

Effects of five exotic invasive plants extracts on the survival of the invasive snail *Pomacea canaliculata* (Lamarck)

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ABSTRACT

The toxicity of cold water (25±1°C) and boiling water extracts of 5-invasive plants including *Mikania micrantha* (Kunth), *Wedelia trilobata* (Linnaeus), *Ageratum conyzoides* (Linnaeus), *Ambrosia artemisiifolia* (Linnaeus) and *Spartina alterniflora* (Loisel) was examined on mortality of golden apple snails (*Pomacea canaliculata* Lamarck). The cold water extracts were more toxic than boiling water extracts. The toxic potential of tested water extracts followed the order: *M. micrantha* > *W. trilobata* > *A. conyzoides* > *A. artemisiifolia* > *S. alterniflora*. The toxicity of water extracts varied with concentration. Among the 5-plants tested, *M. micrantha* proved most toxic to snails.

Key words: *Ageratum conyzoides*, *Ambrosia artemisiifolia*, biocontrol, Golden apple snail, invasive plants, *Mikania micrantha*, mortality, *Pomacea canaliculata*, *Spartina alterniflora*, toxicity, *Wedelia trilobata*

INTRODUCTION

Golden apple snails (GAS, *Pomacea canaliculata* Lamarck) were introduced into the Asian countries in the 1980s from their native habitat in South America for commercial purposes (7,8,21), but this proved a market failure and the snails were abandoned into rivers, canals and paddy fields (5,15). These snails proliferated quickly because of the absence of a native enemy and caused huge losses in rice production as snails feed on the rice seedlings (18,23,26). They also became the natural intermediate hosts for the parasite of *Angiostrongylus cantonensis* (24) and threatened the biodiversity of Asian wetland (4,9). Hence, the golden apple snail is now considered as one of the 100 worst invasive alien species by the Invasive Species Specialist Group of IUCN (19).

Molluscs can be controlled by molluscicides of synthetic or plant origin (13,18) but the use of synthetic molluscicides causes serious environmental pollution and is hazardous to human health. Hence low cost, environmental friendly toxic materials of plant origin [*Chenopodium quinoa*, *Sapindus mukorossi*, *Oldenlandia affinis* and *Viola odorata*] to control this snail (10,24,22) have been examined. Many plant extracts contain bioactive compounds with anti-feedant, anti-germination, insecticidal or anti-bacterial properties etc. (11,12,20,27). However, little information is available about the

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molluscicidal nature of toxic substances from alien invasive plants. In South China, alien invasive plants such as *M. micrantha*, *W. trilobata*, *A. conyzoides*, *A. artemisiifolia* and *S. alterniflora* have significantly damaged the native ecosystem (14,28,29). These plants have dispersed widely on the farmlands, field ridges and on hillsides and have become the malignant weeds endangering crop production. As an amphibian animal, the golden apple snail and the above-mentioned invasive plants usually coexist in the crop lands for certain periods, when the water in paddy fields is drained off after the crop harvest. Moreover, the snails usually lay their eggs on the stems of some weeds growing along field ridges. In this study, the toxic activities of cold water and boiling water extracts of these five invasive plants on the snail were examined with the objective of identifying plants possessing anti-molluscan properties.

MATERIALS AND METHODS

Aerial parts of the 5-invasive plants viz. *M. micrantha*, *W. trilobata*, *A. conyzoides*, *A. artemisiifolia* and *S. alterniflora* were collected in the vegetative stage from the Research and Teaching Farm, South China Agricultural University (23°14'N, 113°38'E). Among these, *S. alterniflora* belonged to Poaceae, and the others to Compositae.

I. Preparation of plant extracts

Aerial plant parts were washed with dechlorinated water and air-dried in shade at room temperature ($25 \pm 1^\circ\text{C}$) for 48 h. Thereafter, the plant materials were cut into small pieces, dried at 60°C for 24 h in an oven and pulverized using a plant grinder. The powders (40 mesh) were stored in a desiccator. The Experimental treatments consisted of two factors : Water 2 types (water, boiling water) and Plant materials 4 levels (0,2,5,10 g/L).

Different concentrations (2, 5 and 10 gL^{-1}) of water extract were prepared by soaking 2, 5 and 10 g of ground plant material in dechlorinated water (1L) for 24 h at room temperature ($25 \pm 1^\circ\text{C}$). The solutions were filtered through a 2-layer gauze. Boiling water extract was prepared by boiling 100 g plant material with 1 L dechlorinated water for 1 h; the extract was filtered through a 2-layer gauze and the residue was resuspended in 0.50 L dechlorinated water and boiled again for 30 min. It was then filtered, the two extracts were combined and the volume made to 1 L with dechlorinated water. Different concentrations of boiling water extract were obtained by diluting the extract to 2, 5, 10 g l^{-1} concentrations, respectively. This range was determined by a preliminary test in which the toxic effects of the extracts on the snail in 24 h were determined using a series of concentrations ranging from 0-10 g l^{-1} .

II. Golden apple snails (GAS)

Golden apple snails (GAS) were collected from the paddy fields of South China Agricultural University. Prior to their use in the trials, snails were acclimatized for one week to laboratory conditions in plastic basins with dechlorinated water (6 L each) and were fed with Chinese cabbage (*Brassica pekinensis*). Dechlorinated water in basins was renewed daily. Snails were grouped into 4-groups based on height measured with a Vernier caliper and classified according to their shells height (H): GAS I ($2 \text{ mm} \leq H \leq 10 \text{ mm}$),

GAS II (10 mm < H ≤ 17 mm), GAS III (17 mm < H ≤ 27 mm) and GAS IV (27 mm < H ≤ 34 mm). Only active snails were chosen for the toxicity test. Group I and II, snails were juveniles and were sexually undifferentiated. Group III and IV snails were either male or female recognized by their humped operculum (6).

Snail mortality was determined as described by Abdelgaleil *et al.* (1) and Huang *et al.* (10). Snails whose pleopoda were exposed outside and in a static state were considered dead (1, 10). These suspected dead snails were also checked by stimuli with a stainless steel needle and by slightly pulling with a small tweezer. Only snails whose pleopoda easily slid out of the shell without any reaction to stimuli were confirmed dead.

III. Toxicity assay

Toxicity of different concentrations of water extract and boiling water extract was determined using dechlorinated water as negative control. No snail died in water (control) during the experimental period (120 h). For the tests, 10 snails of each size (I, II, III and IV) were placed in a plastic basin (22.5 cm top internal diameter, 15.5 cm bottom internal diameter and 8.0 cm height) containing 1 L of various solutions and the basins were sealed with one layer of gauze to prevent the snails from escape. Each test had three replicates. Dead snails were recorded at 48 h and 120 h and removed from the basins. During the experimental period, snails were not fed and the temperature was maintained at 25±1°C.

Toxicity was determined as the survival ratio of snail under experimental concentrations and expressed as

$$\text{Survival ratio}(\%) = \left(1 - \frac{\text{number of dead snails}}{\text{number of tested snails}}\right) \times 100$$

Number of dead snails was obtained by counting the dead snails of different sizes or total dead snails. Number of tested snails was 10 for each size and 40 individuals in total.

Toxicity index is a fundamental concept used in the method of whole range evaluation of different strengths. It was calculated to reflect the overall effects across the different experimental concentrations. It was calculated as Under:

$$\text{Toxicity index}(\%) = \frac{\text{Inhibition area}}{\text{Total area}} \times 100$$

The computation of inhibition area, total area and the inhibition index was done with softwares using a mathematical integration function combined with experimental concentrations. In this study, the toxicity index was calculated using software WESIA (Whole-range Evaluation of the Strength of Inhibition in Allelopathic-bioassay) (17).

IV. Statistical analysis

Dose-response data were analyzed using the whole-range assessment method (2). The whole-range assessment was done by using the program WESIA (Whole-range Evaluation of the Strength of Inhibition in Allelopathic-bioassay) developed by Liu *et al.* (17). In this study, the toxicity index was calculated as per the whole-range assessment

method, which was a summary of overall biological response of GAS to the plant extracts tested. Snail mortalities were represented by mean values and standard error. One-way analysis of variance (ANOVA) was applied to determine the significant differences in the different treatments using SPSS 13.0 software.

RESULTS AND DISCUSSION

Toxicity indices of water extracts of invasive plants

I. *M. micrantha*: The values of toxicity index in treatments of water extract followed the order : GAS I > GAS IV > GAS III > GAS II, while, in the treatment of boiling water extract, they followed order : GAS III > GAS I > GAS II > GAS IV (Table 1). The toxicity index of boiling water extract on GAS size-III was 3 times higher than that on GAS size-IV. GAS size-I and III was vulnerable to water extract and boiling water extract, respectively. However, the values of toxicity indices were quite different between the water extract and boiling water extract treatments. For GAS size-IV, the toxicity index of water extract was 22 times higher than boiling water extract. Hence boiling water extract method is not suitable to determine the toxicity of plant components, perhaps because the boiling destroys the active toxic chemicals and hence, weakens the toxic potential of tested plants. This however needs further examination, because plants extraction duration for two methods was different. The extraction time in boiling treatments perhaps was not adequate to prepare the extract.

Table 1. Toxicity index of extracts on different sizes of Golden apple snails (GAS) at 48 h

GAS size	<i>M. micrantha</i>		<i>W. trilobata</i>		<i>A. conyzoides</i>		<i>A. artemisiifolia</i>		<i>S. alterniflora</i>	
	Water extract	Boiling water extract	Water extract	Boiling water extract	Water extract	Boiling water extract	Water extract	Boiling water extract	Water extract	Boiling water extract
I	87.8	4.5	73.3	47.3	59.8	20.2	33	14.6	6.3	0.7
II	78.7	4.4	64.7	30	48.0	4.4	31.9	7.5	5.3	2.2
III	79.7	11.2	57.7	32.5	48.3	5.8	25.9	6.7	7.5	2.2
IV	82.1	3.7	56.7	41.5	43.5	31.9	16.4	14.7	16.4	12.7

Note: Snails are divided into 4-groups according to their shells height (H): GAS I (2 mm <= H <= 10 mm), GAS II (10 mm < H <= 17 mm), GAS III (17 mm < H <= 27 mm) and GAS IV (27 mm < H <= 34 mm). The tested concentrations of water extract and boiling water extract are 2 g l⁻¹, 5 g l⁻¹, and 10 g l⁻¹.

II. *W. trilobata*: The toxicity index values in treatments of water extract followed the order : GAS I > GAS II > GAS III > GAS IV. The toxicity index of water extract on GAS size-I was 1.5 times higher than boiling water extract. In treatment of boiling water extract, toxicity index values of *W. trilobata* extract was in order : GAS I > GAS IV > GAS III > GAS II. For water extract and boiling water extract treatments, it was similar and the highest toxicity index was observed in GAS size-I. In this study, based on size, the GAS were divided into 4-groups [I, II, III, and IV]. Generally small snails are more vulnerable to plant toxins than larger ones. Young snails of *Biomphalaria alexandrina* are sensitive to plant extract of *Euphorbia splendens*, *Atriplex stylosa* and *Guayacum officinalis* (3).

III. *A. conyzoides*: The toxicity index values in treatments of water extract were in order: GAS I > GAS III > GAS II > GAS IV. Toxicity index of water extract on GAS size-I was 1.4 times higher than on size-IV. In the treatment of boiling water extract, the toxicity index were in order of : GAS IV > GAS I > GAS III > GAS II. The toxicity index of boiling water extract on GAS size-I and size-IV were quite different than on GAS size-II and size-III. Heating treatment can not extract enough toxic substances from *A. conyzoides*, perhaps due to deactivation of effective chemicals or insufficient extraction process.

IV. *A. artemisiifolia*: The toxicity index values in treatments of water extract were in order of : GAS I > GAS II > GAS III > GAS IV. The toxicity index of water extract on GAS size-IV was nearly half of that on GAS size-I. In the boiling water extract treatment, toxicity index values were in order of : GAS IV > GAS I > GAS II > GAS III. The toxicity indices of boiling water extract on GAS size-I and IV were nearly twice than those on GAS size-II and size-III. Further, the toxicity indices were quite different between the two extraction methods. Snails of size-IV were more tolerant to water extract of *W. trilobata*, *A. conyzoides*, *A. artemisiifolia* than of size-II and size-III, indicating that larger snails are more resistant to the active components in plant extracts. Although one expects that larger snails expose greater surface area for toxicant contact, hence, should be more susceptible but this is not the case herein. It is presumed that toxicity is not related only to only size but to perhaps other unidentified factors.

V. *S. alterniflora*: The toxicity index in the treatments of water extract were in order of : GAS IV > GAS III > GAS I > GAS II. The toxicity of water extract of *S. alterniflora* on GAS I was different than other four plants. Obviously, snails of size-I were also more vulnerable to water extract of all plants, except of *S. alterniflora*. In treatment of boiling water extract, the values were in the order of GAS IV > GAS II, GAS III > GAS I. The toxicity index of boiling water extract on GAS size-II was the same as that of size-III. Compared with other plants, the effects of water extract and boiling water extract of *S. alterniflora* against snail size-I to size-IV were very weak. However, the toxicity index of its water extract and boiling water extract on GAS size-IV was higher compared with other sizes of GAS, showing their strong effects on GAS of size-IV. Joshi *et al.* (13) reported that GAS with 15-20 mm length were most resistant to extract of *Chenopodium quinoa*. In this study, we also found that the size-II showed greater resistance to water extract of *S. alterniflora* than other sizes.

The toxic potential of water extract of the tested invasive plants was in order of: *M. micrantha* > *W. trilobata* > *A. conyzoides* > *A. artemisiifolia* > *S. alterniflora* (Table - 2). *M. micrantha* was most toxic to GAS. Toxicity index of water extract of *M. micrantha* was 11-times higher than *S. alterniflora*. Further, toxicity index was quite different between the water extract and boiling water extract of *M. micrantha*. For boiling water extract, the toxicity of tested plants was in order of : *W. trilobata* > *A. conyzoides* > *A. artemisiifolia* > *M. micrantha* > *S. alterniflora* and the toxicity index was very lower than water extract of these five plants. *S. alterniflora* extracts showed the least toxicity among the tested plant species while *M. micrantha* being the strongest. The toxic characteristics of many plant extracts on mollusks are often affected by plant species, mollusk species, methods of extract preparation, tested concentrations and sampling part of plants (10,22). We compared the toxicity indices of 5- plants and found that water extract of *M. micrantha* was the most toxic.

Table 2. Toxicity index of five plant extracts on Golden apple snails at 48 h

Plant species	Water extract	Boiling water extract
<i>M. micrantha</i>	82.0	7.0
<i>W. trilobata</i>	61.8	37.3
<i>A. conyzoides</i>	51.4	16.1
<i>A. artemisiifolia</i>	24.3	10.6
<i>S. alterniflora</i>	7.3	3.9

Note: The tested concentrations of water extract and boiling water extract are 2 g l⁻¹, 5 g l⁻¹, and 10 g l⁻¹.

GAS Mortalities

I. *M. micrantha*: The 100 % mortality occurred within 120 h in all 3- concentrations of water extract. However, none of the 3- concentrations of boiling water extract of *M. micrantha* completely killed the GAS at 120 h (Fig. 1). It is obvious that water extract of *M. micrantha* can be used as a potential molluscicide. Some plants [*Chenopodium quinoa*, *Sapindus mukorossi*, *Oldenlandia affinis* and *Viola odorata*] extract are used to kill GAS (10,24,22). In this study, water extract of *M. micrantha* may contain bioactive compounds with molluscicidal properties, which is worth a further examination.

II. *W. trilobata*: The mortalities of GAS at 48 h in water extract (2.0 g l⁻¹), boiling water extract (2.0 g l⁻¹) and boiling water extract (5.0 g l⁻¹) were lower than other treatments (Fig. 2). However, at 120 h > 90% of GAS were killed in water extract (5.0 g l⁻¹), water extract (10.0 g l⁻¹) and boiling water extract (10.0 g l⁻¹); the mortality of GAS in both water extract (2.0 g l⁻¹) and boiling water extract (2.0 g l⁻¹) was < 40%. These results indicated that the toxicity of *W. trilobata* depended on concentrations of active chemicals in extract. Current extract methods may be not suitable for the release of toxic chemicals in *W. trilobata*.

III. *A. conyzoides* : At 120 h, 100% mortality occurred only in water extract (10.0 g l⁻¹) (Fig. 1). The mortality in water extract (2.0 g l⁻¹) was significantly higher than in boiling water extract (2.0 g l⁻¹). No treatments killed the GAS completely at 48 h. The mortality of GAS at 120 h in boiling water extract (2.0 g l⁻¹), boiling water extract (5.0 g l⁻¹) and boiling water extract (10.0 g l⁻¹) was greatly enhanced compared with 48 h. The toxic effect of chemicals in boiling water extract of *A. conyzoides* was closely related to the exposure time of GAS in tested solutions.

IV. *A. artemisiifolia*: The mortality in water extract (2.0 g l⁻¹), water extract (5.0 g l⁻¹), boiling water extract (2.0 g l⁻¹) and boiling water extract (5.0 g l⁻¹) was low (< 20%) at 48 h (Fig. 2). Neither the water extract nor the boiling water extract of different concentrations killed the GAS completely at 120 h, although mortality in water extract (10.0 g l⁻¹) was higher than other treatments. With *A. artemisiifolia*, however, the mortalities in boiling water extract (2.0 g l⁻¹) and boiling water extract (5.0 g l⁻¹) at 120 h were higher than in water extract (2.0 g l⁻¹) and water extract (5.0 g l⁻¹) respectively (Fig. 1). This suggests that extraction of toxic material from this plant was better at higher temperature. It is thus, likely that the toxic constituents in this plant had different thermal stability compared with others.

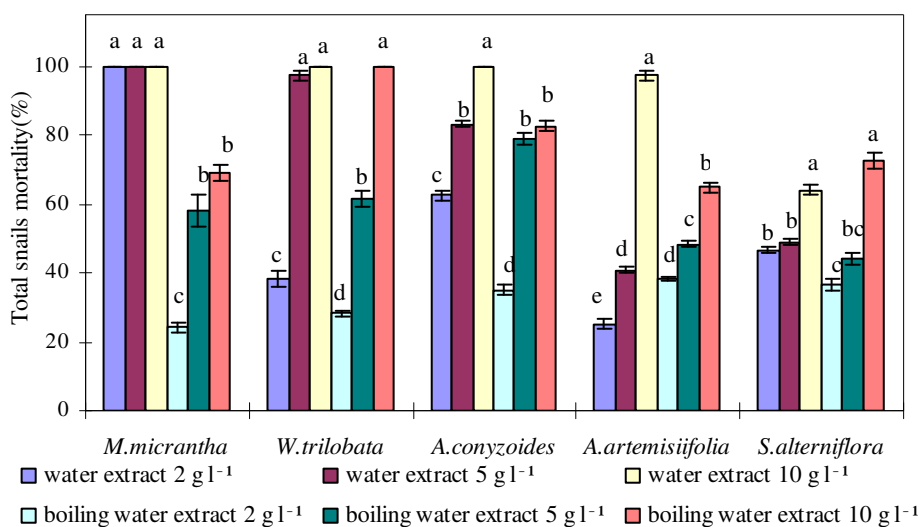


Figure 1. Mortalities of GAS in water extract and boiling water extract of five invasive plants at 120 h. Different letters over columns are significant at p=0.05, Tukey's HSD multiple range test. The letters 'a'-'e' indicate the significant difference among different treatments for the same plant.

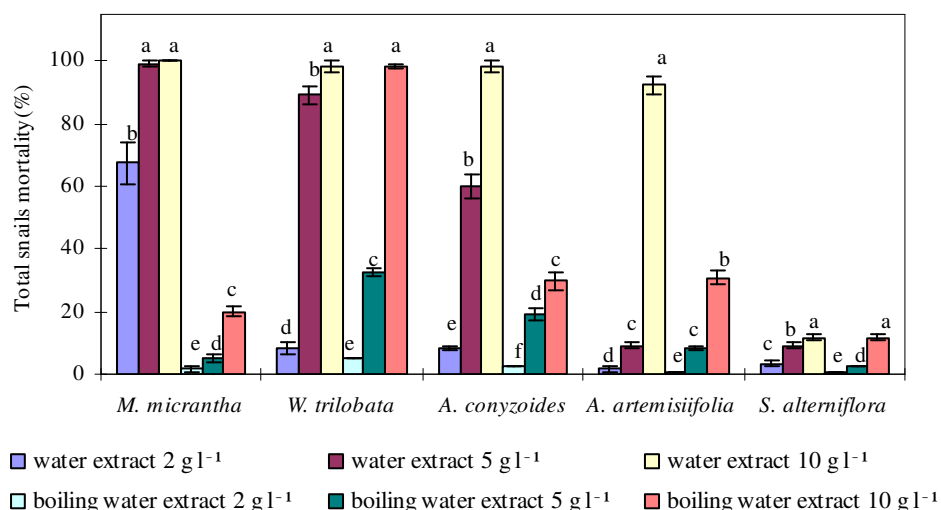


Figure 2. Mortalities of GAS in water extract and boiling water extract of five invasive plants at 48 h. Different letters over columns are significant at p=0.05, Tukey's HSD multiple range test. The letters 'a'-'f' indicate the significant difference among different treatments for the same plant.

V. *S. alterniflora* : The mortality of GAS in both water extract and boiling water extract treatments at 120 h did not exceed 80% (Fig.1). The mortality in all treatments at 48 h were also low (< 20%), suggesting weak toxicity of these extracts. *S. alterniflora* is also an invasive species in China and it inhibits the seeds germination and seedlings growth of *Scirpus mariqueter* in the invaded soil due to its allelopathic effects through the root exudates in soil (16). However, water extract and boiling water extract of *S. alterniflora* were not toxic to snails in this experiment. Perhaps, the GAS was insensitive to the allelopathic chemicals released from *S. alterniflora*.

The water and boiling water extracts caused different GAS mortalities at 48 h and at 120 h. With *M. micrantha* and *W. trilobata*, the mortality in 3-concentrations of water extract was always higher than boiling water extract up to 120 h except boiling water extract (10.0 g l⁻¹) of *W. trilobata*, which gave 100% mortality at 120 h. With *A. conyzoides* at 48 h, all 3- concentrations of water extract were more toxic than boiling water extract.

Furthermore, in 5- plants materials studied, three concentrations of water extracts of *M. micrantha* caused greater mortality than others. Preparation of cold water extracts is an easy and convenient method for farmers to use in the fields than other chemical treatments. Farmers can collect *M. micrantha* and other alien plants tested herein and use the dry powder directly as a molluscicide in local paddy fields. Based on the comparison of toxicity index at 48 h and mortality at 120 h in this study, the optimum recommended amount of *M. micrantha* in fields is 400 Kg/ha (2 g l⁻¹).

CONCLUSIONS

The harvesting of *M. micrantha* and other alien plants to control GAS would achieve two objectives: (i) To control expansion of this harmful invasive snail species and (ii) to control the invasive snails by incorporating the plant material in paddy fields. When the water level in the paddy fields is 2 cm high, the *M. micrantha* powder can be directly added to the paddy fields at the recommended amounts. However, further study is needed to examine, if the direct use of these plant materials may have toxic effects on other native snails in paddy fields.

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