

A preliminary study on the anti hatching of freshwater golden apple snail *Pomacea canaliculata* (Gastropoda: Ampullariidae) eggs from *Barringtonia racemosa* (Magnoliopsida: Lecythidaceae) seeds extract

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Abstract. The objective of the present study was to evaluate the alternative anti hatching of *Pomacea canaliculata* eggs using *Barringtonia racemosa* seed extract. Five concentrations of *B. racemosa* extract (20, 40, 60, 80, 100 ppm) and two types of solvent (methanol and water) were tested in this study. The Anova test showed that the concentration of *B. racemosa* seed extract was affected significantly on the hatchability of *P. canaliculata* eggs ($p < 0.01$). The results showed that the hatchability of *P. canaliculata* eggs were decreased with increasing the concentration of *B. racemosa* seed extracts, where the lower hatchability was found at 80 ppm, but it was not significant different with 100 ppm. It was concluded that the *B. racemosa* seed extract can be used to control hatchability of *P. canaliculata* eggs.

Key Words: *Barringtonia racemosa*, *Pomacea canaliculata*, anti hatching, secondary metabolites.

Introduction. The freshwater golden apple snails, *Pomacea canaliculata* (Lamarck, 1822) distribution is basically tropical and subtropical, including the Amazonas and La Plata basin (EstebeNET & Martin 2002). This snail was introduced to Indonesia as aquarium fauna in 1981 (Wahyu 1996), and after more than 20 years, the snail has spread and became very abundant in various habitats such as marshes, ponds, irrigations, lakes and rice fields in almost all places in Indonesia (Marwoto & Isnainingsih 2011). Presently, *P. canaliculata* is considered as a serious rice pest in Indonesia (Suharto 2002). The biology, impact and management of freshwater golden apple snails as agricultural pests and biological control agents has been extensively reviewed by Cowie (2006).

There were at least three methods have been applied to control this pest, for example biologically (Teo 2001; Yusa 2001), mechanically (Wada et al 1999; Takahashi et al 2002) and chemically. In regard to chemical method, the synthetic pesticides were commonly used to control freshwater golden apple snail (De la Cruz et al 2001). However, these pesticides caused resistance to the pests and harmful to the environment and people. Therefore, some researchers have been studied the alternative pest control of freshwater golden apple snails (Joshi et al 2008). One of the potential natural product for freshwater golden apple snail control was *Barringtonia racemosa* locally known as *penteut ie*. According to Musman (2010) the seed extracts of *B. racemosa* has a molluscicidal activity to *P. canaliculata* due to the presence of saponins and flavonoids which had a significant effect on mortality of the freshwater golden apple snails.

Several studies have been reported that hatching of the snail eggs can be inhibited by reducing the air supply to embryos in the egg (Wu et al 2005; Kurniawati et

al 2007), decreasing of temperature of incubation water (Schnorbach 1995; Taylor et al 1996; Horn et al 2008), or by blocking the penetration of water into the egg (Pizani et al 2005). However, no studies on using *B. racemosa* extracts as anti hatching of *P. canaliculata* eggs were reported. Hence, the objective of the present study was to examine *B. racemosa* seed extracts as anti hatching eggs of *P. canaliculata*.

Material and Method

Collection of *P. canaliculata* eggs and *B. racemosa* seeds. The eggs of *P. canaliculata* were collected in paddy field and irrigation channel of Gampong Gla Deyah (coordinate 5° 32' 14" N 95° 22' 6" E), Aceh Besar District, Aceh Province, Indonesia. The matured *B. racemosa* fruits were obtained from Gampong Lam Neuhen (coordinate 5° 31' 55" N 95° 23' 58" E), Aceh Besar District, Aceh Province, Indonesia. The plant was identified and confirmed by Botanist of Syiah Kuala University, Indonesia. The voucher specimen number is (Br/ff/LN/16213).

Extractions

Extraction of *B. racemosa* seed in methanol solvent. Fruits of *B. racemosa* were pared and the seed was thin-sliced using a knife, and sliced seeds were dried under indirect sunlight for 5 days. The dried slice seed was blended to make powders with a sieve size of 40 meshes. The powders were weighed as much as 100 g, and then put into a jar (5 L in volume). A total of one liter of absolute methanol was poured slowly into the jar and stirred homogeneously, and then the mixture was left for 24 hours. The mixture was separated with Whatman #1 paper. The filtrate was evaporated using an evaporator vacuum to obtain viscous crude extracts. The extracts were dried in a desiccator for eight hours.

Extraction of *B. racemosa* seed in water solvent. A total of 200 g of seeds were thin-sliced, and then the slices were blended to make pulp. The pulp was boiled within 100 mL water for 15 minutes, the decoction was filtered using Whatman #1 paper. The filtrate was evaporated through an evaporator vacuum to obtain viscous crude extracts. The specific gravity of condensed extracts was determined and then they were stored in a refrigerator for two days.

Exposure of extraction on the eggs. The study took place in outdoor at Gampong Gla Deyah from October till November 2012. Five concentrations of *B. racemosa* extracts were tested i.e. 20, 40, 60, 80 and 100 ppm with four replications. Water was utilized to dissolve the tested extracts. The solutions were filled into a spray bottle (5 mL in volume) as much as 2 mL on each concentration. A total of 48 unit aquarium glasses (45 cm x 30 cm x 35 cm) was used for this experiment, and the aquariums were filled with tap water (4 L). A gauze wire (30 cm x 20 cm in size) was set up in the middle of the aquarium above water level and the *P. canaliculata* eggs were laid onto the gauze wire (Figure 1). The observation of hatching eggs was done every day at 10.00 am for 30 days.



Figure 1. *P. canaliculata* eggs laid on a gauze wire in an aquarium.

Statistical analysis. All data were subjected to one way analysis of variance (ANOVA) followed by Tukey's multiple range test using SPSS software 18.0 versions.

Results and Discussion. The Anova test showed that the *B. racemosa* extracts extracted in methanol and water affect significantly on hatchability of *P. canaliculata* eggs ($p < 0.01$), where the hatchability of *P. canaliculata* eggs were decreased with increasing concentration of *B. racemosa* extract both in the methanol and water solvents. The Tukey test showed the concentration of 100 ppm were resulted in lower hatchability compared to other concentrations, however this result was not significantly different at a concentration of 80 ppm in both solvents (Table 1).

The inhibition of egg hatchability may be due to the active substances in the *B. racemosa* extracts. Egg capsule of the snail is formed by a calcium carbonate layer (Cowie 2006), the layer serves as a membrane for gas exchanges from the inside of the egg to the environment or vice versa. In addition, the egg shell acts as an effective barrier against losing of water during the incubation period in the air without blocking the supply of air (Turner 1998).

The study revealed that increasing the concentration of *B. racemosa* seed extracts were decreasing the hatchability of *P. canaliculata* eggs. The decreasing of egg hatchability was assumed due to the extract covering the surface of the egg capsule blocking the air supply and cause the embryo to die. This is in agreement with the Rawlings (1999) and Bigatti et al (2010) who reported that the number of embryos of Neo Gastropod molluscs decreased when the air supply decreasing. In addition, Wu et al (2005) studied the morpholine (C_4H_9NO) to inhibit hatching of *P. canaliculata* eggs. They reported that this material inhibit hatching of *P. canaliculata* eggs effectively, however, morpholine shows chronic effects in humans especially toxic to lungs and mucous membranes (Shea 1939), while the *B. racemosa* seed extracts are environmental friendly and non toxic to human and therefore suitable for controlling *P. canaliculata*.

Table 1

Hatchability of *P. canaliculata* eggs sprayed with the methanol and water extracts of *B. racemosa* seeds. Values with the same letter indicate treatments that were not significantly different ($p < 0.01$)

Conc. of the extract (ppm)	Number of eggs	Number of hatched eggs	The mean of hatching egg (%) sprayed with the methanol extract of <i>B. racemosa</i> seeds	Number of eggs	Number of hatched eggs	The mean of hatching egg (%) sprayed with the water extract of <i>B. racemosa</i> seeds
0	1047	1047	100 ^c	1121	1043	92.93 ^e
20	1437	141	9.92 ^b	1081	500	46.06 ^d
40	1125	75	7.89 ^b	1454	282	19.92 ^c
60	1692	115	6.81 ^b	994	69	7.21 ^b
80	1725	0	0.00 ^a	1227	10	0.72 ^a
100	1671	0	0.00 ^a	1626	0	0.00 ^a

Conclusions. The hatchability of freshwater golden apple snail eggs decreased with increasing of concentration of *B. racemosa* extracts. Therefore, the extracts of *B. racemosa* seeds can be used to inhibit hatchability of *P. canaliculata* eggs. The best concentration of the solution was 80 ppm and 100 ppm and the practice solvent was tap water.

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