. sativum* bulb powder (garlic) and *E. splendens* latex (Christ thorn). Given the small number of studies described during the current review, which seeks to find natural products with molluscicidal activity, there is a need for further studies to seek *A. fulica* control. In addition, there is a need to stimulate a standardization of tests involving *A. fulica* mollusks to define a limit concentration to act on mollusks that does not cause ecotoxic effects, extract application methods, mollusk length and quantity for the test, period of analysis, or parameters analyzed (biochemical and morphological). There is a need to realize tests to identify the substances present in the extracts concurrently with *A. fulica* mortality tests. Finally, together with these tests, we consider it interesting to perform tests evaluating the extract ecotoxicity on terrestrial organisms, aiming to analyze the possible consequences of these compounds to the environment.

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