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Sublethal Exposure of *Biomphalaria glabrata* and *Indoplanorbis exustus* Eggs to Crude Extracts of *Brassaia actinophylla* and Niclosamide

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=국문요약=

*Biomphalaria glabrata*와 *Indoplanorbis exustus* 충란에 대한 *Brassaia actinophylla* 추출물과 Niclosamide의 아치사 처리

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*Biomphalaria glabrata*와 *Indoplanorbis exustus*의 알에 대한 식물성 살패제 *Brassaia actinophylla* (Araliaceae)의 메탄올 추출물을 아치사 농도로 처리하였을 때 효과를 평가하였다. 그 결과 *B. actinophylla*를 10 mg/l 농도로 처리하였을 때, 두 종류의 패류의 배(embryo)에서 모두 변화를 보였다. Niclosamide를 0.004 mg/l로 처리하였을 경우 역시 *B. glabrata*와 *I. exustus*의 배 발생을 억제하였다. 따라서 *B. actinophylla*는 주혈흡충증의 중간숙주인 패류 관리에 있어 난세포 치사제로 이용될 수 있을 것이다.

Key words : *Brassaia actinophylla*, *Biomphalaria glabrata*, *Indoplanorbis exustus*, Ovicidal agent

INTRODUCTION

Snail control is an important aspect of the control of schistosomiasis. It is an essential element of an integrated approach to prevent this positive infection. The realization that plants showing intrinsic molluscicidal activity could provide a cheap and readily available means of snail control has led to the screening of many plant species, several of which have shown considerable promise as the source of control agents (Kloos and McCullough, 1987; Farnsworth *et al.*, 1987; Kuo, 1987). *Brassaia actinophylla* is considered as one of the most promising plant molluscicide (Upatham *et al.*, in press) together with *Tetrapleura tetraptera*, *Phytolacca dodecandra* and *Millettia thonningii* (Kloos and McCullough, 1987; Evan *et al.*, 1986). Adewunmi and Marquis (1981) reported the lack of acute ovicidal activity of aridan in the extract of *T. tetraptera*.. Adewunmi (1991) noted the knock-down effect on the embryos of *Biomphalaria glabrata* by the continuous exposure to aridanin (10 mg/l), an extract of *T. tetraptera*.

The objective of the present investigation is to study the effects of methanol extracts of *B. actinophylla* and niclosamide on development of eggs of schistosomiasis-mediating snails, *B. glabrata* and

Table 1. Effect of methanol extract of *B. actinophylla* on the hatching of eggs of *I. exustus*

Concentration (mg/l)	Number of eggs	Number of eggs hatched on days after exposure						Hatching rate(%)
		6	7	8	9	10	11	
0 (Control)	67	0	0	44	22	1	0	100.0
2	70	0	0	21	37	8	0	94.3
4	67	0	0	44	19	0	0	88.7
6	73	0	0	33	21	0	0	74.0
8	63	0	0	17	27	0	0	69.8
10	68	0	0	5	18	11	3	54.4
12*	67	0	0	0	7	15	3	37.3
14*	72	0	0	4	9	3	0	22.2
16*	58	0	0	0	3	0	0	5.2
18*	65	0	0	0	0	0	0	0
20*	88	0	0	0	0	0	0	0
22*	77	0	0	0	0	0	0	0

* = lethal concentration

Indoplanorbis exustus.

MATERIAL AND METHODS

Two species of snails *B. glabrata* (Say) and *I. exustus* (Deshayes) were separately reared in polyethylene bags with dechlorinated tap water and were aerated. They were fed with lettuce leaves. Occasionally, fresh dechlorinated tap water was added to stimulate oviposition. Egg masses were laid on polyethylene sheet and were collected daily by cutting out a small piece with one egg mass attached and transferred to a Petri dish for examination.

Toxicity experiments with methanol extracts of *B. actinophylla* and niclosamide [Bayluscide 250 EC (Bayer) (2,5 dichloro-4-nitrosalicylanilide 25% w/v)] were performed by exposing the egg masses of *B. glabrata* and *I. exustus* at sublethal and lethal concentrations. Sublethal concentrations of niclosamide and methanol extract *B. actinophylla* were lower than 0.005 and 11 mg/l, respectively (Upatham *et al.*, in press).

Crystals of methanol extract of *B. actinophylla* were dissolved in dechlorinated tap water to make a stock solution of 50 mg/l. Niclosamide solution was diluted in distilled water to give a stock solution of 0.001 mg/l. Stock solutions of the molluscicides were diluted into a serial dilutions of the respective toxicants as follows : methanol extract of *B. actinophylla*, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20 and 22 mg/l; niclosamide, 0.002, 0.003, 0.004, 0.005, 0.006, 0.007 and 0.008 mg/l.

Egg masses (8-33 eggs) of 0-1 day with no later than blastula stage were exposed to the ranges of methanol extract of *B. actinophylla* and niclosamide concentrations. They were observed daily for abnormal development. The numbers of hatched and unhatched eggs were recorded. Molluscicide solution was renewed every two days. Control group was exposed to dechlorinated tap water. The experiments were done in 3 replications.

RESULTS

Tables 1 and 2 show the effects of methanol

Table 2. Effect of methanol extract of *B. actinophylla* on the hatching of eggs of *B. glabrata*

Concentration (mg/l)	Number of eggs	Number of eggs hatched on days after exposure						Hatching rate(%)
		6	7	8	9	10	11	
0 (Control)	79	0	3	39	32	4	0	100.0
2	81	0	0	20	30	19	6	92.6
4	79	0	12	32	14	0	0	81.7
6	72	0	12	31	15	0	0	80.6
8	86	0	0	5	45	14	0	74.4
10	67	0	0	0	13	29	0	62.7
12*	87	0	0	0	27	22	0	56.3
14*	88	0	0	5	27	13	0	51.1
16*	99	0	0	4	16	13	0	33.3
18*	79	0	0	1	15	8	0	30.4
20*	77	0	0	4	5	0	0	11.7
22*	78	0	0	0	0	0	0	0

* = lethal concentration

extract of *B. actinophylla* on the hatching of eggs of *I. exustus* and *B. glabrata* , respectively. The hatching periods at room temperature (26-28℃) of *I. exustus* and *B. glabrata* are 8-9 days and 7-9 days, respectively (Figs. 3a-c, 4a-c). In general, it is apparent that the increase in concentrations of the methanol extract of *B. actinophylla* causes both the delay and decrease in hatching rates in *I. exustus* and *B. glabrata* (Figs 3e, 4e).

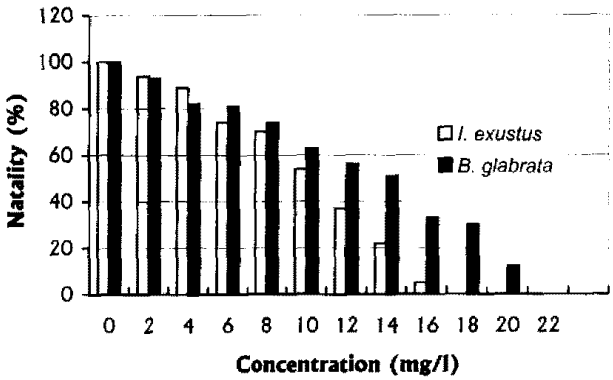

Fig. 1. Natality of snails, *Indoplanorbis exustus* and *Biomphalaria glabrata* exposed to *Brassaia actinophylla*.

Figure 1 shows the natality of snails, *I. exustus* and *B. glabrata*, exposed to the methanol extract of *B. actinophylla*. The concentration of 6-10 mg/l (in *I. exustus*) and 8-10 mg/l (in *B. glabrata*) cause a decrease in hatching rate of eggs in both snails species. The concentrations of 18 mg/l and (in *I. exustus*) and 22 mg/l (in *B. glabrata*) result in the death of all embryos in the eggs.

Table 3 and 4 show the effects of niclosamide on the hatching of eggs of *I. exustus* and *B. glabrata*, respectively. Niclosamide also causes a decrease in hatching rates of eggs in both snail species at very low concentrations. Figures 2 shows the natality of snails, *I. exustus* and *B. glabrata*, exposed to niclosamide. At the concentrations of 0.005-0.006 mg/l, niclosamide cause a decrease in hatching rates in both species. Niclosamide at the concentration of 0.007 mg/l can kill the embryos of both snail species (Fig. 2).

Figures 3 and 4 show the embryonic development of *I. exustus* and *B. glabrata* respectively : 0 - to 1-day-old eggs (Figs 3a, 4a); 3-day-old eggs with the appearance of torsion stage (Figs 3b, 4b); 7- to

Table 3. Effect of niclosamide on the hatching of eggs of *I. exustus*

Concentration (mg/l)	Number of eggs	Number of eggs hatched on days after exposure						Hatching rate(%)
		6	7	8	9	10	11	
0 (Control)	52	0	0	39	13	0	0	100.0
0.002	72	0	12	35	17	0	0	88.9
0.003	63	0	19	35	6	0	0	87.3
0.004	65	0	5	32	8	0	0	69.2
0.005*	68	0	4	14	9	0	0	38.7
0.006*	64	0	1	6	4	0	0	17.2
0.007*	65	0	0	0	0	0	0	0

* = lethal concentration

9 -day-old eggs shows the final stage of hatching (Figs. 3c, 4c). Figures 3d and 4d show the knock-down effects of *B. actinophylla* on the eggs of *I. exustus* and *B. glabrata*. Dead snails in the eggs are shown in Figs. 3e and 4e.

DISCUSSION AND CONCLUSIONS

The ovicidal activity of niclosamide has been reported at a concentrations of 1.0 mg/l and above (Deschiens and Flock, 1969) and 0.625-0.25 mg/l (Adewunmi, 1991). In the present study, lower concentrations (0.002-0.007 mg/l) were found to be lethal to 0-1 day old snail eggs after 7-9 days for *I. exustus* and 7-8 days for *B. glabrata* for long time exposure. The highest sublethal concentration of niclosamide was 0.004 mg/l and methanol extract

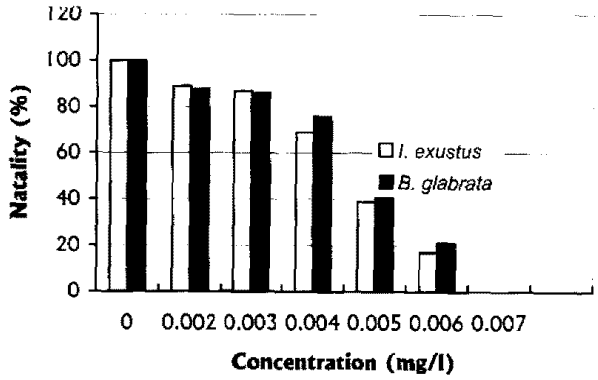

Fig. 2. Natality of snails, *Indoplanorbis exustus* and *Biomphalaria glabrata* exposed to niclosamide.

Table 4. Effect of niclosamide on the hatching of eggs of *B. glabrata*

Concentration (mg/l)	Number of eggs	Number of eggs hatched on days after exposure						Hatching rate(%)
		6	7	8	9	10	11	
0 (Control)	52	0	32	20	0	0	0	100.0
0.002	67	0	24	35	0	0	0	88.1
0.003	71	0	19	42	0	0	0	85.9
0.004	79	0	19	41	0	0	0	75.9
0.005*	78	0	8	26	0	0	0	43.6
0.006*	78	0	3	13	0	0	0	20.5
0.007*	71	0	0	0	0	0	0	0

* = lethal concentration

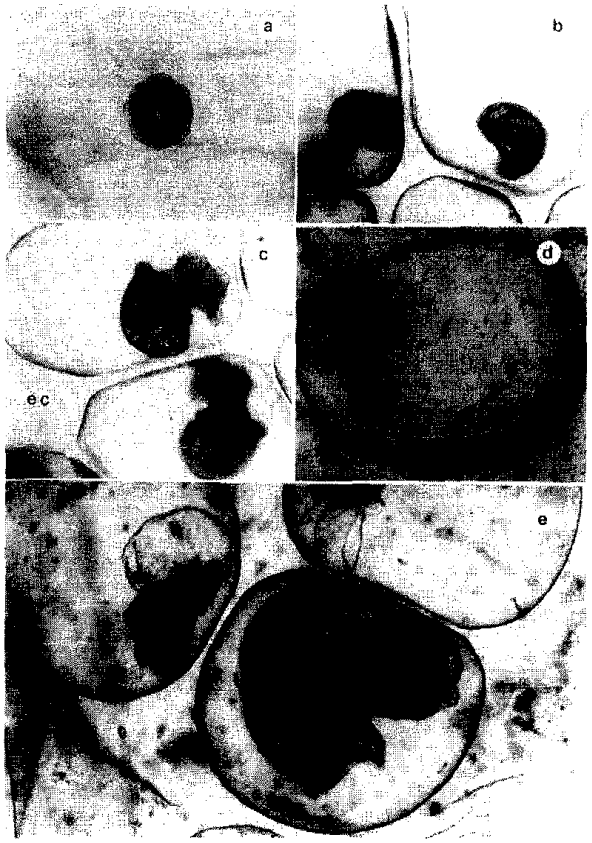

Fig. 3. Embryonic development of snail *I. exustus* : a. 0- to 1- day-old eggs; b. 3- day-old eggs with appearance of torsion stage; c. 7- to 9- day-old eggs showing final stage of hatching; d. Knock-down effect of *B. actinophylla* e. Dead snails in the eggs. ec = egg capsule.

Fig. 4. Embryonic development of snail *B. glabrata* : a. 0- to 1- day-old eggs; b. 3- day-old eggs with appearance of torsion stage; c. 7- to 9- day-old eggs showing final stage of hatching; d. Knock-down effect of *B. actinophylla*; e. Dead snails in the eggs. ec = egg capsule.

of *B. actinophylla* was 10 mg/l. It is found that at the highest sublethal concentration of of niclosamide (0.004 mg/l) and methanol extract of *B. actinophylla* (10 mg/l) the natality of embryos of *I. exustus* and *B. glabrata* were 69.2% and 75.9% respectively whereas those of *B. actinophylla* were 54.4% and 62.7% respectively. The effect of methanol extract of *B. actinophylla* at sublethal concentration (2-10 mg/l) to both snail species is not significant difference at the 95 percent confidence interval. Also there is no significant different effect of niclosamide to both snail species.

Although the lethal concentration of niclosamide is much lower than that of methanol extract of *B. actinophylla,* but comparing with the sublethal concentration of both molluscicides, methanol extract of *B. actinophylla* had more little effective than niclosamide for inhibition of hatching of snail eggs (Figs 1, 2).

By observing, the embryos were killed at different stages of development. At high concentrations of sublethal concentration, the embryos died before the development of trochophore stage, wheareas at low concentrations, the embryos died at the prehatched stage within 10 days of continuous exposure. Furthermore, both niclosamide and methanol extract of *B. actinophylla* were able to kill the early developed snails (cleavage stage) in a short period

of continuous exposure. It appears that only the fully developed snails at the prehatched stage were dead. The hatching process had weakened the protective ability of egg masses of the snails and allowed the entry of molluscicides which were then able to kill the embryos in the egg masses (Adewunmi, 1991).

In conclusions, *B. actinophylla* has a potential use as an ovicidal agent in the control of the snail intermediate host of schistosome. This will give us a better choice by using crude extract of *B. actinophylla* at low concentration for the long time exposure to control population of snail as it can kill embryos of those snails.

SUMMARY

The effects of methanol extracts of plant molluscicide, *Brassaia actinophylla* (Araliaceae) had been assessed on development of eggs of snails, *Biomphalaria glabrata* and *Indoplanorbis exustus* at sublethal concentrations. Results revealed that the administration of 10 mg/l of *B. actinophylla* caused some alterations in embryos of both species. Niclosamide (0.004 mg/l) also arrested the development of embryos in *B. glabrata* and *I. exustus*. It can be concluded that *B. actinophylla* has a potential use as an ovicidal agent in the control of the snail intermediate hosts of schistosomiasis.

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