

## Evaluation of teaseed cake as a piscicide for the culture of giant freshwater prawn, *Macrobrachium rosenbergii*

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### ABSTRACT

Results from acute toxicity tests indicated that teaseed cake is more toxic to silver barb (*Puntius gonionotus*) and marble goby (*Oxyleotris marmorata*) than to giant freshwater prawn. Giant freshwater prawn can tolerate up to 50.0 mg l<sup>-1</sup> of teaseed cake without any mortality after 24 h, while concentrations of 18.0 and 28.0 mg l<sup>-1</sup> of teaseed cake can completely eliminate silver barb and marble goby within 3 and 4 h, respectively. The 24-h LC<sub>50</sub> of teaseed cake on 0.05, 4.70 and 23.10 g prawn were 135.0, 62.0 and 61.0 mg l<sup>-1</sup>, respectively, while the 24-h LC<sub>50</sub> of teaseed cake on 0.45, 3.26 and 7.00 g silver barb were 2.8, 10.0 and 10.1 mg l<sup>-1</sup>, respectively. Meanwhile, the 24-h LC<sub>50</sub> of teaseed cake on 1.43 and 45.05 g marble goby were 16.0 and 22.0 mg l<sup>-1</sup>. Application of teaseed cake at the concentration of 30 mg l<sup>-1</sup> after reducing water level to half the depth of water storage level and letting it stand for 6 h before refilling to normal level did not have any effect on growth and survival rate of giant freshwater prawn after a 4-week experimental period. Results from this study indicated that selective eradication of competitive and predatory fishes in giant freshwater prawn culture ponds can be done by lowering water level in the ponds to half the volume, applying 30 mg l<sup>-1</sup> of teaseed cake, letting it stand for 6 h and then refilling the ponds.

### INTRODUCTION

Teaseed cake, the residue of *Camellia* sp. seeds after oil extraction, contains saponin, a water soluble glucoside which destroys red blood cells. Teaseed cake may vary in saponin content. It has been reported to be more toxic to finfish than to shrimp in brackish water and salt water (Terazaki *et al.*, 1980; Minsala and Chiu, 1986). The higher sensitivity of finfishes to the glucoside has made teaseed cake an effective piscicide in marine shrimp ponds. Recommended levels for use in eradicating undesirable fish in

shrimp ponds are 10-25 ppm of pond water (Cook, 1976) or 1.1 ppm of crude saponin, which is equivalent to 21 ppm of pond water if the teaseed cake contains 5.2% saponin (Terazaki *et al.*, 1980).

In freshwater the application of 25 mg l<sup>-1</sup> of teaseed cake in earthen ponds resulted in 28.0, 61.0, 58.0, 34.0 and 64.7% mortality of *Clarias* sp., *Cyprinus carpio*, *Gambusia* sp., *Oreochromis niloticus* and *Puntius gonionotus*, respectively (Chiayvareesajja *et al.*, 1997). However, there is no report on the toxicity of teaseed cake on *M. rosenbergii*. Saponin at the rate of as low as 0.9 mg l<sup>-1</sup> has been

reported to decrease the respiratory protein level and acid-base balance, and to modulate the immune system of *M. rosenbergii* (Yeh *et al.*, 2006).

The giant freshwater prawn, *Macrobrachium rosenbergii*, is a commercially important species cultured extensively throughout Southeast Asia and in some other regions. One of the major problems concerning freshwater prawn culture is the intrusion of predatory and competitive fish species into culture ponds. The intrusion of competitors results in more demand for feed. Carnivorous fish prey on prawn and also compete for feed. In Thailand, the major competitor in freshwater prawn ponds is silver barb (*Puntius gonionotus*) and major predatory fish is marble goby (*Oxyleotris marmorata*). Both are native to freshwater in Asia-Pacific region.

In the present study, we examined the possibility of using teaseed cake as a selective piscicide, for periodic eradication of competitive and predatory fishes in freshwater prawn culture ponds. The evaluation was done by studying the resistance of giant freshwater prawn and two species of freshwater fishes commonly found in prawn ponds to toxicity of teaseed cake. Sub-lethal effect of teaseed cake on growth and survival rate of giant freshwater prawn were also studied.

## MATERIALS AND METHODS

### Test animals

Giant freshwater prawn (*M. rosenbergii*) and two species of freshwater fish commonly found in giant freshwater prawn culture ponds, silver barb (*Puntius gonionotus*) and

marble goby (*Oxyleotris marmorata*), were obtained from private farms. Test animals were acclimated in the laboratory for one week before bioassay testing. Three different sizes of giant freshwater prawn and silver barb and two different sizes of marble goby were used for 24 h acute toxicity tests. Average wet weights of giant freshwater prawn used for the experiments were 0.05, 4.70 and 23.10 g. Average wet weights of silver barb used were 0.45, 3.26 and 7.00 g. Average wet weights of marble goby used were 1.43 and 45.05 g. Giant freshwater prawn with average wet weight of 1.1 g was used for long-term toxicity tests.

### Lethal effect of teaseed cake on test species

The short-term lethal effect tests were conducted in 40 L aquaria containing 30 L freshwater for 0.45, 3.26 and 7.00 g silver barb, 0.05 and 4.70 g giant freshwater prawn and 1.43 g marble goby. The tests for 23.10 g giant freshwater prawn and 45.05 g marble goby were conducted in 250 L aquaria containing 200 L freshwater. Dechlorinated tap water with pH of 8.4, alkalinity of 253 mg L<sup>-1</sup> as CaCO<sub>3</sub> and total hardness of 99 mg L<sup>-1</sup> as CaCO<sub>3</sub> was used for the experiments.

Test animals were transferred from holding tanks into test aquaria and were acclimated for 1 h before the application of teaseed cake. Stocking densities were 10 prawns/aquarium for giant freshwater prawn, 20 fish/aquarium for 0.45 and 3.26 g silver barb and 1.43 g marble goby, and 6 fish/aquarium for 45.05 g marble goby. Each aquarium was aerated with an air stone. There were three replicates for each test. Experimental period was 24 h.

Range-finding tests were carried out, using 50% stocking density of the definitive test, to determine the concentrations of saponin that should be used in the definitive test. There was no feeding during the experimental period. Observations were made at 1 h intervals from 1 to 6 h and then at 24 h. The  $LC_{50}$  values and their 95% confidence limit were calculated using probit analysis (Litchfield and Wilcoxon, 1949; APHA *et al.*, 1992)

### **Effect of teaseed cake on survival and growth rates of freshwater prawn**

The concentration of 30 mg L<sup>-1</sup> of teaseed cake was determined for use for the long-term effect test considering the result of the first experiment in which 100% of all size of silver barb and marble goby were killed at a teaseed cake concentration of 28 mg L<sup>-1</sup> within 6 h, while 100% survival rate of all sizes of giant freshwater prawn were observed at a teaseed cake concentration of 50 mg L<sup>-1</sup> after 24 h.

The experiment was conducted in 2 m<sup>3</sup> outdoor concrete ponds with a water depth of 1.0 m. Giant freshwater prawns were stocked at the rate of 50 prawns/pond. Ground water with pH of 8.5, alkalinity 98.0 mg l<sup>-1</sup> as CaCO<sub>3</sub> and total hardness 61.0 mg L<sup>-1</sup> as CaCO<sub>3</sub> was used for the experiments. The water in experimental ponds was continuously aerated by air stones with a blower. The prawns were transferred into experimental ponds and acclimated 24 h before the application of teaseed cake. The experiments consisted of the treatment with 30 mg L<sup>-1</sup> teaseed cake and control with no application of teaseed cake. There were three replicates in each treatment.

Prior to the application of teaseed cake, the water level in the experimental ponds were reduced to 0.5 m. After 6 h the ponds were filled to 1.0 m level. Prawns were fed with pelleted feed at recommended feeding rates twice a day. Twenty percent of pond water was exchanged weekly. Water quality was analyzed weekly for dissolved oxygen, pH, alkalinity, total hardness, and chlorophyll *a*. Dissolved oxygen was measured with YSI Model 95 oxygen meter while water pH was measured with YSI Model 63 pH meter. Alkalinity, total hardness and chlorophyll *a* were analyzed according to methods recommended by APHA *et al* (1992). After 4 weeks, prawns were harvested, counted and weighed. Average survival rate and average weight of experimental prawns were statistically compared.

## **RESULTS**

### **Lethal effect of teaseed cake on giant freshwater prawn**

The mortality rates of giant freshwater prawn exposed to different concentrations of saponin are shown in Table 1. The rate of response of prawn to teaseed cake demonstrates that medium and big prawns with average weights of 4.70 and 23.10 g respectively were more sensitive to saponin than small prawn with an average weight of 0.05 g. All 0.05 g prawns exposed to 200 mg L<sup>-1</sup> teaseed cake died after 24 h, and 30.0, 80.0 and 96.7% of the prawns exposed to 125, 150 and 175 mg L<sup>-1</sup> teaseed cake died after 24 h. However, no mortality occurred among 0.05 g prawns exposed to 100 mg L<sup>-1</sup> teaseed cake after 24 h. All the

medium-sized (4.70 g) and big-sized (3.10 g) prawns exposed to 50 mg L<sup>-1</sup> teaseed cake survived after 24 h. Mortality rates of prawns exposed to 56, 62 and 68 mg L<sup>-1</sup> teaseed cake were 6.7, 50.0 and 93.3%, respectively for 4.70 g prawn, and 16.7, 80.0 and 96.7% for 23.10 g prawn. All medium- and big-sized prawns exposed to 75 mg L<sup>-1</sup> teaseed cake died after 24 h. The 24-h LC<sub>50</sub> of teaseed cake on 0.05, 4.70 and 23.10 g prawn were 135.0, 62.0 and 61.0 mg L<sup>-1</sup>, respectively (Table 4).

Table 1. Mortality rates (%) of giant freshwater prawn exposed to different concentrations of teaseed cake for 24 h

Size (g)	Teaseed cake concentration (mg L <sup>-1</sup> )				
	100	125	150	175	200
0.05	0	30.0±5.8	80.0±5.8	96.7±3.3	100

  

Size (g)	Teaseed cake concentration (mg L <sup>-1</sup> )				
	50	56	62	68	75
4.70	0	6.7±3.3	50.0±5.8	93.3±3.3	100
23.10	0	16.7±3.3	80.0±5.8	96.7±3.3	100

### Lethal effect of teaseed cake on silver barb and marble goby

All 0.45 g silver barb exposed to 10.0 mg L<sup>-1</sup> teaseed cake died after 2 h (Table 2). Mortality rates of 0.45 g fish exposed to 1.0, 1.8, 3.2 and 5.6 mg L<sup>-1</sup> teaseed cake were 11.7, 15.0, 41.7 and 71.7%, respectively. No mortality of 0.45 g silver barb exposed to 0.56 mg L<sup>-1</sup> teaseed cake was observed after 24 h.

Table 2. Mortality (%) rates of silver barb exposed to different concentrations of teaseed cake for 24 h

Size (g)	Teaseed cake concentration (mg L <sup>-1</sup> )				
	0.56	1.0	1.8	3.2	5.6
0.45	0	11.7±1.7	15.0±2.9	41.7±3.3	71.7±1.7

  

Size (g)	Teaseed cake concentration (mg L <sup>-1</sup> )				
	5.6	8.0	10.0	13.0	18.0
3.26	0	18.3±1.7	40.0±2.9	95.0±2.9	100*
7.00	0	6.7±3.3	63.3±3.3	80.0±2.9	100**

\* all fish died within 2 h

\*\* all fish died within 3 h

Silver barb with average weights of 3.26 and 7.00 g were less sensitive to saponin than 0.45 g silver barb. All fish of both sizes (3.26 and 7.00 g) exposed to 18.0 mg L<sup>-1</sup>



teaseed cake died after 2 - 3 h while those exposed to 5.6 mg L<sup>-1</sup> teaseed cake survived after 24 h. Mortality rates of silver barb exposed to 8.0, 10.0 and 13.0 mg L<sup>-1</sup> teaseed cake after 24 h were 18.3, 40.0 and 95.0% for 3.26 g fish and 6.7, 63.3 and 80.0% for 7.00 g fishes, respectively. The 24-h LC<sub>50</sub> of teaseed cake on 0.45, 3.26 and 7.00 g silver barb were 2.8, 10.0 and 10.1 mg L<sup>-1</sup>, respectively (Table 4).

All 1.43 g marble goby exposed to 25.0 mg L<sup>-1</sup> teaseed cake died within 4 h (Table 3). Mortality rates of the 1.43 g fish exposed to 15.0 and 20.0 mg L<sup>-1</sup> teaseed

cake after 24 h were 50.0 and 80.0%, respectively. No mortality was observed with 1.43 g marble goby exposed to 10.0 mg L<sup>-1</sup> teaseed cake after 24 h. All 45.05 g marble goby exposed to 28.0 mg L<sup>-1</sup> teaseed cake died after 4 h. Mortality rates of 45.05 g fish exposed to 18.0, 21 and 25.0 mg L<sup>-1</sup> teaseed cake after 24 h were 33.3, 55.5 and 66.7%, respectively. No mortality was observed with 45.05 g marble goby exposed to 15.0 mg L<sup>-1</sup> teaseed cake after 24 h. The 24 h LC<sub>50</sub> of teaseed cake on 1.43 and 45.05 g marble goby were 16.0 and 22.0 mg L<sup>-1</sup>, respectively (Table 4).

Table 3. Mortality rates (%) of marble goby exposed to different concentrations of teaseed cake for 24 h

	Teaseed cake concentration (mg L <sup>-1</sup> )			
Size 1.43 g	10.0	1.0	1.8	25.0
	0	11.7±1.7	15.0±2.9	100*
	Teaseed cake concentration (mg L <sup>-1</sup> )			
Size 45.05 g	15.0	18.0	21.0	25.0
	0	33.3±0.0	55.5±5.7	66.7±9.6
				100*

\* all fish died within 4 h

Table 4. 24-h LC<sub>50</sub> values (mg L<sup>-1</sup>) and 95% confidence limits for teaseed cake on giant freshwater prawn, silver barb and marble goby

Species/Average weight (g)	24-h LC <sub>50</sub> (mg L <sup>-1</sup> )	95% Confidence limit	
		Lower limit	Upper limit
Giant freshwater prawn			
0.05	135.0	129.8	140.4
4.70	62.0	60.2	63.9
23.10	61.0	59.8	62.2
Silver barb			
0.45	2.8	2.4	3.2
3.26	10.0	9.5	10.5
7.00	10.1	9.4	10.8
Marble goby			
1.43	16.0	15.4	16.6
45.05	22.0	21.4	22.7

Table 5. Application rates of teaseed cake ( $\text{mg L}^{-1}$ ) that caused 0% and 100% 24-h mortality rate of giant freshwater prawn, silver barb and marble goby.

Size (g)	0% 24-h mortality rate	100% 24-h mortality rate	Time for total kill
Giant freshwater prawn			
0.05	100.0	200.0	>6-24 h
4.70	50.0	75.0	>6-24 h
23.10	50.0	75.0	6 h
Silver barb			
0.45	0.56	10.0	2 h
3.26	5.6	18.0	2 h
7.00	5.6	18.0	3 h
Marble goby			
1.43	10.0	25.0	4 h
45.05	15.0	28.0	4 h

### Effect of teaseed cake on growth and survival rates of giant tiger prawn

Average weights and average survival rates of giant freshwater prawn in ponds with and without the application of teaseed cake are shown in Table 6. After 4 weeks, the prawns in control ponds grew from an average initial weight of 1.1 g to 2.9 g with an average survival rate of 86.0%, whereas

those in ponds treated with teaseed cake grew from an average initial weight of 1.1 g to 3.0 g with an average survival rate of 85.0%. One-way analysis of variance indicated that there was no significant difference ( $P < 0.05$ ) between average weights and average survival rates of prawns in control ponds and prawns in ponds treated with teaseed cake.

Table 6. Average weight (g) and average survival rate (%) of experimental prawns after 4-week experimental period. Initial weight 1.1 g

Treatment	Average survival rate (%)	Average weight (g)
Control	86.0 $\pm$ 0.7 <sup>a</sup>	2.9 $\pm$ 0.1 <sup>b</sup>
30 $\text{mg L}^{-1}$ teaseed cake	85.0 $\pm$ 5.2 <sup>a</sup>	3.0 $\pm$ 0.3 <sup>b</sup>

Values in the same column followed by different letters are significantly different ( $P < 0.05$ ).

Table 7. Water quality in experimental ponds (sampling period 13:00-14:00 p.m.).

Treatment	Water quality				
	pH	Alkalinity ( $\text{mg L}^{-1}$ as $\text{CaCO}_3$ )	Total hardness ( $\text{mg L}^{-1}$ as $\text{CaCO}_3$ )	Dissolved oxygen ( $\text{mg L}^{-1}$ )	Temperature ( $^{\circ}\text{C}$ )
Control	8.4-8.7	92.0-104.5	53.0-60.5	8.4-12.6	27.0-29.0
30 $\text{mg L}^{-1}$ teaseed cake	8.6-9.0	98.0-110.0	52.0-62.0	8.5-14.6	27.5-29.0

## DISCUSSION

Teaseed cake, the residue of *Camellia* sp. seeds after oil extraction contains 5.2-7.2% saponin (Terazaki *et al.*, 1983), a glucoside which is highly toxic to fish. It is widely used as a piscicide in shrimp ponds. Saponin has been reported to decrease hemocyanin levels and affect the acid-base balance, feeding, growth and molting frequency of *Marsupenaues japonicus* (Chen and Chen, 1996). Likewise it has been reported to damage gills and erythrocytes and affect oxygen uptake and hemoglobin levels in fish (Roy *et al.*, 1990; Homechaudhuri and Banerjee, 1991).

Results from this study indicated that teaseed cake is more toxic to freshwater fish than to giant freshwater prawn. Giant freshwater prawn can tolerate up to 50.0 mg L<sup>-1</sup> of teaseed cake without any mortality after 24 h, while concentrations of 18.0 and 28.0 mg L<sup>-1</sup> of teaseed cake can completely eliminate silver barb and marble goby within 3 to 4 h.

Giant freshwater prawn is more sensitive to saponin than marine shrimp. The 24-h LC<sub>50</sub> of saponin for *Penaeus merguensis*, *Marsupenaues japonicus* and *P. carinadus* are 50.4, 27.08 and 75 mg L<sup>-1</sup>, respectively (Tang, 1961; Terazaki *et al.*, 1980; Chen *et al.*, 1996). Yeh *et al.* (2006) reported that saponin at a concentration of 0.9 mg L<sup>-1</sup> decreased respiratory protein levels and acid base balance, and modulated the immune system of giant freshwater prawn. This concentration is equivalent to 12.5-17.3 mg L<sup>-1</sup> of teaseed cake. However, the result of this study indicated that application of teaseed cake at 30 mg L<sup>-1</sup> after lowering the water level to half of the water depth and

letting it stand for 6 h before refilling to normal level did not have any effect on growth and survival rate of giant freshwater prawn after a 4-week experimental period.

The application of 25 mg L<sup>-1</sup> teaseed cake in freshwater earthen ponds resulted in 28.0, 61.0, 58.0 and 34.0% mortality of *Clarias* sp., *Cyprinus carpio*, *Gambusia* sp. and *Oreochromis niloticus*, respectively after 24 h (Chiayvareesajja *et al.*, 1997). They also reported a 64.7% 24 h mortality of 11.3 cm silver barb in earthen ponds treated with 25 mg L<sup>-1</sup> teaseed cake, which is higher than the concentration that killed 100% of 0.45-7.00 g silver barb in this experiment. Causes of this difference may be either from the different saponin content of teaseed cakes used or the different sizes of the experimental fish.

Recommended levels of teaseed cake for use in eradicating undesirable fish in shrimp ponds are 10-25 mg L<sup>-1</sup> (Cook, 1976) or 1.1 mg L<sup>-1</sup> of crude saponin which is equivalent to 21 mg L<sup>-1</sup> of teaseed cake containing 5.2% saponin (Terazaki *et al.*, 1980). Minsalan and Chiu (1986) recommended the dosage of 15 mg L<sup>-1</sup> teaseed cake for the elimination of fish in shrimp ponds.

Teaseed cake can be used as a piscicide for selective elimination of fish in giant freshwater prawn ponds because it is much more toxic to freshwater fish than to giant freshwater prawn. The concentration of 30 mg L<sup>-1</sup> of teaseed cake is recommended for this purpose. Selective eradication of competitive and predatory fish in giant freshwater prawn culture ponds can be done periodically by decreasing water level in the ponds to half its volume, applying 30 mg L<sup>-1</sup> teaseed cake, and letting it stand for 6h before refilling the ponds.

## CONCLUSION

According to the results of this study, teaseed cake is more toxic to silver barb and marble goby than to giant freshwater prawn. Giant freshwater prawn can tolerate up to 50.0 mg L<sup>-1</sup> of teaseed cake without any mortality after 24 h while the concentrations of 18.0 and 28.0 mg L<sup>-1</sup> teaseed cake can completely eliminate silver barb and marble goby within 3 and 4 h. The application of teaseed cake at the concentration of 30 mg L<sup>-1</sup> after decreasing the water level to half the depth of water storage level and letting it stand for 6 h before refilling to normal level did not have any effect on growth and survival rate of giant freshwater prawn after 4 weeks. Results from this study indicate that teaseed cake can be used as a piscicide for selective elimination of fish in giant freshwater prawn ponds. The concentration of 30 mg L<sup>-1</sup> of teaseed cake is recommended. Selective eradication of competitive and predatory fishes in giant freshwater prawn culture ponds can be done periodically by following the protocol described in this paper.

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