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MOLLUSCICIDAL EFFECTS OF ENDOD (*PHYTOLACCA DODECANDRA)* ON FASCIOLA TRANSMITTING SNAILS

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ABSTRACT: A study was conducted to investigate the molluscicidal effects of crude water suspension of unripe green Endod (*Phytolacca dodecandra*) berries (Type 44) on different developmental stages of *Lymnaea natalensis* and *Lymnaea truncatula.* Concentration of 20 ppm for exposure period of 24 hours induced 100 % mortality in young *L. natalensis* and *L. truncatula* snails, while higher concentration was required for mature snails of both species**.** The LC_{90} on *L. natalensis* with shell height of 5-9.8 mm., 10–2.9 mm. and 13–18.5 mm. was 19.8, 21.1, 22.8 ppm, respectively. With regards to *L. truncatula* the LC_{90} for snails with a shell height of 3.4-5.4 mm and 5.7–8.2 mm was 19.5 and 21 ppm, respectively. Study on time-concentration relationship revealed that exposing snails for one hour required a concentration of 48.9 and 72.5 ppm in young (6–12.4 mm.) and adult (12.5–18.5 mm.) *L. natalensis* to cause 90% mortality, respectively. In the case of *L. truncatula* snails (4.5–7.4 mm) 54.5 ppm was needed to produce 90 % mortality. However, both snail species were killed at about a concentration of 20 ppm between 6 and 24 hours of exposure times.

Key words/phrases: Endod, fascioliasis, *Lymnaea natalensis*, *Lymnaea truncatula,* Molluscicide

INTRODUCTION

Fasciola is a helminthic termatode parasite causing widespread mortality and morbidity in cattle, sheep and goats throughout the world (Blood and Radostits, 1989). Snails of the family *Lymneaidae* exclusively transmit the parasite (Over, 1982). Both *Fasciola hepatica* and *Fasciola gigantica* are found in Ethiopia and are transmitted by *Lymnaea truncatula* and *Lymnaea natalensis,* respectively (Graber, 1975). Various reports indicate that it is a serious problem of livestock production in Ethiopia causing considerable economic losses. A rough estimate of the economic loss due to decreased productivity caused by bovine fascioliasis is about 350 million Birr (Bahru Gemechu and Ephraim Mamo, 1979). Ngategize *et al*. (1993) also estimated an annual economic loss of 48.4 million Birr due to ovine fascioliasis in the Ethiopian highlands. Applying management tactics such as rotational grazing, fencing off, grazing by alternative hosts and treatment of infested animals with flukicidal drugs and the use of molluscides to kill snail intermediate hosts are among the methods for the control of fascioliasis (Blood and Radostits, 1989). The use of management tactics is impractical in countries like Ethiopia where most of the livestock are kept in communal grazing land. Chemotherapy has its limitations such as low efficacy against all stages of fluke, drug resistance and unaffordability for the vast majority of financial resource poor farmers (Blood and Radostits, 1989). Commercial molluscicides also have their own drawbacks like high price, toxicity to valuable aquatic organisms, loss of potency under different environmental conditions, repellence to snails at low concentration and low efficacy (Aklilu Lemma, 1984).

Endod (*Phytolacca dodecandra*), the Ethiopian soapberry plant, has long been known for its traditional use as soap for washing clothes. The discovery of molluscicidal properties of Endod led to extensive subsequent studies, which revealed endod to be the best plant derived molluscicide out of 1000 species so far tested (Legesse Weldeyohanis *et al.*, 1987). The main factors that make endod very promising in the control of snail-borne diseases are its high mulluscicidal potency, low mammalian toxicity, rapid biodegradability, stability under different environmental conditions, the potential for large scale cultivation and the adaptability of the plant to different agroecology (Aklilu Lemma and Yau, 1974; Legesse Weldeyohanis *et al.,* 1987).

The extraction of the active molluscicidal component of endod has been done by three main methods: water extraction, alcohol extraction and fermentation process. Both alcohol extraction and fermentation process produce high yield of the active material but they have limitations of high cost and lack of reproducibility, respectively. The water extraction process gives a less active product and the cost is very low (Aklilu Lemma *et al.,* 1972; Aklilu Lemma and Heyneman, 1975; Tesfaye Lemma and Aklilu Lemma, 1977). Lambert *et al.* (1991) recommended the application of simple water extract of partially ground berries. The use of crude powder of unripe green berries without any extraction process suspended in water is economically feasible and can be used directly by farmers.

Most of the bioassays done on snails were using alcohol and water extracts on schistosoma transmitting snails. Limited references are available on works done on crude endod powder particularly on fasciola transmitting snails. There is also scanty information on the effect of endod on different age groups of *Lymnaea* snails. Aklilu Lemma (1970) reported endod killed *L. natalensis* at higher concentration compared to schistosoma transmitting snail species. He also reported that 6 hrs exposure needs higher concentration than 24 hrs of exposure to get 90% mortality in other snail species while nearly equal concentration was needed in the case of *L. natalensis* at both exposure times.

The present study was conducted to observe the effect of endod on different age groups of fasciola transmitting snails and assessing time-concentration relationships.

MATERIALS AND METHODS

Study location

The study was conducted at the Institute of Pathobiology (IPB), Addis Ababa University.

Snails

L. natalensis snails were collected from Lake Zeway, 160 kms South of Addis Ababa. They were kept in aquaria containing aged tap water fed with boiled and dried lettuce. *L. truncatula* snails were collected from Kordida, a small river 100 kms north of Addis Ababa, along the Addis Ababa - Debre Birhan road. The snails were kept in the laboratory by feeding them with blue green algae (*Nostock vascorum*) grown on petridishes. The limited experience on laboratory feeding of snails suggests that *L. natalensis* being a fresh-water snail thrives better when fed lettuce whereas *L. truncatula* being amphibious snail performs better on algae. Both snail species were kept in laboratory for an adaptation period of three to four days prior to initiation of the study. The test snails were checked for the presence of trematode infection and only two *L. truncatula* snails, which were excluded from the trial, were found shading the cercariae. The active snails were then selected and grouped based on their size (by measuring the snail shell height). A total of 1365 snails (945 *L. natalensis* and 420 *L. truncatula*) were used to investigate the effect of endod on developmental stages of snails, while 1637 snails (801 *L. natalensis* and 836 *L. truncatula*) were used for determination of time-concentration relationship.

Endod

Unripe green berries of endod (Type 44), grown in the premises of IPB, were dried for three days in the sun and ground using an electric grinder.

Study design

One gram of powder of endod was soaked in 500ml of clean tap water for 16–18 hrs, which was then reconstituted to one liter to form a stock solution of 1000 Parts Per Million (ppm). This stock solution was diluted with clean tap water to give the desired weight to volume concentration expressed in ppm. Nine to ten snails were then placed in plastic trays containing 200 ml of serial dilutions of endod and the same volume of tap water for the control group. Following specified period of exposure, the snails and plastic trays were thoroughly washed with tap water and the snails were kept for recovery period of 24 hrs in aged tap water. The test was replicated 3-5 times based on the availability of snails for each purpose. The pH of the solution ranged between 6.45-6.55 throughout the study period. Identification of dead snails was based on the absence of motility and failure to react when the pads were pricked with needle.

Statistical analysis

To estimate the lethal concentration (LC_{50} and LC_{90}) the data were summarized using a scientific calculator following the procedure described by Healy (1988). The procedure is based on the principle of fitting a logit model to the data. Simple linear regression analysis is performed taking logit of the proportion as dependent variable and the logarithm of the dose as independent variable. In this case the null hypothesis is that the regression line fits well to the data. Expected proportion is obtained after getting expected logit using the regression result and the lethal concentrations are determined with their corresponding chi-square values by which the goodness-of-fit of the regression line to the data is assessed.

RESULTS

The death rates for snails exposed for 24 hours to different concentrations of endod are shown in Table 1. A hundred per cent mortality was observed in young snails at a concentration of 20 ppm for both *L. natalensis* and *L. truncatula*, while a higher concentration was needed to produce the same effect in mature snails. The LC values of endod on different developmental stages of snails show that both species and all stages tested are nearly equally susceptible with slight decrease in susceptibility with the increase in age (Table 2). The difference in susceptibility among developmental stages of *L. natalensis* increases at short exposure time (Table 3).

Table 1. Effect of endod on different developmental stages of snails after 24 hours of exposure.

Species	Shell height (mm)	Total no of snails per each concentration	Percentage mortality in the following concentrations (ppm)						
			0	7.5	10	12.5	15	17.5	20
L. natalensis	5-9.8	45	2.2	0	29	40	69	82	100
	10-12.9	45	0	0	24	42	62	82	89
	13-18.5	45	4.4	0	15.6	26.7	51	72	80
L. trucantula	3.4-5.4	30	6.7	10	30	43	70	80	100
	5.7-8.2	30	3.3	10	33	43	57	73	90

Table 2. Susceptibility of different developmental stages of *L. natalensis* and *L. truncatula* to endod after 24 hours of exposure.

Species	Shell height (mm)	LC_{50} (ppm)	LC_{90} (ppm)	X^2	d.f
	5-9.8	12.6	19.8	1.91	3
L. natalensis	10-12.9	13.1	21.1	2.63	3
	13-18.5	14.5	22.8	0.86	3
	3.4-5.4	12.1	19.5	0.94	3
L. truncatula	5.7-8.2	12.6	21.0	2.94	4

Table 3. Mortality rates of *L. natalensis* exposed to different concentrations of endod at 1,3,6 and 24 hours of exposure.

Exposure time (hrs)	Concentration (ppm)	No. of Snails	Mortality			
			Young snails (6–12.4)		Mature Snails (12.5–18.5mm)	
			No. Dead	%	No. Dead	%
	0	27	1	3.7	0	0
	10	"	5	18.5	2	7.4
	15	"	6	22.2	5	18.5
	20	"	9	33.3	6	22.2
1	25	"	13	48.2	8	29.6
	30	"	18	66.7	15	55.6
	35	"	23	85.2	18	67
	0	30	0	0	0	0
	10	"	4	13.3	2	6.7
3	15	"	8	26.7	7	23.3
	20	"	13	43.3	13	43.3
	25	"	21	70	18	60
	30	"	26	86.7	22	73.3
	0	27	1	3.7	0	0
	10	"	5	18.5	3	11.1
6	12.5	"	13	48.2	11	40.7
	15	"	19	70.4	15	55.6
	17.5	"	22	81.5	19	70.4
	20	"	27	100	24	88.9
	0	45	3	6.7	2	4.4
	10	"	9	20	8	17.8
24	12.5	"	17	37.8	13	28.9
	15	"	31	68.9	24	53.3
	17.5	"	37	82.2	34	75.6
	20	"	45	100	42	93.3

More than 40 ppm was required to induce 90% mortality when snails were exposed for 1 and 3 hours while about 20 ppm was sufficient to get the same effect in 6 and 24 hours of exposure time (Tables 3 and 4). The estimated LC values show that increasing the exposure period reduces the amount of endod required. However, LC_{50} and LC_{90} values for both species of *Lymnaea* were similar both at 6 and 24 hours of exposure (Table5).

Table 4. Mortality rates of *L. truncatula* (4.5–7.4 mm) exposed to different concentrations of endod at 1,3,6 and 24 hours of exposure.

Exposure Time(hrs)	Concentration (ppm)	No. of Snails	Mortality	
			No. Dead	%
	0	30	0	0
	20	"	5	16.7
	25	"	10	33.3
	30	"	13	43.3
1	35	"	15	50
	40	"	20	66.7
	45	"	25	83.3
	50	"	27	90
	0	30	0	0
	15	"	7	23.3
3	20	"	10	33.3
	25	"	15	50
	30	29	19	65.5
	35	30	25	83.3
	40	"	28	93.3
	0	30	1	3.3
	10	"	4	13.3
	12.5	"	11	36.7
6	15	"	18	60
	17.5	"	22	73.3
	20	"	28	93.3
	0	30	2	6.7
	7.5	"	4	13.3
	10	"	9	30
24	12.5	28	12	42.9
	15	30	16	53.3
	17.5	"	21	70
	20	29	25	86.2

Table 5. Time-concentration relationship of endod against *L. natalensis* and *L. truncatula*.

Exposure time (hrs)	Species	LC_{50} (ppm)	LC_{90} (ppm)	X^2	d.f
1	*L. natalensis* (6-12.4mm)	21.3	48.9	6.41	4
	(12.5-18.5mm)	30.5	72.5	2.3	4
	L. truncatula (4.5-7.4mm)	31	54.5	2.7	5
3	*L. natalensis* (6-12.4)	19.2	36.9	2.5	3
	(12.5-18.5mm)	22	44.1	0.03	3
	L. truncatula (4.5-5.4mm)	23	41	2.5	4
6	*L. natalensis* (6-12.4mm)	12.8	18.9	0.08	3
	(12.5-18.5mm)	14.2	21	1.15	3
	L. truncatula (4.5-5.4mm)	13.9	21.1	0.14	2
24	*L. natalensis* (6-12.4mm)	12.8	18.5	1.2	2
	(12.5-18.5mm)	13.7	20	3.4	3
	L. truncatula (4.5-5.4mm)	12.4	21.7	3.5	4

DISCUSSION

Snails vary in susceptibility to molluscucides for various reasons such as physiological differences between species, presence of trematode infection, difference in composition of water medium (hardness, pH, total dissolved solutes) (Aklilu Lemma and Yau, 1974). The results of the present work show that at about the same concentration both *L. natalensis* and *L. truncatula* are equally susceptible to endod. Aklilu Lemma (1970) found that LC_{90} value of endod on adult *L. natalensis* to be 28.9 ppm, which is higher than the present finding (22.8 ppm). This variation may be due to differences in type of endod used or in strain of snails. Aklilu Lemma and Yau (1974) observed susceptibility of *Biomphalaria* to endod and copper sulfate increases with increasing age, whereas susceptibility to pentachlrophenol decreases with increasing age. Although there was no marked difference, there was a slight decrease in susceptibility as the age (size) increases. This difference in susceptibility of different developmental stages of snails may be attributed to physiological and biological changes associated with the development of snails. The practical significance of the present finding is that in the field application of endod to control fasciolasis, it is better to apply when the populations of young snails dominate.

The results obtained from the study of time-concentration relationship is significant since it provides valuable information on the duration of time that endod should be applied in the field. At higher concentrations only a short period of exposure was required to induce 90% mortality. However, LC_{90} values for both snail species were identical both with 6 and 24 hours of exposure time. The 90% mortality result of *L. natelensis* for the exposure period of 6 and 24 hours reported by Aklilu Lemma (1970) is in agreement with the present finding. Concerning *Bulinus* and *Biomphalaria* snails Aklilu Lemma, in the same study, reported that at the exposure time of 6 hours 3 or more times concentration needed at 24 hours was required to produce 90% mortality. This unique property of *Lymnaea* snails may be due to their intrinsic nature whose tissues may allow absorption of a lethal dose in short period of time. Provided that there is no scarcity of endod, it is possible to use higher concentrations for short period. Field application of endod in slow moving water bodies does not need maintaining lethal concentration for 24 hours, since application for 6 hours have similar effect at the same concentration. The lethal effect of endod to small fish is a disadvantage shared with all molluscicides in use for control of schistosomiasis (WHO, 1965; Makhubu *et al.*, 1987). However, edible fish rarely breed in small streams and canals where fasciola transmitting snails *(L. natalensis*) breed (Aklilu Lemma, 1984). Additional data are needed to evaluate the effects of endod for this and other possible environmental hazards**.** *L truncatula,* intermediate host of *Fasciola hepatica*, is an amphibious snail living in shallow ponds, wet lands, small streams and temporary collection of water particularly where conditions are cool and humid (Brown, 1980). The snail activity in these areas is limited to short rainy seasons (Goll and Scott, 1979). Therefore, application of endod to such areas at the specific season when snails flourish is feasible since endod has low mammalian toxicity and biodegraded rapidly (Makhubu *et.al.*, 1987; Aklilu Lemma, 1984). Endod being a widely grown, relatively non-toxic natural product requiring minimal technological inputs and handling is more reliable in control of fascioliasis than commercial molluscicides. It has also been shown that the molluscicidal potency of endod remains fairly stable over a wide range of pH, temperature, UV-irradiation and in various concentrations of organic and inorganic materials (Aklilu Lemma and Yau, 1974). Further detailed studies should be conducted on ecology and biology of snail intermediate hosts and appropriate methods and period of applications of endod for control of fascioliasis in different agro-ecological zones.

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