

THE MOLLUSCICIDAL ACTIVITIES OF SOME *EUPHORBIA MILII* HYBRIDS AGAINST THE SNAIL *INDOPLANORBIS EXUSTUS*

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Abstract. The objective of this study was to observe the molluscicidal activities of *Euphorbia milii*, known as “poysean” in Thailand, against *Indoplanorbis exustus*. Latex from 12 different *E. milii* hybrids was screened for its molluscicidal activities. *Indoplanorbis exustus* were exposed for 24 and 48 hours to the latex at various concentrations ranging from 6 to 25 ppm and mortality rates were recorded. Eight hybrids of latex were effective. The six most effective hybrids were *E. milii* Dang-udom, *E. milii* Arunroong, *E. milii* Raweechotchuong, *E. milii* Srisompote, *E. milii* Sri-umporn and *E. milii* Tongnopakun, which killed all snails after 24 hours of exposure. Under the same conditions, latex of *E. milii* Dowpraket and *E. milii* Promsatid killed 50% of the snails. Such results indicate that these 6 hybrids seem promising as natural molluscicidal agents.

INTRODUCTION

In Southeast Asia, especially India and Thailand, *Indoplanorbis exustus* snails can harbor metacercariae and cercariae of several kinds of flukes, such as the bovine blood fluke, *Schistosoma spindale* (Ito *et al*, 1962; Papasarathorn *et al*, 1963; Harinsuta *et al*, 1965; Kullavanijaya and Wongwaisavawan, 1993), and intestinal echinostomiasis (Bhaibulaya *et al*, 1964, 1966; Yokogawa *et al*, 1965). Human infections are acquired from consumption of the raw snails infected with metacercariae or cercariae. The people in northeastern Thailand are fond of eating raw food, including fish, prawns and snails. Thus, these diseases still remain prevalent in Thailand.

Control measures for these diseases may be achieved by destroying the intermediate host. However, controlling the intermediate host is difficult because these snails have high reproduction potential (Krull, 1933) and can survive in different habitats (Malek, 1985). Many plant species have been tested as molluscicides all over the world, as indicated by Kloos and Mc-Cullough (1987), Kuo (1987) and Jurberg *et al* (1989). *Euphorbia splendens* is a plant with great potential for use in the control of schistosomiasis vector snails.

Euphorbia milii is a famous pot-house plant that originated in Madagascar. The orange-red flowering clones are common in tropical and subtropical areas, including Thailand. A copious quantity of poisonous, milky sap of *E. milii* contains diterpene esters. The latex

of Crown-of-Thorns (*Euphorbia milii* Des Moul. ex Boiss var *hislopii*, syn. *E. splendens*) seems to be one of the most interesting plant molluscicides that has been tested so far. It is active against mollusks that are intermediate hosts of *Schistosoma* trematodes (Vasconcellos and Schall, 1986; Mendes *et al*, 1997; Schall *et al*, 1998). The phytochemical fractionation of latex of *E. splendens* showed that milliamine L, one of eight substances derived from active fractions, was 100 times as active as miclosamide (Zani *et al*, 1993), a chemical compound used for schistosomiasis snail vector control. Recent studies revealed that 0.5 ppm of *E. milii* latex showed molluscicidal activities under laboratory conditions and a concentration as low as 5.0 and 12.0 ppm under field conditions (Vasconcellos and Schall, 1986; Mendes *et al*, 1997; Schall *et al*, 1998). Recently, the molluscicidal activities of *E. milii* latex have also been studied in various species of fluke snail vectors such as *Biomphalaria* spp (Vasconcellos and Schall, 1986; Baptista *et al*, 1992; Mendes *et al*, 1997; Schall *et al*, 1998), *Bulinus* spp (Vasconcellos *et al*, 1993) and *Lymnaea columella* (Vasconcellos *et al*, 2003).

E. milii has been grown in Thailand for a very long time; the plants are known as “poysean” (Chinese for “eight saints”) and are regarded as bringing good fortune (lucky plants). It believed that the first plants were brought to Thailand by Chinese immigrants. For the past 20-30 years growers in Thailand have developed an array of hybrids with much larger flowers than found in previous cultivars, along with a seemingly infinite variety of color combinations, most of these having local Thai names.

The objective of this study was to observe the molluscicidal activities of common strains of *Euphorbia milii* hybrid plants against *Indoplanorbis*

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exustus, a vector of intestinal and blood flukes in Thailand (Bhaibulaya *et al.*, 1964, 1966; Yokogawa *et al.*, 1965; Kullavanijaya, 1993).

MATERIALS AND METHODS

Plants used

Twelve hybrids of *E. milii* hybrids from a private garden in Bangkok (*E. milii* Dang-udom, *E. milii* Kularbkelang, *E. milii* Arunroong, *E. milii* Raweechotchuong, *E. milii* Srisompote, *E. milii* Sriumporn, *E. milii* Tongnopakun, *E. milii* Dowpraket, *E. milii* Promsatid, *E. milii* Mingkhon, *E. milii* Yondkumpon, and *E. milii* Soyrtchane) were used in this experiment.

Snail collection

A total of 410 *Indoplanorbis exustus* were collected from lotus pools in Bangkok and Nakorn Pathom Province; snails with shell sizes that varied from 5 to 9 mm in diameter were used in the experiment.

Latex preparation

The latex was collected on the same day the experiment was performed. Branches of *E. milii* hybrids were cut about 10 cm below the apical meristem of each branch. The raw latex was collected in a closed container to avoid coagulation.

The concentrations used in the bioassays were prepared from raw latex, in successive dilutions with distilled water. Using one ml of natural latex and filling it up with 1,000 ml of distilled water, we obtained the concentration of 1,000 mg/l (= 1,000 ppm, stock solution). After the preparation of the stock solution of 1,000 mg/l, successive dilutions of the aqueous extract for *E. milii* Dang-udom and *E. milii* Kularbkelang, were prepared to obtain the final concentrations of 6, 8, 10, 12, 14, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25 mg/l in the final volume of 500 ml in each solution. The other *E. milii* hybrids were prepared from raw latex, in final dilutions with 500 ml distilled water of 22 ppm (mg/l). We used a control with pure distilled water without latex, with the same volume of the solution.

Period of exposure and recovery

Forty-one samples of *Indoplanorbis exustus* were exposed to concentrations in 500 ml with 10 snails in each flask over 24 hours. The flasks were covered with a plastic screen to allow air flow and prevented the snails from escaping. The space between the solution and the screen allowed the animals to leave the solution without leaving the container. During this period, the flasks containing the concentrations were kept at room temperature and the snails were not fed.

After exposure, the snails were removed from the flasks, rinsed with distilled water for removal of residues from the shell and moved to the containers with the solvent (distilled water), in the same volume as the initial one, for another period of 24 hours (recovery period) and fed with small bits of fresh lettuce. After this 24 hours period that is 48 hours after the beginning of the test, the dead and the living snails were counted.

Characterization of the snails' death

The deaths of the snails during the tests were confirmed by the change in the shell color and absence of muscle contractions. In general, the cephalopodal mass was distended in a distinguished fashion.

RESULTS

The molluscicidal activities of aqueous extracts obtained from the natural latex of *E. milii* Dang-udom on *Indoplanorbis exustus* is presented in Table 1. The LC₅₀ of *E. milii* Kularbkelang was over 25 ppm within 48 hours. The data demonstrates the difference in toxicity between the two hybrids. The mortality rate caused by *E. milii* Dang-udom is higher than that caused by the other. The LC₅₀ and LC₉₀ within 48 hours of *E. milii* Dang-udom were 18 ppm and 20.5 ppm, respectively.

In Table 2, ten hybrids of *E. milii* were screened for their molluscicidal activities against *Indoplanorbis exustus*. The snails were exposed to the latex of *E. milii* hybrids at a concentration of 22 ppm for 24 hours. It was shown that most snails were retracted into their shells after treatment with the latex and a reddish brown solution was observed around them before they died. The molluscicidal activities of ten *E. milii* hybrids were compared. The results demonstrated that *Indoplanorbis* snails were susceptible to the latex of *E. milii*, with mortality rates ranging from 10 to 100%. The five most effective hybrids of *E. milii* were: Arunroong, Raweechotchuong, Srisompote, Sriumporn and Tongnopakun. The latexes prepared from three *E. milii* hybrids – Mingkwan, Yodkumpon, and Soyrtchane – were slightly toxic to *Indoplanorbis* snails. Thus, the results revealed that the efficacy of *E. milii* latex on *Indoplanorbis* snails was varied, depending on hybrids.

DISCUSSION

Similar to the results from previous studies on the toxicity of latex from other hybrids of *E. milii* (Oliveira-Filho and Paumgarten, 2000; Giovanelli *et al.*, 2001), the latex from *E. milii* Dang-udom showed highly

Table 1
Molluscicidal action of the latex of *E. milli* Dang-udom against *Indoplanorbis exustus*.

Concentration of <i>E. milli</i> Dang-Udom (mg/l)	Number of <i>I. exustus</i>				Mortality (%)
	Before application		After application		
	Alive	Dead	Alive	Dead	
0	10	0	9	1	10
6	10	0	9	1	10
8	10	0	8	2	20
10	10	0	9	1	10
12	10	0	6	4	40
14	10	0	6	4	40
16	10	0	5	5	50
18	10	0	5	5	50
19	10	0	5	5	50
20	10	0	2	8	80
21	10	0	0	10	100
22	10	0	0	10	100
23	10	0	0	10	100
24	10	0	0	10	100
25	10	0	0	10	100

Table 2
The molluscicidal activity of ten *E. milii* hybrid latex at a concentration of 22 ppm (mg/l) against *Indoplanorbis exustus*.

Group	Number of <i>Indoplanorbis exustus</i>				Mortality (%)
	Before application		After application		
	Alive	Dead	Alive	Dead	
Control	10	0	10	0	0
<i>E. milii</i> Arunroong	10	0	0	10	100
<i>E. milii</i> Raweechotchuong	10	0	0	10	100
<i>E. milii</i> Srisompote	10	0	0	10	100
<i>E. milii</i> Sri-umporn	10	0	0	10	100
<i>E. milii</i> Tongnopakun	10	0	0	10	100
<i>E. milii</i> Dowpraket	10	0	3	7	70
<i>E. milii</i> Promsatid	10	0	4	6	60
<i>E. milii</i> Mingkhon	10	0	7	3	30
<i>E. milii</i> Yondkumpon	10	0	8	2	20
<i>E. milii</i> Soyatchanee	10	0	9	1	10

effective molluscicidal activity against *Biomphalaria glabrata* – LC₉₀ = 2 ppm within 48 hours and 100% mortality at the concentration of 3 ppm (data not shown). Many researchers have reported the toxicity of *E. milii* on mollusks, but most of them conducted studies on the genus *Biomphalaria*. In Thailand, where the habitat of *Indoplanorbis* spp is distributed throughout the country, no studies on the toxicity of *E. milii* on this mollusk have been reported.

The present study indicates that crude latex of *E. milii* hybrids are promising and very potent plant molluscicides for killing *I. exustus* snails. One of the greatest advantages of *E. milii* is that it requires only a small volume of plant material during plant multiplication and extraction stages, as well as a small volume of extracted product needed for stock. Handling the plant requires some care due to the numerous thorns along its stems, and with possible

squirting of the crude latex into the eyes. Adoption of safety measures, such as wearing appropriate gloves and goggles during handling, is advised.

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