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Assessment of molluscicidal activity of essential oils from five Azorean plants against *Radix peregra* (Müller, 1774)

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ABSTRACT

The molluscicidal activity of essential oils from two endemic (*Juniperus brevifolia*; *Laurus azorica*) and three introduced (*Hedychium gardnerianum*; *Pittosporum undulatum*; *Psidium cattleianum*) Azorean plants against the snail *Radix peregra* was studied under laboratory conditions. Essential oils from leaves of *H. gardnerianum*, *L. azorica* and *J. brevifolia* presented promising molluscicidal activity on both adults and juveniles stages of *R. peregra*. The molluscicidal activity of these essential oils was found to be both time and concentration dependent. Lethal concentrations (LC_{50}) varied between 15.4 (*L. azorica*) and 44.6 ppm (*H. gardnerianum*) for juveniles and from 45.3 (*H. gardnerianum*) to 54.6 ppm (*J. brevifolia*) for *R. peregra* adults. Ovicidal effect, calculated as percentage of egg hatching, at 100 ppm concentration, was observed in essential oils from *P. undulatum* flowers (4.2% of hatching) and leaves of *H. gardnerianum* (4.9%), *L. azorica* (7.4%) and *J. brevifolia* (17.7%). The present study is the first attempt to assess the molluscicidal potential of some Azorean plants essential oils against a Lymnaeidae snail. In fact, the *H. gardnerianum*, *L. azorica* and *J. brevifolia* can offer natural alternative tools for the control of *R. peregra* population, but more research is needed in order to determine the mode of action of these oils and determine the side effects on the ecosystem where this freshwater snail occurs.

1. Introduction

Fascioliasis has a worldwide distribution, being found in the subtropical and tropical regions of the world, including Latin America, USA, UK, Ireland, Europe, Middle East, Asia and Africa. Even though, the intermediate host belongs, normally, to the Lymnaeidae family, *Fasciola hepatica* reveals a high ability to parasite a large host diversity, e.g. *Lymnaea humilis*, *Lymnaea bulimoides*, *Lymnaea cubensis*, *Lymnaea viatrix* and *Lymnaea neotropica*, *Lymnaea columella* (in Latin America), and *Lymnaea bulimoides* (in North America) (Cruz-Mendoza et al., 2004; Bargues et al., 2007; Lima et al., 2009). In Europe (including Azores) the common liver fluke, *F. hepatica*, its a parasite whose primary intermediate host is the freshwater snail *Galba* (=*Lymnaea*) *truncatula* Müller, 1774.

In the Azores, the disease was first detected in 1962 in São Miguel Island, but since 1983 the Veterinary Services of the island, based on the number of animals slaughtered in controlled municipal slaughterhouse, confirmed the growth of disease.

It subsequent years, studies of Mendonça (1987) and Furtado and Cunha (2002), based on data from Ponta Delgada's slaughterhouse, refer an average infection of 7.4 and 7.6% in 1983 and 1998, respectively. In 2009, it decreases to 2.58% (unpublished data).

The most effective method of eradication of fascioliasis is through the use of fasciolicide of easy application that no let residues in meat and milk, or through control of its intermediate host (Moens, 1982). On this last system the combination of prophylactic and structured methods on the host, through integrated control processes (mechanical, environmental, biological and chemical) could be the most effective approach. In Azores, several studies have addressed the distribution of *G. truncatula* in São Miguel Island (Backhuys, 1975; Mendonça, 1989a; Martins, 1991) as well as their control, both biological and ecological measures (Cunha, 1993), chemical and via the molluscicidal activity of floral species (Mendonça, 1989b).

The investigation of the molluscicidal properties of plants has recently been greatly expanded, with more than 1400 species studied thus far. Plant extracts have been used to control the snail population with the advantage that, besides being less toxic in nature, they can be degraded faster than the synthetic molluscicides that have also high cost, possible build up of snail resistance, and toxicity in nontarget organisms (Jaiswal and Singh, 2008; Radwan et al., 2008). Many plant products have been found to have a high molluscicidal potential (Singh et al., 1996a, 2006; Lahlou and Berrada,

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2001; Singh and Singh, 2005; Silva et al., 2006; Adenusi and Odaibo, 2008; El-Kamali et al., 2010; Kumar et al., 2010; Larhsini et al., 2010; Upadhyay and Singh, 2011).

In the Azores there are approximately 1000 vascular plant species. These plants are either part of the primitive vegetation in which 72 species are endemic, or have been recently introduced. Some of the plants, which were introduced for cultivation or ornamental purposes, are now naturalized and are spreading outside of the locations where they were originally planted. This study is a fraction of a larger project to investigate new environment-oriented tools to control trematode vectors in the Azores.

Particularly, this study focuses in the molluscicidal and ovicidal activity of essential oils from two Azorean endemic and three introduced plants against *R. peregra*, and thus a potential intermediate host for *F. hepatica*.

2. Materials and methods

2.1. Plant material and distillation of the essential oils

Leaves of the Azorean endemic plants *Juniperus brevifolia* (Seub.) Antoine (Cupressaceae) and *Laurus azorica* (Seub.) Franco (Lauraceae) were collected in protected locations near Nordeste (São Miguel Island) during the Summer of 2008. *Hedychium gardnerianum* Sheppard ex Ker-Gawler (Zingiberaceae), also locally known as "conteira", was collected during the Summer of 2008, in Sete Cidades (São Miguel Island) and its leaves used for essential oil preparation. Leaves and flowers of *Pittosporum undulatum* Vent. (Pittosporaceae) were collected in the campus garden of the University of the Azores during the flowering period. Finally, *Psidium cattleianum* Sabine (Myrtaceae) fruits were purchase from a local producer near Ponta Delgada. After collection, each plant material was placed in plastic bags and immediately brought to the laboratory, where it was stored at −20 °C. Specimens were identified by specialist from the Herbarium of Carlos Machado Museum in Ponta Delgada.

A minimum amount of 2 kg of fresh vegetal material was used for the preparation of essential oils. Essential oils were obtained from the different plant parts by hydrodistillation during 4 h, using a modified Clevenger apparatus according to the method described by Medeiros et al. (2003). The stock solutions were dissolved in ethanol at the concentrations of 100 mg essential oils per mL (w/v).

2.2. Animal material

Adult snails of *R. peregra* (1.34 cm ± 0.20 cm in length) were collected locally from Furnas (São Miguel Island) during the months of April and May 2009. The snails were allowed to acclimatize under laboratory conditions for 72 h before experiments. Some snails, not used in the assay, were maintained in polyethylene aquariums (35.7 cm × 23.5 cm × 13.4 cm) containing 3 L of spring water (19 ± 1 °C, pH 7.3–7.4), with air pumps working permanently. In order to prevent any contamination, three times per week the aquaria was cleaned, removing excrements and dead snails. Lettuce leaves (*Lactuca sativa* L.) were used as foodstuff. Laboratory-bred cultures of adult snails were kept for reproduction to obtain eggs and juveniles. The egg masses were observed under the light microscope to ascertain and confirm initial viability and extent of egg embryonation. Only viable eggs were used for ovicidal experimentation. A second group of eggs were kept for hatching to obtain juvenile snails (48–96 h after eclosion) which were further assayed in the same conditions as the adults.

2.3. Molluscicidal activity assay

The molluscicidal activity of essential oil samples was evaluated according to the method described by Schall et al. (1998) against juveniles and adults of *R. peregra*. Ten juveniles or adult snails were randomly transferred into glass beakers and submerged in 50 mL of spring water containing oil sample (50 μL of the stock solutions) at a final concentration of 100 ppm concentration for 24 h and maintained at room temperature (23 ± 1 °C) under natural light–dark photoperiod. The beakers were covered with a plastic film (with small holes to permit aeration) to prevent snails from falling out. None of the snails were fed during this period. At the end of the exposure period, the surviving snails were washed and rinsed in spring water to remove essential oil solution and transferred into new beakers containing an identical volume of spring water for a 24 h phase of recovery. During this period, they were fed with fresh lettuce leaves. Control experiments were executed similarly, and simultaneously as the treatments. At the end of the recovery period the snail's mortality was determined, and confirmed by absence of heartbeat (in juveniles) and lack of reaction by probing the snails with a needle to elicit typical withdrawal movements (in adults). Four replicates of 10 snails were used for both essential oil samples and controls in this single-dose screening assay.

2.4. Determination of lethal concentration (LC_{50}) and lethal time (LT_{50})

The essential oil samples, whose mortality was higher than 70%, were tested at six different concentrations (juveniles: 10, 20, 30, 40, 45, 60 ppm; adults: 30, 40, 50, 60, 70, 80 ppm) under the same conditions described above. Four replicates of 10 snails per concentration were used for this assay and statistic parameters lethal concentration LC_{50} and LC_{90} were determined.

To investigate time/mortality relationship (after time of exposure), the adults snails were exposed during 4, 8 and 16 h to the essential oil sample at 50 ppm concentration (equivalent to the LC_{50} values), below the equal circumstances described above. Snail's mortality was assessed every 2 h until 90% mortality was achieved. Each treatment was repeated three times using 10 snails.

2.5. Assay for activity against egg masses

To evaluate the ovicidal effect according to the method described by Adenusi and Odaibo (2008), *R. peregra* egg masses containing 35–40 eggs each (48–72 h old), were permanently exposed for 24 h to 100 ppm essential oil solutions (dissolved in less than 5% ethanol). One egg mass per replicate with a total of three replications was used. After the exposure period, the egg masses were washed and rinsed several times in spring water to remove the sample solution and transferred to beakers for incubation until hatching. When the desired developmental time was reached, the egg masses were examined daily and the number of unhatched eggs was counted and the ovicidal activity calculated as percentage hatching. An embryo in an egg mass was considered dead if its cells became opaque or disaggregated or if unhatched at the end of the experiment.

Two sets of controls were used. One set of egg masses was placed in water and a second group dipped in ethanol 5% (v/v) to determine the mortality derived from the solvent used to dissolve the essential oils solutions.

2.6. Analysis data

Single-concentration mortality and ovicidal data were subjected to an analysis of variance procedure after arcsine transformation. Means with significant variance and *F*-statistic were

Table 1
Mortality (%) of *Radix peregra* when exposed for 24 and 48 h at a concentration of 100 ppm of essentials oils from Azorean plants.

Essential oils (parts used)		n^A	Juveniles	Adults	
			24 h (±SEM)[B]	24 h (±SEM)[B]	48 h (±SEM)[B]
Control	*Untreated*	40	0.0 ± 0.00^b	0.0 ± 0.00^b	2.5 ± 2.50^c
	Ethanol	40	2.5 ± 2.50^b	0.0 ± 0.00^b	5.0 ± 2.90^c
Endemic	*Juniperus brevifolia* (leaves)	40	100.0 ± 0.00^a	97.5 ± 2.50^a	100.0 ± 0.00^a
	Laurus azorica (leaves)	40	100.0 ± 0.00^a	100.0 ± 0.00^a	100.0 ± 0.00^a
Introduced	*Hedychium gardnerianum* (leaves)	40	100.0 ± 0.00^a	97.5 ± 5.00^a	100.0 ± 0.00^a
	Pittosporum undulatum (flowers)	40	100.0 ± 0.00^a	10.0 ± 0.00^b	10.0 ± 0.00^c
	Pittosporum undulatum (leaves)	40	100.0 ± 0.00^a	10.0 ± 5.80^{b,*}	50.0 ± 17.3^b
	Psidium cattleianum (fruits)	40	100.0 ± 0.00^a	7.5 ± 4.80^{b,*}	26.7 ± 8.70^b

[A] Number of snails test.
[B] The means (±SEM) of mortality in the same column followed by the same letters are not significantly different based on ANOVA one-way; Least Significant Differences test for juveniles (*F-test* = 6.639, *df* = 31) and adults (24 h: *F-test* = 28.203, *df* = 31; 48 h: *F-test* = 126.786, *df* = 31).
[*] The means (±SEM) of mortality followed by the same symbol are significantly different based on ANOVA one-way; Least Significant Differences test for *P. undulatum* leaves (*F-test* = 8.271, *df* = 7) and *P. cattleianum* fruits (*F-test* = 7.146, *df* = 7).

separated by least significant differences test (LSD) using SPSS statistic package, software version 15.0 for Windows (SPSS, 2006).

Data from concentration-mortality and time-mortality assays from all replicates were used to calculate the LC_{50} (the concentration required to kill 50% of the snails), and LT_{50} (the exposure time required to obtain 50% mortality of snails) values, after correction to mortality data according to Abbot (1925). Then, this data were analyzed using the Statistical Analysis System (SAS, 1982). χ^2 test was used to assess the goodness-of-fit of the model to the data (Finney, 1971). Differences among snails mortality were considered significant when the values from lethal concentration or lethal time failed to overlap the 95% confidence limits (CL).

3. Results

3.1. Snail's susceptibility

The toxicity of the essential oils from two endemic and three introduced plants common in Azorean archipelago against juveniles (2–3 d olds) and adult stages of *R. peregra*, are shown in Table 1. The results showed that essential oils from leaves of *H. gardnerianum*, *L. azorica* and *J. brevifolia* were the most effective against juveniles and adults of *R. peregra*, followed by *P. undulatum* (leaves) and *P. cattleianum* (fruits). Essential oil from *P. undulatum* (flowers) was the least effective. The lethal effect showed on the juveniles of *R. peregra* was higher than the effect on adult snails. Actually, all samples exhibited considerable appetency for killing juvenile snails (100% mortality at 24 h), showing no statistical differences between them but different from the control ($P < 0.05$). However, during the exposure period (24 h) adults mortality ranged from 7.5% to 100% with significant difference between the samples ($P < 0.05$) where *H. gardnerianum*, *L. azorica* and *J. brevifolia* revealed a mortality rate higher than 97.5%. After the recovery period (after 48 h from the beginning of the assessment), the mortality ranged from 10% to 100% ($P < 0.05$). In adults, the mortality registered at 24 h was significantly lower than the one observed after the recovery period in *P. undulatum* (leaves) ($P < 0.05$), and *P. cattleianum* (fruits) ($P < 0.05$).

3.2. Dose–response

The three essential oils that showed the highest molluscicidal activity against both juveniles and adult stages of *R. peregra* at a concentration of 100 ppm were selected to be tested at lower concentrations in order to determine their respective LC values. χ^2 values were not significant ($P > 0.05$), for any essential oil, indicating that the data fit the assumptions of the Probit model. Slopes of the Probit regression lines observed for the concentration-mortality curves are significant among essential oils. The essential oils toxicity was dose dependent against two stages of *R. peregra* (Table 2).

3.3. Laurus azorica

presents the lowest slope (3.6) and *H. gardnerianum* the highest one (10.5) for adult snails. The LC_{50} values calculated for the essential oils, at end of recuperation period, ranged from 45.3 to 54.6 ppm. Based on the nonoverlap of 95% confidence limits of the LC_{50}, these essentials oils were grouped in two classes, the first

Table 2
Estimated LC_{50} concentrations of essential oils Azorean plants, against juveniles and adults of *Radix peregra*.

Essential oils	Plant part	n^A	Concentration interval (ppm)	LC_{50} (ppm)	Limits[B]		Slope value	H^C
					LCL	UCL		
Juveniles								
Hedychium gardnerianum	Leaves	40	20; 30; 45; 60	44.6^a	40.3	51.9	4.40 ± 0.64^b	0.92
Juniperus brevifolia	Leaves	40	10; 20; 30; 40	27.9^b	26.8	28.8	13.5 ± 1.15^a	0.93
Laurus azorica	Leaves	40	10; 20; 40; 60	15.4^c	13.9	16.8	3.30 ± 0.28^b	1.37
Adults								
Hedychium gardnerianum	Leaves	40	30; 40; 60; 70	45.3^b	43.5	47.2	10.5 ± 0.77^a	1.68
Juniperus brevifolia	Leaves	40	30; 40; 60; 70	54.6^a	51.5	57.7	6.50 ± 0.79^b	0.28
Laurus azorica	Leaves	40	30; 40; 60; 70	52.2^a	47.2	60.9	3.60 ± 0.62^c	1.34

[A] Number of snails test excluding controls.
[B] 95% Confidence limits expressed in number of ppm of essential oil required to kill the snails. LC values and slopes within a column followed by the same letter are not significantly different based on nonoverlapping 95% CL.
[C] *H*, heterogeneity factor (χ^2:*df*).

Table 3
Estimated LT_{50} and LT_{90} of essential oils Azorean plants, against adults of *Radix peregra*.

Essential oils (parts used)	4 h Exposition				8 h Exposition				16 h Exposition			
	LT_{50} (95% CL)[B]	LT_{90} (95% CL)[A]	Slope (±SEM)	H^B	LT_{50} (95% CL)[B]	LT_{90} (95% CL)[A]	Slope (±SEM)	H^B	LT_{50} (95% CL)[B]	LT_{90} (95% CL)[A]	Slope (±SEM)	H^B
Hedychium gardnerianum (leaves)	49.3^c (46.1–52.2)	81.3^c (72.2–100.1)	5.9 ± 0.89^a	0.38	54.9^a (51.6–59.4)	92.8^b (79.3–125)	5.6 ± 0.92^a	0.49	18.0^a (14.8–21.1)	63.3^a (50.3–89.2)	2.4 ± 0.28^a	0.81
Juniperus brevifolia (leaves)	203.4^a (107–993)	25606.7^a (3131–7216996)	0.6 ± 0.14^c	0.62	50.2^a (46.9–108)	110.7^b (95.5–127)	3.9 ± 0.35^a	0.62	4.25^b (2.36–5.8)	20.4^b (16.8–27.6)	1.9 ± 0.57^a	0.60
Laurus azorica (leaves)	77.9^b (68.6–93.4)	299.9^b (202.3–624.1)	2.9 ± 0.34^b	0.89	60.4^a (55.5–66.7)	184.7^a (148–256)	2.6 ± 0.24^b	0.54	18.1^a (16.0–20.6)	60.1^a (46.5–89.4)	2.5 ± 0.29^a	1.56

[A] LT_{50}, LT_{90} values and 95% confidence limits (CL) expressed in number of hours to kill the snails. LT values and slopes within a column followed by the same letter are not significantly different based on nonoverlapping 95% CL.
[B] *H*, heterogeneity factor (χ^2:*df*).

includes *H. gardnerianum* (45.3 ppm of LC_{50}) and the second includes *L. azorica* and *J. brevifolia* (52.2 and 54.6 ppm, respectively).

For juveniles snails, *L. azorica* presents the lowest LC_{50} value (15.4 ppm) which is followed with the lowest slope (3.3). On the other hand, *J. brevifolia* has the highest slope (13.5) and and *H. gardnerianum* the highest LC_{50} (44.6 ppm). The slopes were all statistically different.

3.4. Time-response

The estimated lethal time (LT_{50}) for essential oil samples ranged from 4.3 to 203.2 h in the three stipulated times (4, 8 and 16 h) (Table 3). Probit regression parameters for the time-mortality data indicated the model was valid for all samples. Slope estimated for all of the Probit regression lines were significantly different and ranged from 0.6 to 5.9. Based on the nonoverlap of 95% confidence limits, significant differences were established among LT_{50} values. These differences occur in a different way in the three times observed. At 4 h expososure *H. gardnerianum* shows the lowest LT_{50} (49.3 h). At 8 and 16 h exposition *J. brevifolia* presents the lower LT_{50} (50.2 and 4.3 h, respectively).

3.5. Ovicidal effects

The egg hatch rate ranged from 4.2% to 97.2%, for *P. undulatum* (flowers) and ethanol 5% (v/v), respectively, with significant differences among treatments ($P < 0.05$). Based on the ovicidal activity of essential oils at 100 ppm, the results were classified into two different groups. *P. undulatum* flowers (4.2% of eclosions), leaves of *H. gardnerianum* (4.9%), *L. azorica* (7.4%) and *J. brevifolia* (17.7%) exhibit high activity and showed no significant differences between them. The second group, with a reduced or much reduced activity, was formed by fruits of *P. cattleianum* (89.2%) and leaves of *P. undulatum* (55.7%) (Table 4).

4. Discussion

Our observations on the field revealed that in São Miguel Island the density of *G. truncatula* decreases drastically, when compared with data from Martins (1991). This circumstance may be due to veterinary programs implemented in the last two decades that aiming to control the helminthiasis in the island. Concomitantly to a reduction of the population density of this snail, there is an increase in *R. peregra* population density, in the same places where *G. truncatula* was previously found. This phenomenon could be due to an opportunistic recovery of other molluskan species (e.g. *R. peregra*) as a consequence of a massive use of synthetic molluscicides (Godan, 1983; Agarwal and Singh, 1988).

The decrease in the average of cattle infection rate, recorded in 2009, could be associated with changes in population density of these two Lymnaeidae species in the island, since the prevalence of infection in snails depends on the possibilities of a natural encounter between snails and trematodes. However, the ability to parasitize different Lymnaeidae species and the high density of an alternative intermediate host might contribute to sustain the larval development of this trematode (Rondelaud, 1993; Dreyfuss et al., 2000). This unspecificity of *F. hepatica* is well documented in the work of Correa et al. (2010), where 26 lymnaeid species, including *R. peregra*, are referred as natural or experimentally infected with this trematode. Even in Ireland, where *G. truncatula* was the only recorded intermediate host of *F. hepatica,* the evidence of infection in *R. peregra* was also observed by Relf et al. (2009), helping to explain the presence of infection in animals grazing in habitats where *G. truncatula* is absent.

Table 4
Percentage of hatching eggs of *Radix peregra* when exposed for 24 h to 100 ppm of essentials oils from five Azores plants.

Essential oils (parts used)		n^A	% Of hatching (±SEM)[B]
Control	*Untreated*	106	97.0 ± 2.1[a]
	Ethanol	111	97.1 ± 0.4[ab]
Endemic	*Juniperus brevifolia* (leaves)	110	17.7 ± 8.5[d]
	Laurus azorica (leaves)	119	7.40 ± 5.4[d]
Introduced	*Hedychium gardnerianum* (leaves)	107	4.90 ± 1.0[d]
	Pittosporum undulatum (flowers)	110	4.20 ± 2.5[d]
	Pittosporum undulatum (leaves)	108	55.60 ± 12.7[c]
	Psidium cattleianum (fruits)	105	89.2 ± 3.0[b]

[A] Number of eggs test.
[B] The means (±SEM) of mortality in the same column followed by the same letters are not significantly different based on ANOVA one-way; Least Significant Differences (*F-test* = 47.586, *df* = 23).

The molluscicidal activity of the essential oils from Azorean endemic and introduced plants against *R. peregra* was established in the present work. In previous studies, Mendonça et al. (1993) demonstrated that the aqueous and organic extract of *H. gardnerianum* and *P. undulatum* also has molluscicidal activity against *G. truncatula*. Furthermore, Warren and Peters (1968) verified that *Schistosome cercariae* is killed by a substance released from the stems of *H. coronarium*.

Our results indicate that from the six essential oils tested, only *H. gardnerianum*, *L. azorica* and *J. brevifolia* are potential source of botanical molluscicides, with toxicity LC_{50} being less than 55 ppm against juveniles and adults *R. peregra*. These values are, according to Singh et al. (1996b), in the range of high molluscicidal activity. On the other hand, *P. cattleianum* (fruits) and *P. undulatum* (flowers and leaves) showed molluscicidal activity against juveniles at a concentration of 100 ppm. For the same concentration, the essential oils from leaves of *H. gardnerianum*, *L. azorica* and *J. brevifolia* and flowers of *P. undulatum* were able to inhibit egg masses hatching.

Toxic effects of *H. gardnerianum*, *L. azorica* and *J. brevifolia* essential oils are time and dose dependent, as it is evidenced from regression between exposure period and LT_{50} of different treatments. The time dependent toxic effect of plant products may be either due to the uptake of the active moiety, which progressively increases the internal dose and the biologically active dose, or the active compounds metabolized by snails into a new substance with higher toxicity, as it was suggested by Kumar and Singh (2006).

According to Isman (2000), some essential oils have a broad spectrum of biological activities, and their action is due to the compound (s) that occurs in greater quantity in the extract and/or to a synergistic effect between compounds. The identity of the active principles of *J. brevifolia, L. azorica,* and *H. gardnerianum* was previously reported, and according to Silva et al. (2000), Pedro et al. (2001) and Medeiros et al. (2003), the monoterpene fraction predominates in the three oils, being α-pinene the main constituent of *H. gardnerianum* and *L. azorica* and limonene of *J. brevifolia*. Molluscicidal activity of these two compounds, α-pinene and limonene, was described by Lahlou (2004), as well as differences in bioactivity between the enantiomers, thus enabling different modes of action of molluscicides may conceivably be a multicomponent process, affecting more than one system. One of the systems studied by Jaiswal et al. (2008) and Kumar et al. (2009) is the effect of some natural compounds on various enzyme activities, essential for the physiological functioning of mollusks.

In conclusion, this is the first evaluation study of essential oils from Azorean plants against the snail *R. peregra*, a potential alternative intermediate host of *F. hepatica* in natural habitats. Further studies are required to determine the mode-of-action of these essential oil products and the damage they cause to the snail's tissues and the side effects that may exist for the wildlife that surrounds them, through semi-field trials.

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