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REGULAR ARTICLE

Molluscicidal activity of the aqueous extracts from *Solanum mammosum* L., *Sapindus saponaria* L. and *Jatropha curcas* L. against *Pomacea canaliculata*

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Abstract

Biomolluscicides development is of great interest in pest management to avoid the effects on the environment caused by synthetic molluscicides. The Species: *Solanum mammosum* L., *Sapindus saponaria* L. and *Jatropha curcas* L. have demonstrated molluscicidal properties against *Pomacea canaliculata*. This study was carried with mixtures of aqueous extracts of these plants in order to check for a synergistic effect between them. Extracts obtained from each species were evaporated and lyophilized. By using a simplex centroid design were obtained ten mixes for testing. All of them were diluted to 500ppm and tested on adult snails for 24 hours of exposure followed by 24 hours of recovery on tab water. Those mixes that showed the best results of mortality were chosen to determine the median lethal dose. The formulations *S. saponaria* (100%) and *S. mammosum* - *S. saponaria* (50%-50%) showed the best results of mortality with LD50 values of 24.04 ppm and 17.78 ppm respectively, with no significant differences between them (p> 0.05). These results showed that a synergistic effect is not produced in the activity of these plants when the extracts mixture was performed.

Key words: Bioassay, molluscicide, *Pomacea canaliculata*

Introduction

In Ecuador there are 410.000 hectares of rice crops, of which 200.000 are affected by the presence of apple snail (*Pomacea canaliculata* Lamarck) (El Telégrafo, 2012). This has caused severe damage to the agricultural sector of the country, causing a decline in profitability and rice production, becoming one of the major threats to food sovereignty (Barona, 2012). Lack of knowledge of how to tackle this kind of pests has resulted in the indiscriminate and inappropriate use of agrochemicals making an impact on the ecosystem to unbalance the native fauna, which has aggravated the problem (Salcedo, 2013).

An alternative in sustainable agriculture to pest control is the use of biopesticides, which are derived from natural materials, plants, microorganisms and minerals, highly effective against targeted pests and generally pose little or no risk to people or the environment (EPA, 2013).

Interest on plant molluscicides has increased in recent years, focusing on the ability to demonstrate that its use represents a lower cost and higher availability compared to the use of synthetic chemicals (Kloos and McCullough, 1982).

Solanum mammosum L. (teta de vaca), a native plant from South America in the Solanaceae family, part of the *Solanum* genus, has shown molluscicidal activity due to the presence of various steroidal alkaloids in the form of glycosides (solanine, solanidine, solasonine and solamargine). Preliminary studies into structure - activity indicate that the molluscicidal properties of fruit of *Solanum mammosum* L. depend on the type of aglycone and glycosidic bond possessing (Lim, 2013).

Sapindus saponaria L. (jaboncillo), from the family Sapindaceae, genus *Sapindus* is a shrub distributed throughout the tropics and subtropics of the world (Abreu, 2005), it is considered as one of the most promising plant molluscicides (Kloos and McCullough, 1982). Phytochemicals studies on *Sapindus saponaria* L. reveal the presence of triterpene saponins in fruit pericarp (Abreu, 2005), with pharmacological properties such as hemolytic, antimicrobial, antitumor, insecticides, and molluscicides (Vincken et al., 2007; Chan, 2007).

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Jatropha curcas L. (piñón), from the family Euphorbiaceae, genus *Jatropha* is a perennial shrub native to South and Central America, but cultivated in many tropical regions, including Africa and Asia (Gübitz et al., 1996). Studies on the seed husks of *Jatropha curcas* L. have shown toxic activity against snails (Liu et al., 1997); making it a promising plant molluscicide (Rug et al., 1996; Liu et al., 1997).

Toxicity of *Jatropha curcas* L. in molluscs has been attributed to the presence of phorbol esters (Liu et al., 1997), bioactive diterpenes that have a wide range of effects on cells (Goel et al., 2007).

In this research different formulations were tested using aqueous extracts of fruits of *Solanum mammosum* L. and *Sapindus saponaria* L. and the aqueous extract of the seed husks of *Jatropha curcas* L., against the mollusc *Pomacea canaliculata* Lamarck, in order to check whether a summation or synergistic effect on the molluscicidal activity occurs when mixtures of extracts is done.

Materials and Methods

Harvest

A sample of plant material was taken for botanical identification, which was botanized at the National Herbarium of Ecuador (QCNE), Quito, with key CIBE006 (*J. curcas*); CIBE012 (*S. mammosum*) and CIBE018 (*S. saponaria*) and a control is retained within the existing herbarium Bioproducts Laboratory in CIBE-ESPOL, Guayaquil, Ecuador.

Extraction

Ripe fruits of *Solanum mammosum* L. and *Sapindus saponaria* L. were collected in Guayas during the months of July and August. *Jatropha curcas* L. seeds were collected in Manabi during the months of June and July.

Solanum mammosum L. fruits were cut into small pieces and placed in the oven at 60ºC for 24 hours. The dried material was subjected to a process of manual grinding to get fine dust particles. The aqueous extracts were made using the powder obtained from the fruits of *teta de vaca*, the fruit pericarp of *jaboncillo* and the seed husks of piñón, which were processed separately. Extraction was carried out by decoction of plant materials in proportion of 10% in water during 20 minutes. The aqueous extract of each plant was evaporated to 87ºC and 400 mmHg. Finally, the concentrated extract was lyophilized to 120 x 10^{-3} mbar and 47ºC below zero. The yields for *Solanum mammosum* L., *Sapindus saponaria* L. and *Jatropha curcas* L. were of 0.05 ± 0.01 g/L, 15 ± 1.0 g/L and 0.05 ± 0.01 g/L respectively.

Phytochemical screening

For the phytochemical screening, 30 grams of dried material was mixed with 90 mL of distilled water and it was macerated for 48 hours. The aqueous extracts were divided into fractions for subsequent determination of secondary metabolites which may be present in the aqueous extract (Miranda and Cuellar, 2002).

Surface tension of aqueous extracts

The surface tension of all bioactive formulated to 500 ppm was measured using a tensiometer of ring and plate (Tensiometer Kruss K6). Subsequently the data were analyzed by ANOVA and Tukey's multiple range to determine whether there were significant differences between the surface tensions of the formulations. Further, a linear regression analysis was performed to determine whether the relationship between the decrease of the surface tension of water and mortality of the snail *Pomacea canaliculata* Lamarck. Data were analyzed using the statistical program Minitab v.16.

Molluscicide assay

The molluscicides mixtures, from the extracts lyophilized from *Solanum mammosum* L, *Sapindus saponaria* L and *Jatropha curcas* L, was performed using a simplex centroid design using the Statgraphics Centurion XVI statistical program. The treatments were performed in triplicate per treatment and four replications were performed.

The period of exposure of the snails in the bioactive formulated was 24 hours followed by 24 hours of recovery period, the snails were removed from the raised and deposited in distilled water. Each treatment was carried out a control test using 10 snails in 1000 mL of distilled water.

Determination of LD50

Mortality of snails was evaluated at concentrations of 400, 300, 200, 100 and 50 ppm for the mixtures that showed the best results of mortality in snails. Additional doses of 10 and 1 ppm were evaluated for formulations of *Sapindus saponaria* L. 100% and *Solanum mammosum* L. - *Sapindus saponaria* L. 50-50%.

The median lethal dose (LD_{50}) of each bioactive formulation was estimated from the graphical method based on the percentage mortality of *Pomacea canaliculata* Lamarck snail versus the logarithm of the concentration of the extract used.

Results and Discussion

Table 1 shows the results obtained from the phytochemical screening the aqueous extracts of *Solanum mammosum* L., *Sapindus saponaria* L and *Jatropha curcas* L. Abundant presence of alkaloids in *Jatropha curcas* L. and *Solanum mammosum* L. was evident, the test was negative for *Sapindus saponaria* L. Presence of phenols and tannins were recorded in *Solanum mammosum* L., and presence of flavonoids and reducing compounds in all the extracts. The presence of saponins was observed in extracts of *Solanum mammosum* L. and *Sapindus saponaria* L. Of this group of compounds tested, has been identified that the saponins, tannins, alkaloids and flavonoids have molluscicidal activity (Perry, 1980; Kloos and McCullough, 1982; Huang et al., 2003).

Referring to the surface tension, statistically significant differences ($p < 0.05$) in reducing the surface tension of the formulations were found. However, as shown in Figure 1, there is not a statistical relationship between the percentage of mortality and the surface tension of the extract.

Using the simplex centroid design, ten formulations were obtained. To these, the test was performed at a concentration of 500 ppm and formulations giving best answer mortality were selected.

According to Table 2, the formulations 1, 2, 5, 6, 8 and 9 were selected.

Table 1. Phytochemical screening of aqueous extracts of plant molluscicides

Assay (metabolites)	*S. mammosum* L.	*S. saponaria* L.	*J. curcas* L.
Dragendorf (Alkaloids)	+	-	+++
Mayer (Alkaloids)	+	-	+++
Wagner (Alkaloids)	+	-	+++
Cl3Fe (Phenols / tannins)	+	-	-
Shinoda (Flavonoids)	+	+	+
Fehling (Reducing compounds)	+	+	+
Espuma (Saponins)	+	+++	-
Mucilage	-	-	-

+++ Abundant; +Positive; - Negative

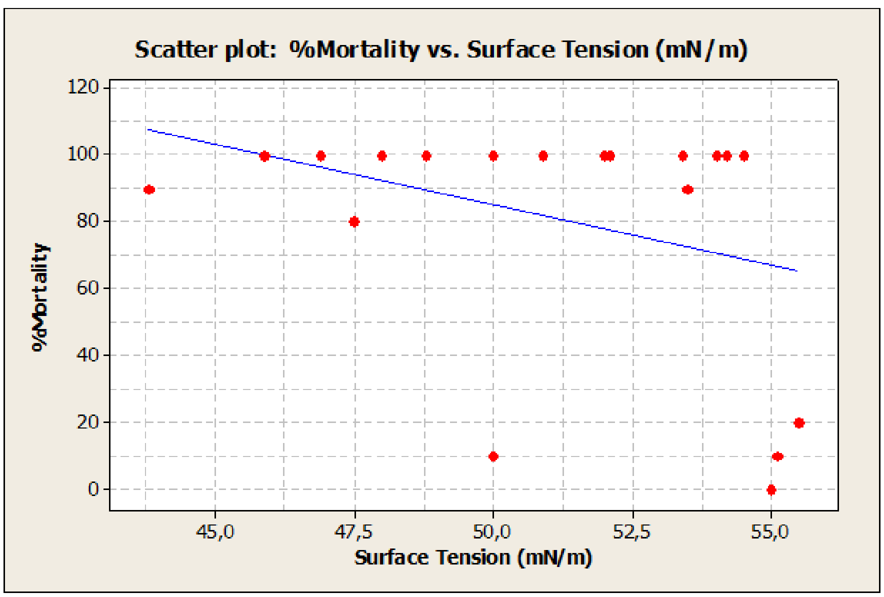

Figure 1. Scatter plot: %Mortality vs. Surface Tension (mN/m).

Table 2. Mortality (%) of the extracts on the snail *Pomacea canaliculata* Lamarck to 24 h of exposure and 24 h of recovery.

Mixtures	*Solanum mammosum* L (%)	*Sapindus saponaria* L. (%)	*Jatropha curcas* L. (%)	500 ppm
1	50	50	0	97,50
2	0	50	50	95,00
3	0	0	100	22,50
4	0	100	0	97,50
5	33,33	33,33	33,33	97,50
6	50	0	50	100
7	100	0	0	100
8	100	0	0	100
9	0	100	0	100
10	0	0	100	7,50

Subsequently, tests were conducted dose of 400, 300, 200, 100, 50 ppm of the selected formulations. Statistically significant differences between the applied doses of each formulation employed and the effect to lethality test ($p < 0.05$) were found. The best mortality results were obtained with the formulations *Sapindus saponaria* L. (100%) and *Solanum mammosum* L. - *Sapindus saponaria* L. (50%-50%) which showed no statistically significant difference for all doses used (Table. 3). Other formulations showed a tendency to decrease more drastically the lethal effect when decreasing the concentration of the extract, compared with the formulations *Sapindus saponaria* L. (100%) and *Solanum mammosum* L. - *Sapindus saponaria* L. (50%-50%). The formulation *Solanum mammosum* L. (100%) was the one with largest decrease in the lethal effect compared to the other formulations.

Means in the same column or row followed by the same capital or lowercase letters respectively are not significantly different ($p > 0.05$).

Figure 2 shows the graph of the determination of the median lethal dose selected for all formulations, except the formulation *Solanum mammosum* L - *Jatropha curcas* L (50% -50%) this due to the fact of the poor results obtained with lower concentrations of 500 ppm.

Table 3. Mortality (%) at different doses of the mixtures selected.

Mixtures	400 ppm	300 ppm	200 ppm	100 ppm	50 ppm
SOL:SAP(50:50)	97.50 Aa	95.50 Aa	90.83 Ab	84.17 Ac	83.33 Ac
SAP (100)	96.67 Aa	91.67 Aa	85.83 Ab	77.50 Ac	75.00 ABc
SOL (100)	88.33 ABa	79.17 Aa	42.60 BCb	9.17 Bc	5.00 Dc
SOL:SAP:JAT(33:33:33)	85,00 ABa	85.00 Aa	79.17 ABb	45.83 ABc	41.67 BCc
SAP:JAT (50:50)	76.67 Ba	75.83 Aa	57.50 ABb	39.17 ABc	33.33 CDc
SOL:JAT(50:50)	19,17 Ca	10.83 Ba	6.67 Cb	0.00 Bc	0.00 Dc

SOL:SAP(50:50): *Solanum mammosum* L. - *Sapindus saponaria* L. (50%-50%); SAP (100): *Sapindus saponaria* L. (100%); SOL (100): *Solanum mammosum* L. (100%); SOL:SAP:JAT(33:33:33): *Solanum mammosum* L. - *Sapindus saponaria* L. – *Jatropha curcas* L. (33%-33%-33%); SAP:JAT(50:50): *Sapindus saponaria* L - *Jatropha curcas* L. (50%-50%); SOL:JAT(50:50): *Solanum mammosum* L - *Jatropha curcas* L. . (50%-50%).

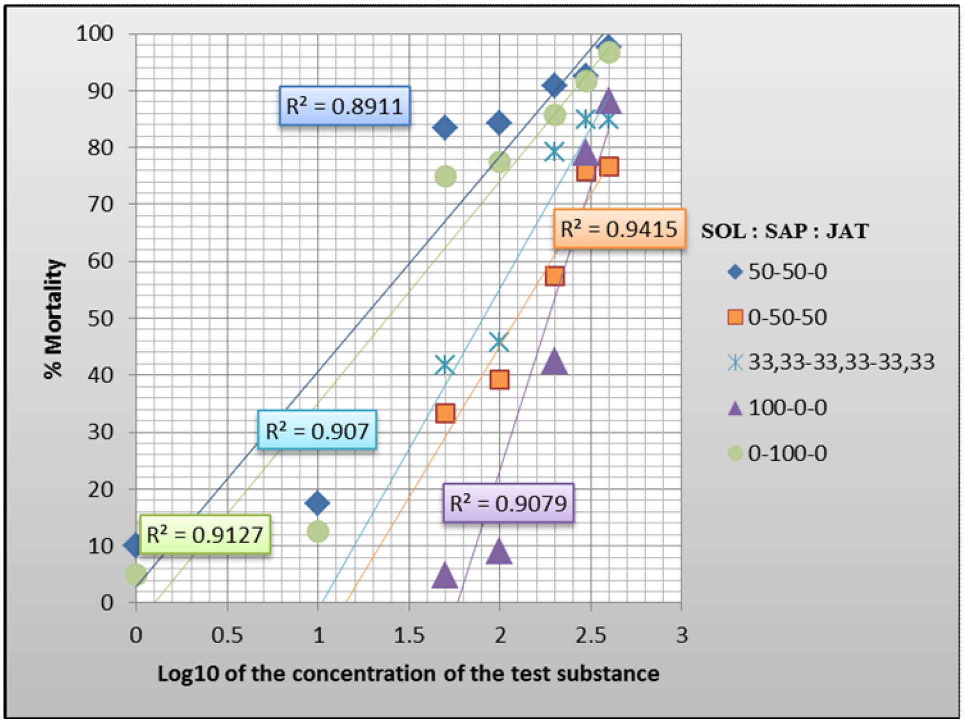

Figure 2. Determination of the DL_{50} for the mixtures selected.
SOL: *Solanum mammosum* L.; SAP: *Sapindus saponaria* L.; JAT: *Jatropha curcas* L.

LD_{50} values for *Sapindus saponaria* L. (100%) and *Solanum mammosum* L. - *Sapindus saponaria* L. (50% -50%) were of 24.04 ppm and 17.78 ppm, respectively (table 4). Huang et al. 2003, reported LD50 values on *Pomacea canaliculata* Lamarck of 22 ppm at 48 hours, using extracts of *Sapindus mukorossi* L., this value is similar to the value obtained with the best formulations used in this work.

Table 4. Median Lethal doses of the Mixtures.

Mixtures	LD_{50} (ppm)
SOL(50):SAP(50)	17.78
SAP (100)	24.04
SAP(50):JAT(50)	124.06
SOL(100)	184.92
SOL(33.33):SAP(33.33):JAT(33.33)	80.93

SOL: *Solanum mammosum* L.; SAP: *Sapindus saponaria* L.; JAT: *Jatropha curcas* L

Considering the chemical composition detected in the phytochemical screening and the information of those species available in the literature (Herrera et al., 2007; Braz Filho, 2010), *S. saponaria* presents, among other compounds, a complex mixture of saponins with oleanane skeleton. *S. mammosum* is characterized by the presence of steroidal glycoalkaloid, which solanine [**3**] and solasonine [**4**], are referred to as the major (Figure 3).

The compounds with molluscicide effect attributed to *Sapindus saponaria* L. are acyclic triterpenoid saponins and sesquiterpene oligoglycosides (Rouquayrol, 1984; Ribeiro et al., 1995; Alfonso et al., 2000; Ojewole, 2004; Herrera et al., 2007; Tsuzuki et al., 2007; Pellegrini et al., 2008). The molluscicidal activity of the saponins may be due to its characteristic effect on soft detergent membranes mollusks (Francis et al., 2002), probably an interaction between saponins with the sterols present in cell walls of gills mollusks (San Matíns et al., 2009). Use of molluscicidal with active compounds of saponins may be limited because of the behavior of the respiratory patterns *Pomacea canaliculata* Lamarck snail under various contents of dissolved oxygen in the water, using its siphon and breathing lungs instead of gills, in places where crops are irrigated with highly polluted water (San Matíns et al., 2009). Diversify the possibilities of use of *Sapindus saponaria* L. by mixing it with a plant molluscicide containing alkaloids, another compound with molluscicidal activity as *Solanum mammosum* L. may be attractive in the control of the species *Pomacea canaliculata* Lamarck.

Conclusions

Mixtures of different plants with molluscicidal properties were tested to potentiate their action against the snail *Pomacea canaliculata* Lamarck. The formulations *Sapindus saponaria* L. (100%) and *Solanum mammosum* L. - *Sapindus saponaria* L. (50% -50%) gave the best results in mortality with LD50 values of 24.04 ppm and 17.78 ppm respectively, with no significant statistical differences between each other, to a confidence level of 95%. No synergistic effect between the mixture of the aqueous extracts of *Solanum mammosum* L., *Sapindus saponaria* L. and *Jatropha curcas* L., was found. However, mixing *Sapindus saponaria* L. with *Solanum mammosum* L. can be useful in areas where the effectiveness of *Sapindus saponaria* L. is restricted by environmental conditions, such as low availability of oxygen in the water.

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