Preliminary Assessment of the Molluscicidal Potency of Crude Seed Oil Extract of *Azadirachta indica*, *Acacia albida* and *Balanite aegyptiaca* Plants on *Lymnae natalensis* (snails)

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Abstract

The best way to prevent Fascioliasis is through the reduction in population of the Lymnaeid snail intermediary host. Plant based molluscides had over the years shown remarkable promises. Presented here are a series of experiments evaluating the potency of crude seed oil extracts of Azadirachta indica, Acacia albida and Balanite aegyptiaca against Lymnae natalensis, an intermediary host of Fasciola gigantica collected from Katagum Local Government Area of Bauchi State, Northeastern Nigeria. The results indicated that, molluscicidal potency of seed oils of A. indica, A. albida and B. aegyptiaca were concentration dependent. Mortality rate increases with corresponding increase in concentration of the extracts. Comparatively, seed oil extracts of B. aegyptiaca showed higher molluscicidal tendencies, followed by A. indica and lower with A. albida. The results of the LC_{50} and LC_{90} values of different seed oils on Lymnae natalensis varied accordingly. All the three plant species seed oils tested showed various level of molluscicidal potency against Lymnae natalensis after 24 hours of exposure. However, highest molluscicidal potency was found in A. indica with LC_{50} values of 6.328 and LC_{90} values of 7.925 while a relatively low molluscicidal potency was observed in A. albida and B. aegyptiaca with similar LC_{50} values of 14.348 and LC_{90} values of 17.475 respectively. Results obtained suggest that, all the three plant species seed oils possessed molluscicidal potency with significant difference in LC_{50} and LC_{90}. Comparative assessment and field trials for seed oil extracts of these abundant plant species were recommended.

Keywords: Potency, Molluscicidal, Crude, Seeds, Oil, Extracts.

INTRODUCTION

Reducing Lymnoid snails population remained one of the best ways of preventing Fascioliasis (Scott, 2016). Over the years, there has been growing concern and interest on the use of plant parts as candidate molluscicides for the control of freshwater snail intermediary host of trematode parasites including *Fasciola spp*. Quite a number of plants molluscicides have been tested through laboratory researches, although none has yet been used extensively in an endemic country. World Health Organization (1983) documented that, an appealing aspect of natural plant products is that, while they may be highly toxic, they degrade very rapidly when released into the environment. Since 1930, over 1000 plant species including about 600 in China have been tested for molluscicidal activities. WHO (1983) further reported that, some 42 molluscicidal compounds have been isolated from different plants.

These include saponins, flavanoids, alkaloids and terpenoids which had shown encouraging results.
Several plant extracts with molluscicidal effects had been evaluated from Nigeria. Majority of these extracts potency where evaluated against the snail intermediary host of *Schistosoma haematobium*; *Bolinus (Phy) globosus*. Azare, Okwute and Kela (2007) reported Lc50 values of 40.42 and 48.07 for evaporated and unevaporated crude water leaf extracts of *Alternanthera sesselis* on *Bolinus globosus* collected from different parts of Nigeria. Efficacy of ethanolic leaf extracts of *Carica papaya* and *Terminalia catappa* were also evaluated against *B. globosus* with promising results (Adetunji and Salawu 2010).

Olafintoye (2010) investigated molluscicidal activities of ethanoic and methanoic extracts of leaf, stem, bark and roots of *Securidaca longepedunculata* and *Tephrosia brateolata,* Lc50 range values of 0.15-0.60 ppm was recorded. Molluscicidal effects of *Talinum triangulare* and *Zingiber officinale* on *Bulinus spp.* were also documented (Okeke and Obachuku 2011; Labe, Inabo and Yakubu 2012).

Recently, molluscicidal activity of aqueous extracts of leaves, stem bark and roots of desert date (*Balanite aegyptiaca* Del.) against *Lymnae natalensis* was conducted (Abdullahi et al. 2018). The results indicated that snail species were more susceptible to leaves extracts than stem and root extract of the same plant.

This study i) evaluates the molluscicidal properties of the three (3) plants seeds oil, ii) compares molluscidal potency of seed oils from the three plants, and iii) recommends further steps to be taken to ensure sustainability of the use of these plants seed oil as candidate molluscicides.

METHODOLOGY

Collection of plant materials

Seeds and fruits of *Azadirachta indica, Balanite aegyptiaca* and *Acacia albida* were collected from Katagum Local Government Area of Bauchi state, Northeastern Nigeria. They were identified and authenticated by a plant Taxonomist and voucher specimens were deposited at the Herbarium section of the Biological Sciences Department, Federal University Dutse, Nigeria.

Mature ripe fruits were washed with tap water and the epicarp removed using sterile surgical blade. The mesocarps of the fruits were then scraped or crushed manually. The seeds were then collected and air dried for 7 days in the laboratory. The seeds of individual plant species were then placed into a Gopal Automatic MS oil filter press extraction machine. The filtered oil was stored in the laboratory in screw-tired glass bottles.

Sampling and isolation of snails

Snail sampling was conducted around edges of River Jama'are, freshwater ponds of Sakuwa in Zaki LGA, Gadau, Gamawa, slow moving streams of Fago in Shira and lakes and irrigation canals of villages spread across the study area. Between April 2017 to September 2018 (Early and late rain season), a total of 511 adult snails were collected using scoop net adopted from Adamu *et al.,* (2000). The snails collected were taken to the laboratory in large glass bottles with perforated lids for aeration. Majority of the snails were identified to have fresh water characteristics. 230 *Lymnae natalensis* snails Intermediary host of Fascioliasis

were identified. The snail samples for the toxicity test were maintained in an aquarium. They were left to acclimatize to laboratory condition before being used for the potency test.

Preparation of the stock solution and dilution

Different concentrations used in this bioassay were prepared from the oil obtained from the seeds of *A. indica, B. aegyptiaca* and *A. albida*. 10, 30, 50, 70 and 90% concentrations were prepared by diluting the pure seed oils in distilled water. For each series of test, there was a control group placed in unchlorinated water, without seed oil.

Molluscicidal potency test

The different concentrations of seed oil extracts of *A. albida, A. indica* and *B. aegyptiaca* were tested against adult *Lymnae natalensis* snails according to the method recommended by WHO (1983). To each concentration of seed oil for investigation, 5 adult snails were immersed in a beaker and allowed to stay for 24 hours under room temperature. After 24 hours of exposure, motility and mortality status of the snails were observed and recorded. Mortality of the test snails were confirmed by the change in the shell color and failure of the flesh portion to withdraw into shell upon mechanical stimuli.

Statistical analysis

The Lc50 was calculated by using Probit analysis (using SPSS) to find out the economical dose of leaf extract.

RESULTS AND DISCUSSION

Results

The results indicated that, molluscicidal potency of seed oils of *A. indica*, *A. albida* and *B. aegyptiaca* were concentration dependent. Mortality rate increases with corresponding increase in concentration of the extracts.

For the different concentrations of 10%, 30%, 50%, 70% and 90%, mortality rates of *Lymnae natalensis* (table 1-3) indicated (0%, 40%, 60%, 60% and 100%) for *Acasia albida*; (20%, 60%, 80%, 100% and 100%) for *Azadirachta indica*; and (40%, 40%, 100%, 100% and 100%) for *Balanite aegyptiaca* seed oil extracts. These results suggest that all the three plant species seed oils possess molluscicidal potency.

Table 1: Molluscidal potency of *Acacia albida* seed oil on mortality rates of *Lymnaea natalensis*

Inoculation time (hours)	Conc. (%)	Total № Tested	№ Dead	% mortality	LC_{50}	95% confidence interval	LC_{90}	95% confidence interval
24	10	5	0	0.00	14.348	0.192-26.420	17.475	0.456-29.944
24	30	5	2	40.00				
24	50	5	3	60.00				
24	70	5	3	60.00				
24	90	5	5	100.00				

Comparatively, seed oil extracts of *B. aegyptiaca* (table 3) showed higher molluscicidal tendencies, followed by *A. indica* and lower with *A. albida*. With 10% seed oil concentration, 40% snail mortality was observed for *B. aegyptiaca*, 20% mortality for *A. indica* while 0% mortality was observed using *A. albida* seed oil.

0% mortality was observed for all the different volumes used for unchlorinated water as control experiment.

The results of the LC_{50} and LC_{90} values of different seed oils on *Lymnae natalensis* were presented in Table 1-3. The LC_{50} value and LC_{90} varied accordingly. All the three plant species seed oils tested showed various level of molluscicidal potency against *Lymnae natalensis* after 24 hours of exposure. However, highest molluscicidal potency was found in *A. indica* with LC_{50} values of 6.328 and LC_{90} values of 7.925 while a relatively low molluscicidal potency was observed in *A. albida* and *B. aegyptiaca* with similar LC_{50} values of 14.348 and LC_{90} values of 17.475 respectively.

Table 2: Molluscidal potency of *Azadirachta indica* seed oil on mortality rates of *Lymnaea natalensis*

Inoculation time (hours)	Conc. (%)	Total № Tested	№ Dead	% mortality	LC_{50}	95% confidence interval	LC_{90}	95% confidence interval
24	10	5	1	20.00	6.328	0.247-13.218	7.925	0.477-15.387
24	30	5	3	40.00				
24	50	5	4	60.00				
24	70	5	5	60.00				
24	90	5	5	100.00				

Table 3: Molluscidal potency of *Balanite aegyptiaca* seed oil on mortality rates of *Lymnaea natalensis*

Inoculation time (hours)	Conc. (%)	Total № Tested	№ Dead	% mortality	LC_{50}	95% confidence interval	LC_{90}	95% confidence interval
24	10	5	2	40.00	14.348	0.192-26.420	17.475	0.456-29.944
	30	5	2	40.00				
	50	5	5	100.00				
	70	5	5	100.00				
	90	5	5	100.00				

Discussion

The study showed that aqueous seed oil extracts of *A. albida*, *A. indica* and *B. aegyptiaca* possessed molluscicidal potencies at different concentrations. It also showed that all the three seed oil extracts of the plants were active at 90% concentration killing 100% of the *Lymnae natalensis* snails when exposed for 24 hours under room temperature. This indicated that they all have promising effects that require further investigations and subsequent field trials as recommended by WHO (1983) guidelines plant molluscicidal trials.

Molluscicidal potency of *A. indica* is stronger than *B. aegyptiaca* and *A. albida*. Several authours had reported similar success results on molluscicidal activities of *B. aegyptiaca* seeds from Africa. Sudan (Ragab,Elmalik and Adam, 2005), Ethiopia (Eshatu et al, 2013) and Nigeria (Abdullahi et al, 2018).

CONCLUSION

In Nigeria where seeds of these three plants species studied are abundant and mainly wasted, field trials of seed oil extracts can save both resources and environment. Seed oil extracted from these plant species had showed promising results. Their potency credentials need further investigations specifically in comparison to extracts from other plant parts such as leaves, stem and roots.

We recommend use of seed oil extracts of these plants for field trials in Nigeria and other African countries where Fascioliasis is prevalent. Further research on effect of seed oils extracts on snail intermediary host of other trematode infections is also recommended. The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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