Research article

Acute Toxicity of Tea Seed Cake on Climbing perch (*Anabas testudineus* (Bloch))

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Received 15 November 2013 Accepted 21 September 2014 (*Corresponding Author)

Abstract Tea seed cake is common used in fish and shrimp farms in Thailand for controlling pond predators such as fish, shellfish or tadpole due to its applicable and affordable price. Climbing perch (*Anabas testudineus* (Bloch)) is one of several problems in aquaculture because it can lead to threatening diseases, the displacement of native species, causing changes to the water quality. Moreover they may effect as destroy or decrease of the productivity. So, acute toxicity of tea seed cake on climbing perch was studied. Statistic bioassay method (48-hr LC_{50}) was a technique to determine the median lethal concentration of the tea seed cake to kill climbing perch within 48 hours. The results showed that limitation of the 48-hr LC_{50} concentration at 95% confidence of tea seed cake on climbing perch (size of approximately 5±2 cm) was 33 (31.16-36.10) ppm. The slope function was 1.361(0.88-2.11) ppm and safe concentration was 1.65 ppm. This result can be used to apply for controlling of fish predator as fish which have accessory respiratory organs for farming application.

Keywords acute toxicity, tea seed cake, climbing perch

INTRODUCTION

Fish predator is one problem of aquaculture farming because it can lead to bring about disease, the displacement of native species, and causing changes quality of product. Similarly, it can cause of decreasing of the production due to raise or mortality. Climbing perch (*Anabas testudineus* (Bloch)) often found in the shrimp pond or fish farming. It is an omnivorous species (Trung, 1999) and consumes various kinds of food such as detritus, aquatic plants, crustaceans, worms, mollusks and insects. Thus, it has been described as carnivore or an insectivore and plays a role of predator (Yakupitiyage et al, 1998). In addition, it is a fast growing species which is able to live in deep or shallow water and can survive in low oxygen condition (Khatune-Jannat et al., 2012). It is also able to migrate between ponds (Trieu and Long, n.d.). Therefore, large numbers of climbing perch in ponds cause slow growth rate or decrease in average productivity. The farmers have tried to eradicate climbing perch with chemicals but many are harmful to the products and also users. Tea seed cake is the residue of *Camellia* sp. seeds after oil extraction that contains 5.2-7.2% of saponin (Minsalan and Chiu, 1986) which is a toxin giving haemolysis of blood. Owing to the eradicable effectiveness of the tea seed cake, many farmers apply it to eliminate predator fishes, shellfish or tadpoles in the fish and shrimp ponds. The effective dosage of crude saponin for eradicating predatory fishes depends on the proportion of body weight, pond's salinity level (Terazaki et al.,

1980) and fish species. In order to provide a guidance of using the tea seed cake, the study of its acute toxicity on climbing perch (*A. testudineus* (Bloch)) is useful for farming application.

OBJECTIVE

An objective of the study was to determine the median lethal concentration of tea seed cake that would result in the eradication of climbing perch (*A. testudineus* (Bloch)) at 48 hours.

METHODOLOGY

Animal preparation: One thousand climbing perch (*A. testudineus* (Bloch)) (size of approximately 5 ± 2 cm) were transported from climbing perch farm in central Thailand to laboratory. They were reared in 500 liter fiber glass tanks that was contained treated fresh water and added aeration for 7 days to acclimatize before bioassay testing. Food did not add over for 24hr before the experiment and throughout testing period.

Chemical preparation: Power tea seed cake that contains saponin 12% as active ingredient is used in the experiment. The tea seed cake solution was prepared in distilled water to dilute concentration for range finding test and definitive test. Each tea seed cake concentration was added one time at the beginning of each test.

Acute toxicity of tea seed cake to climbing perch: Static bioassay method was used to assess the tea seed cake concentration that caused 50% death of climbing perch within 48 hr test period (48-hr LC_{50}) which consist range finding test and definitive test (Rand, 2003).

Range finding test was the determination of the range concentration from the lowest that 100% of test animals dead to the highest that 100% of test animals survived. Variation of the tea seed cake concentration was added at 8 liter of water into 10 liter glass tanks which stocked 10 random selected test animals, and then each treatment was run three replications. Fish were observed and recorded the number of mortalities for 48 hr when they did not have operculum movement and did response while touching with a glass rod. The result of concentration in the first step was used to determine the further concentration in the next step. The experiment for the definitive test was determination of concentration range from the result of previous step on logarithmic scale. The behavioral response and mortality of climbing perch were recorded within 48 hr. Dead fish was immediately removed after recorded.

Temperature, pH and dissolved oxygen were determined with YSI 550A and Sartorius Basic pH Meter PB-11 before and after toxicity test.

Data analysis: 48-hr LC_{50} values and slope function were calculated at 95% confidence limits according to Litchfield and Wilcoxon (1949). Data were plotted on logarithmic-probability paper.

RESULTS AND DISCUSSION

No mortalities were observed in controls and concentration of 19 ppm concentration of tea seed cake during the 48 hr test period. However, mortalities started within 18 hr in the concentration of 25.69 and 34.74 ppm. Meanwhile, the 47 ppm caused mortality of test fish approximately 10.00±1.00% after 6 hr exposure and 100% after 48 hr in all replications. The mortalities were observed increasingly in the high concentration during almost the end of experiment (Table 1). In addition, the 33 ppm (31.16-36.10) was calculated as the 48-hr LC_{50} with 95% of confidence limit for climbing perch (*A.* (Bloch)) of size approximately 3-7 cm in the tea seed cake (Table 2). The result showed high concentration when it was compared with general concentration for usage in fresh or low saline water at 25-30 ppm (Hongkanarat et al., 2011). This is probably due to the tolerance levels of climbing perch in the hard conditions that is an ability to survive in a wide range of chemicals and temperatures owing to its accessory respiratory organs such as a labyrinth organ. The slope function and safe concentration showed that 1.361 (0.88-2.11) and 1.65 ppm, respectively.

Table 1 Accumulative mortality percentage of climbing perch (*A. testudineus* (Bloch)) (mean±SD) after tea seed cake exposure

Duration of exposure to tea seed cake (hr.)	% Accumulative mortality				
	0 ppm	19 ppm	25.69 ppm	34.74 ppm	47 ppm
0	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
6	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	10.00±1.00
12	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	16.67±0.58
18	0.00±0.00	0.00±0.00	3.33±0.58	30.00±0.58	36.67±1.15
24	0.00±0.00	0.00±0.00	10.00±0.58	36.67±1.15	83.33±1.15
30	0.00±0.00	0.00±0.00	13.33±0.58	40.00±1.15	96.67±1.53
36	0.00±0.00	0.00±0.00	16.67±0.58	40.00±0.58	96.67±0.00
42	0.00±0.00	0.00±0.00	20.00±0.58	43.33±0.58	96.67±0.00
48	0.00±0.00	0.00±0.00	23.33±0.58	43.33±0.58	100.00±0.00

Table 2 48-hr LC_{50} values and slope function with lower and upper limit of 95% of tea seed cake on climbing perch (*A. testudineus* (Bloch))

48-hr LC_{50}(ppm)	slope function
33 (31.16-36.10)	1.361(0.88-2.11)

In addition to the mortality, exposed climbing perch to the tea seed cake also showed increase in excretion and released more mucus in high concentration treatment. The nearly died fish has an opaque body color compared with normal fish and slow response or movement. For those of mortality experiencing, no movement of fin and operculum at all and they were observed at the bottom of the ponds. The response is in agree with El-Murr et al. (2014) that is likely due to reflection increase of oxidative stress that showed revealed normocytic normochromic anemia and significantly increases level of SOD and CAT activities and gene expression. The damaged of general tissues in various organs was also detected with high doses of tea seed cake (El-Murr et al., 2014). According to Boyd (1990), saponin compound in tea seed cake could destroy erythrocytes and was highly toxic to fish. Also saponin acts chiefly with lowering the surface tension between water and gills of fish and induces haemolysis, so preventing oxygen uptake by fish and leading to slow dead by oxygen deprivation (Lamba, 1970). Moreover, Roy and Munshi (1989) reported that the perch *A. testudineus* that were exposed for 24 hr with 2-5 mg/l saponin showed increasing of 20% oxygen uptake and increased its number of erythrocyte, amount of hemoglobin and hematocrit.

Temperature and pH in all treatments are not different before and after test. Meanwhile dissolved oxygen showed optimum level before the test and decreasing later after the test (Table 3). Nevertheless, these parameters are not likely a cause of climbing perch mortality because the fish can thrive well in deficient dissolved oxygen waters. Moreover, it has better ability of breathing due to its special organs and tolerance of adverse environmental conditions (Trieu and Long, n.d.). For this reason, the fish mortality in the experiment is more likely caused by the tea seed cake toxicity.

Table 3 Temperature, pH and dissolved oxygen levels before and after toxicity test

Concentration of tea seed cake (ppm)	Temperature (°C)		pH		Dissolved oxygen (mg/l)	
	Before	After	Before	After	Before	After
0.00	28.1±0.00	28.3±0.10	7.7±0.00	7.5±0.00	5.08±0.29	2.69±0.16
19.00	27.9±0.00	28.3±0.06	7.7±0.00	7.5±0.00	5.01±0.25	2.48±0.09
25.69	27.9±0.00	28.3±0.06	7.6±0.00	7.5±0.00	4.95±0.09	2.21±0.06
34.74	27.8±0.00	28.2±0.06	7.7±0.00	7.5±0.00	5.33±0.27	2.17±0.08
47.00	27.9±0.00	28.2±0.06	7.7±0.00	7.6±0.00	5.07±0.14	1.27±0.19

However, different fish species showed different tolerances. The species as highest mortality in 24 hr of tea seed cake exposure was observed in *Puntius gonionotus*, followed by *Cyprinus*

carpio, *Gambusia* sp. and *Clarias* sp., whereas tea seed cake toxicity ended after 12 hr (Chiayvareesajja et al., 1997). Terazaki et al. (1980) reported that the toxicity of saponin weakened with time. In agree with, Hongkanarat et al. (2011) reported that 48-hr LC50 of tea seed cake on Pacific white shrimp (*Litopenaeus vannamei*) post larvae 10 (PL10) was 416.14 ppm at salinity of 30 ppt that showed less toxicity to shrimp larvae. The results of this experiment showed that tea seed cake could be use to control climbing perch in farms. Further study is suggested to study more on the toxicity to other aquatic species for farming application.

CONCLUSION

The study indicates that tea seed cake is a natural product that can be used for eradication of climbing perch (*A. testudineus* (Bloch)) in aquaculture farming. The solution is very applicable control of fish having accessory respiratory organs. Moreover, it is environmental friendly used when apply it following the guideline and thus, giving sustainable utilization.

AKNOWLEDGEMENTS

The authors would like to thank Faculty of Agricultural Technology, Rajamangala University of Technology Thanyaburi which was supported some budget in this experiment.

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