A laboratory assessment of the potential molluscicidal potency of *Jatropha curcas* aqueous extracts

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Preliminary laboratory studies were conducted to determine the molluscicidal potency of *Jatropha curcas*, the physic nut. *Biomphalaria glabrata* and *Bulinus globosus* snails were exposed to varying concentrations of aqueous extracts of crushed *J. curcas* seeds from unripe, ripe and overripe fruits collected from two geographically different sites, (Bindura and Kariba, Zimbabwe). Snail mortalities were compared between different developmental stages of *J. curcas* and between seed collection areas, and LC_{50} and LC_{90} values for the different extracts tested were computed. *Biomphalaria glabrata* was most susceptible to unripe fruit seed extract (with LC_{50} values of 282 and 389 mg l^{-1} being recorded for Kariba-origin and Bindura-origin plants, respectively) and least susceptible to ripe fruit seed extracts (with LC_{50} values of 605 and 708 mg l^{-1} being recorded for Kariba-origin and Bindura-origin plants, respectively). *Bulinus globosus* was most susceptible to overripe fruit seed extract (Kariba-origin plants: LC_{50}, 389 mg l^{-1}) and least susceptible to unripe fruit seed extract (Kariba-origin plants: LC_{50}, 687 mg l^{-1}). The area from which fruits were collected did not influence the potency of *J. curcas*. The potency of *J. curcas* depends on both the developmental stage of the fruit and the species of the target snail. In view of its many other uses, besides as a molluscicide, we recommend further studies on *J. curcas*.

Keywords: *Biomphalaria glabrata*, *Bulinus globosus*, intermediate host species, toxicity, Zimbabwe

Introduction

Jatropha curcas, the physic nut, which is indigenous to South America, is now widely spread in Africa, including Zimbabwe (Heller 1996). The plant has many purposes, being used in the manufacturing of soap, fertilisers, as a homestead hedge, for erosion control and — in recent years — its potential for the production of biodiesel has attracted much attention. It has also been proposed as a potential plant molluscicide (Rug and Ruppel 2000). Because of its potential multipurpose functions, its agrobotanical aspects (Heller 1996), toxicity (Adam 1974; Goel *et al*. 2007) and chemical composition (Adebowale and Adediran 2006) have been documented.

We studied the molluscicidal potency of *J. curcas* in order to explore the possible secondary use of this plant for the control of snails that transmit schistosomiasis. Other plant-based molluscicides that have been considered for this purpose include *Ambrosia maritima* (Alard *et al*. 1991), *Swartzia madagascariensis* (Lwambo and Moyo 1991), *Tetrapleura tetraptera* (Adewunmi 1991) and *Phytolacca dodecandra* (Ndamba *et al*. 1989), the latter having been studied in greater detail (Lemma 1970, Lemma *et al*. 1972). In Zimbabwe the agrobotany, toxicology, molluscicidal potency and community acceptability of *J. curcas* were studied in detail (Ndamba and Chandiwana 1988; Ndamba *et al*. 1994a, 1994b). The Ethiopian variety was found to have greater potency than the Zimbabwean variety and therefore the former was used in community-based trials in which significant reductions in snail populations and incidences of schistosomiasis among school children were recorded. The sustainability of using the plant as a schistosomiasis-control strategy was, however, questionable. Communities did not have incentives to grow the plant as it had no direct economic returns, and organisational problems at a local level also impacted negatively on efforts to cultivate this plant (Ndekha *et al*. 2003).

Our decision to study *J. curcas* was motivated by its comparative advantage (multipurpose functions, particularly its potential for a biofuel) over *P. dodecandra*. Here we report the results of a laboratory-based study that tested the molluscicidal potency of *J. curcas* seeds at different developmental stages and from different geographical locations in Zimbabwe against *Biomphalaria glabrata* and *Bulinus globosus*. Studies on *P. dodecandra* suggested that there was some geographical and inter-country variation in its molluscicidal potency (Ndamba and Chandiwana 1988).

Materials and methods

Snails

Biomphalaria glabrata and *Bulinus globosus* were bred in laboratories at Johns Hopkins University, using specimens obtained from the Biomedical Research Institute, Rockville MD, USA. The snails were fed on lettuce and used in the experiments when they were 5–7 mm in shell height (*B. globosus*) or 5–10 mm in shell diameter (*B. glabrata*) and were about 6–8 weeks old.

Test materials and procedures

Samples of *J. curcas* fruits at different developmental stages (ripe, unripe and overripe) were collected at Kariba and Bindura (which are situated 450 km apart) and packed in separate plastic bags according to developmental stage and area of origin. For the purposes of this study 'ripe', 'unripe' and 'overripe' were considered to be distinctive developmental stages, having been used by Ndamba *et al*. (1994a) in their studies on *P. dodecandra*. Bindura, 70 km north of Harare, is at an altitude of 1 000 m asl, has a mean annual rainfall of 700 mm, and summer temperatures ranging from 25 to 30 °C. Kariba, on the border between Zimbabwe and Zambia at 400 m asl, receives a mean annual rainfall of 400 mm, with summer temperatures ranging from 25 to 42 °C

The fruits were oven-dried at 40 °C and the seeds were crushed using an electric blender. The resultant powder was filtered through a 0.32 µm sieve and kept in dark bottles at 4 °C until the time of use. The test medium (hereafter referred to as 'Kariba-origin' and 'Bindura-origin' samples/plants/extracts) was prepared by adding 1 000 ml of deionised water to 10 g of test powder and stirring (with a magnetic stirrer) for eight hours to make a stock solution of 10 000 mg l^{-1}. Serial dilutions were made from the stock solution to prepare the various test concentrations This preparation method was used by Rug and Ruppel (2000), Ndamba and Chandiwana (1988) and Liu *et al*. (1997) in studies dealing with plant extracts.

Bioassays

Bioassays were done in 500 ml plastic containers. Preliminary experiments showed that snails exposed to the test material crawled out of the water and, if a lid was used to prevent this, they clung to the lid, thereby compromising their exposure time. Therefore, the main experiments used the same outer containers but with an enclosure made from plastic containers (65 mm diameter, 40 mm height) perforated to allow free circulation of test solution while keeping the snails in the solution.

For each developmental stage of seed (ripe, unripe or overripe) and area (Kariba or Bindura), five snails were exposed to concentrations of 0 (control, water only), 78.2, 156.3, 312.4, 625, 1 250, 2 500 and 5 000 mg l^{-1}, prepared from the 10 000 mg l^{-1} stock solution using spring water. For each run there were four replicates per treatment. The snails were exposed to the test material for 24 hours (12 h light and 12 h darkness) after which they were removed, rinsed with spring water, and put into new containers with 500 ml of spring water (under similar enclosure arrangements as those during exposure) in which they were allowed a recovery period of 48 hours (24 h light and 24 h darkness). No food was provided during the exposure and recovery periods. Separate bioassays were undertaken for each snail species. Because of limited supplies of unripe fruits collected from Bindura, bioassays using *B. globosus* were not done for this area.

In the case of *B. globosus,* mortality was assessed visually and, where death could not be confirmed visually, by pricking the soft parts of the snails to observe any recoiling movements. For *B. glabrata* the visual method was also used to assess mortality and the lack of heart beat activity was used as a confirmatory test. Snails that died during the recovery period were removed from the test containers so that they did not affect the living snails. At the end of the recovery period the total number of dead snails, including those removed during the recovery period, were recorded.

Data analysis

Lethal concentrations (LC_{50} and LC_{90}) for the various aqueous extractions tested were computed using SPSS Probit analysis. The efficacy of various extractions was compared between different developmental stages using one-way analysis of variance (ANOVA) and *t*-test for *B. globosus* in the Bindura-origin samples, where only ripe and unripe fruit extracts were used. A p value of <0.05 was taken as the cutoff point for statistical significance. A univariate analysis of variance test, with $\log_{10}$ (number of dead snails + 1) as the dependent variable, was used to test the effects of different developmental stages, area of origin, and species of snails exposed, on the efficacy of the various extractions.

Results

There were higher *Biomphalaria glabrata* mortalities in extractions from seeds of unripe fruits (Figure 1a), but the differences between the different extractions were not significant (ANOVA; $F = 0.620$, $p > 0.05$). For the Kariba-origin fruits there were significant differences (ANOVA: $F = 7.110$; $p < 0.05$) in mortalities of *B. glabrata* among extractions from seeds of fruits at different developmental stages (Figure 1c). The Scheffe *post hoc* test showed that snail mortalities were higher in the unripe fruit extractions than in the ripe and overripe extractions, and that differences between mortalities in ripe and overripe extractions were not significant.

A comparison of *B. globosus* mortalities in extractions from seeds of ripe and overripe fruits (there were no unripe fruits) from Bindura (Figure 1b) showed higher mortalities in overripe fruit extractions than in ripe fruit extractions (*t*-test: $p < 0.05$). For the Kariba-origin fruits, mortalities of *B. globosus* among extractions from seeds of fruits at different developmental stages were significantly different (ANOVA: $F = 10.797$; $p < 0.05$) (Figure 1d). The Scheffe *post hoc* test showed that snail mortalities were higher in the overripe fruit extractions than in the unripe, and ripe fruit extractions; and that differences between mortalities in ripe fruit and unripe fruit extractions were not significant.

The 50% and 90% lethal concentrations are presented in Table 1. For *B. glabrata* the lowest LC_{50} and LC_{90} concentrations were generally for the unripe fruit stage and the

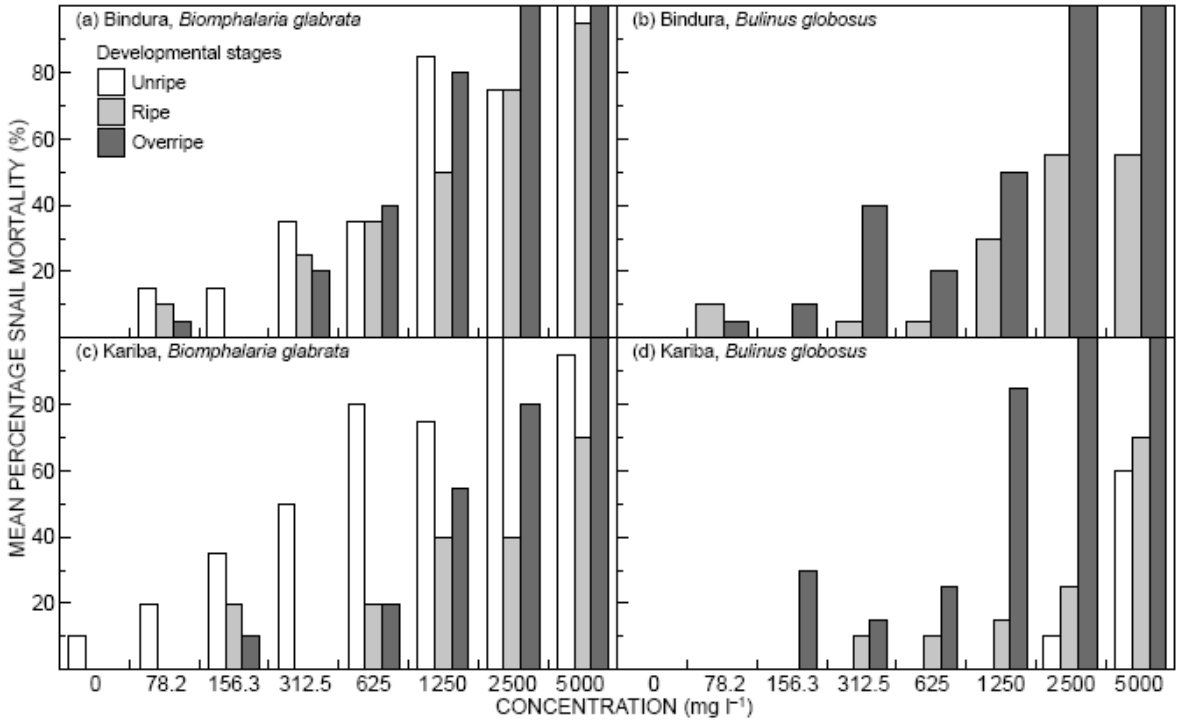

Figure 1: Mortalities of *Biomphalaria glabrata* and *Bulinus globosus* in different concentrations of aqueous extracts of seeds of *J. curcas* at different developmental stages collected from different localities ('Kariba' and 'Bindura' refer to extracts derived from plants originating from Kariba and Bindura, respectively)

Table 1: Toxicity of aqueous extracts of *J. curcas* seeds (LC_{50} and LC_{90} values: mg l^{-1}) to two snail species, from fruits at different developmental stages and from different areas: figures in brackets are confidence intervals; 'Kariba' and 'Bindura' refer to extracts derived from plants originating from Kariba and Bindura, respectively

	Bindura		Kariba	
	LC_{50} (CI)	LC_{90} (CI)	LC_{50} (CI)	LC_{90} (CI)
B. glabrata				
Unripe	389 (326–455)	656 (561–797)	282 (206–351)	560 (471–718)
Ripe	463 (400–537)	708 (616–882)	605 (537–711)	938 (805–1 196)
Overripe	401 (342–462)	549 (483–682)	482 (422–548)	650 (577–798)
B. globosus				
Unripe	–	–	687 (653–754)	794 (736–976)
Ripe	649 (575–773)	981 (838–1271)	661 (598–769)	917 (798–1 169)
Overripe	420 (364–482)	628 (553–765)	389 (349–430)	567 (514–649)

highest values were for the ripe stage, although differences for Bindura-origin plant extracts were not significant. In the case of *B. globosus*, excluding the missing results for Bindura-origin unripe fruit, the lowest LC_{50} concentrations (highest toxicity) were for the overripe stage for both Kariba- and Bindura-origin extracts.

Table 2 shows results of the Univariate Analysis of Variance done on the $\log_{10}$ transformed number of dead snails. There were significant differences in the numbers of snails that died for each snail species and for different developmental stages, with a highly significant interaction between snail species and developmental stage. The area from which the fruits were collected did not influence the potency of *J. curcas* aqueous extracts.

Discussion

The potency of aqueous extracts of *Jatropha curcas* in terms of killing snails was found to be dependent on the developmental stage of the fruit. For *B. glabrata* the unripe stage of the fruit had more potent seed extracts while for *B. globosus* the overripe stage of the fruit had more potent

Table 2: Univariate analysis of variance: tests of between-subjects effects with $\log_{10}$ (dead snails + 1) as the dependent variable

Source	Type III sum of squares	df	Mean square	*F*	Significance (*p*)
Corrected model	4.402[a]	6	0.734	8.409	0.000
Intercept	28.605	1	28.605	327.819	0.000
Snail species	1.667	1	1.667	19.105	0.000
Fruit stage	0.713	2	0.356	4.085	0.018
Area	2.680×10^{-2}	1	2.680×10^{-2}	0.307	0.580
Snail species * fruit stage	2.187	2	1.093	12.529	0.000
Error	30.017	344	8.726×10^{-2}		
Total	69.500	351			
Corrected total	34.419	350			

[a] indicates $r^2 = 0.128$ (adjusted $r^2 = 0.113$)
* denotes an interaction between two factors

seed extracts. Similar observations have been made for *Phytolacca dodecandra*, of which unripe berries are more potent than ripe berries (Ndamba 1993), although no attempt was made to test its overripe berries. The differential potency between the different developmental stages of *P. dodecandra* berries was associated with the abundance of saponins (the active molluscicidal ingredient), which are more abundant in unripe berries than in ripe berries (Ndamba 1993, Ndamba *et al*. 1994a). Although the active ingredient in *J. curcas* has been indicated as phorbol esters (Rug and Ruppel 2000) we did not measure the quantities of these in the different extracts tested. Therefore, it is not possible to relate the observed snail mortalities to varying quantities of the active ingredient in the different extracts.

The LC_{50} and LC_{90} concentrations determined for *B. glabrata* and *B. globosus* indicate that the former species was more susceptible to *J. curcas* poisoning, with higher susceptibility being observed to extracts from seeds of unripe fruits. For *B. globosus*, higher susceptibility was observed when exposed to extracts from the seeds of overripe fruits. The reasons for differences in susceptibility may need to be investigated. Our results indicate that, in the case of *B. glabrata*, the Zimbabwean variety of *Jatropha curcas* is more potent than the Mali variety used by Rug and Ruppel (2000), who reported an LC_{50} concentration of 5 000 mg l^{-1} for aqueous extracts.

There were no differences in mortalities of snails tested against seeds of *J. curcas* collected from Bindura and Kariba, implying that the potency of *J. curcas* may not be influenced by geographical location with respect to these two localities. It may, however, be necessary to test samples from other areas. Ndamba and Chandiwana (1988) also found no differences in the potency of *P. dodecandra* samples from different agroecological zones of Zimbabwe. However, the differences between seeds from Zimbabwe and Mali observed in this study were also observed in the case of *P. dodecandra*, in which the Ethiopian strain was more potent than the Zimbabwean strain.

Comparison of our findings (on *J. curcas* toxicity) with those involving *P. dodecadra,* in which LC_{50} concentrations of less than 10 mg l^{-1} were reported (Ndamba 1993), clearly indicate that the latter species has greater molluscicidal potency. However, the potential economic returns from *J. curcas* give it an advantage over *P. dodecandra*, which has no other commercial value. A community-based study conducted in Zimbabwe mentioned the lack of community motivation to grow *P. dodecandra* as a major drawback to using that plant for controlling schistosomiasis (Ndekha *et al*. 2003). *Jatropha curcas* has many other uses, including the production of oil, fertilisers, bio-diesel, erosion control, and homestead fencing. These may enhance community incentives for growing the plant. Indeed, the Zimbabwe Government is encouraging and assisting rural farmers to grow *J. curcas* on a large scale so as to provide seed to a bio-diesel extraction factory that was commissioned in 2007. In view of these developments we suggest that further preliminary studies on *J. curcas* be conducted before the plant can be recommended for more detailed investigations. Such studies may focus on determining the active ingredient and its distribution within plants during different developmental stages of the fruits, as well as bioassays using *Biomphalaria pfeifferi* (another schistosomiasis-transmitting African snail). Trials to assess the success of field applications are also recommended.

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