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Effect of Indian almond, *Terminalia catappa* leaves water extract on the survival rate and growth performance of black tiger shrimp, *Penaeus monodon* post larvae

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Abstract. This study was performed to determine the effectiveness of different concentration of Indian almond, *Terminalia catappa* leaves water extract in post-larvae (PL) rearing of black tiger shrimp, *Penaeus monodon*. The survival rate and growth performance was done by releasing the PL 3 into the experimental tank until reach PL 20 for 18 days. The survival rate of *P. monodon* PL was significantly higher ($p < 0.05$) in concentration of 3.0 mg mL^{-1} than those PL in controls and other treatments including 1.0 mg mL^{-1}, 2.0 mg mL^{-1} and 4.0 mg mL^{-1}, but the growth rate did not significantly differ among the treatments except for the control. The toxicity test result showed that the LC_{50} of the *P. monodon* PL was 4.7 mg mL^{-1} while expose for 96 hours to *T. catappa* leaves extraction. In conclusion, 3.0 mg mL^{-1} concentration of *T. catappa* leave extraction give positive effect on survival rate and growth performance of *P. monodon* PL, but higher concentration become toxic and can cause high mortality.
Key Words: *Terminalia catappa*, *Penaeus monodon*, post-larvae growth, toxicity, water extraction, LC_{50}.

Introduction. Shrimp farming is one of the important activities in aquaculture due to its unique taste, high nutritive value and persistent demand in world market (Pushparajan & Soundarapandian 2010). Due to increasing demand among consumers, the production has been gradually increasing globally since the 1950s (Flegel et al 2008). About 61.1% of total crustacean through aquaculture are produced in Asian countries with shrimps as the main crustaceans being cultured. In 2011, shrimp production through aquaculture had reached up to 3.85 million tons globally with 22% of the overall production were of black tiger shrimp, *Penaeus monodon* (VASEP 2013) thus making it one of the most important species being cultured commercially in Asian countries. Since the disease outbreak in 1999 causing mass mortality and decreased growth rate of *P. monodon*, the global production of *P. monodon* has been decreasing throughout the Asia Pacific region. Disease outbreak is caused by virus, bacteria and parasites which reduce growth and survival rate of the shrimp larvae due to poor water quality management.

Shrimp farmers suffer high loss due to high mortality rate and growth rate of shrimp in intensive culture caused by stress and culture condition. Also, a high market demand of *P. monodon* had convinced shrimp farmers and investors to increase their pond production. Such event may affect the environmental balances if a proper guideline of intensive pond culture is not followed. Most farmers have been using chemical by adding chlorine hypochlorite (with 60% active substance) in the pond and antibiotic to treat shrimp pathogens to increase the growth rate of the larvae. However, the use of antibiotics was reported not only to be ineffective but they also create more resistant bacteria strains which worsen the situation in shrimp culture (Vici et al 2000) and as well as environmental risk caused by continuous use and elimination to the environment (Zheng et al 2012; Zou et al 2011). Although the use of probiotics has proved to be

effective, it increases production cost which therefore does not interest farmers or hatchery owners (Wickins & Lee 2002; Babayi et al 2004).

Indian almond, *Terminalia catappa* species is known in English under names such as Bengal almond, Singapore almond, Malabar almond, Talisay almond and Tropical almond. The plant has been commonly used among aquarists to promote the natural environment for better health of animals. The extracts of the plant were known to be able to reduce water pH and heavy metal toxicity (Chyau et al 2006) besides being an excellent source of nutrient as well as an antibacterial alternative in ornamental fish culture (Chansue & Assawawongkasem 2008). *T. catappa* contains high degree of organic material, tannins (punicalagin, punicalin, terflavin A and B, tergallagin, tercatain, chebulagic acid, geranin, granatin B, corilagin), several flavanoids, isovitexin, vitexin, isoorientin, rutin and triterpenoiods (ursolic acid, 2α, 3β, 23-trihydroxyurs-12-en-28oic acid) and humic acid (Ahmed et al 2005). The plant has been widely used to treat various type of illness in both traditional and modern medicine as anti-cancer (Yang et al 2010; Chu et al 2007; Zhai et al 2001) and antioxidant agent (Kinoshita et al 2007; Masuda et al 1999), antifungal (Goun et al 2003) and anti-inflammatory agents (Fan et al 2004). In aquaculture, the leaves of *T. catappa* leaves have been used for wound healing (Chansue et al 2004), against bacterial and fungal infection (Chitmanat et al 2005) as well as parasite infection (Chansue & Tangtrongpiros 2005).

T. catappa leaves solution produces hydrollyzable tannin when they are immersed in the water. Tannins have the antibacterial properties (Chung et al 1998). Tannic acid binds strongly to metal ions and calcium inhibiting intestinal bacteria growth (Chansue & Assawawongkasem 2008). Therefore, such property of the extract will prevent the shrimp post larvae (PL) from dangerous metal ions in water. The chelate like sideophore is toxic to the membrane of microorganism. When tannins form chelate complex with ion, there will be no ion available for microorganism to grow under aerobic condition. Therefore, the energy can be used more on growth than immune system against dangerous bacteria (Chansue & Assawawongkasem 2008).

Today, scientific research has identified essential minerals and compounds in plants that are not only required for proper nutrition, but they are responsible for health maintenance and disease prevention. These efforts had been widely done on fish but not well studied on shrimp. In this study different concentrations of *T. catappa* leaves extract were supplemented into the rearing aquarium to determine the effect of the extract on growth and survival rate of *P. monodon* PL between PL 3 to PL 20 stages.

Material and Method

Experimental location. This study was carried out in April 2010 in Institute of Tropical Aquaculture hatchery at Universiti Malaysia Terengganu (UMT).

Samples collections. The red fallen *T. catappa* leaves were collected from Teluk Ketapang Beach nearby the UMT campus. PL 3 of *P. monodon* of Mozambique Specific Pathogen Free (SPF) qualities were supplied by the Overseas Hatchery, Dungun, Terengganu.

Preparation of the extract. Five kg of *T. catappa* leaves were washed, sterilized in autoclave at 121ºC, and dried at room temperature until use. The dried leaves were cut into small pieces (1 x 5 cm) and were collected in the nylon net before been weight at particular measurement. The extraction procedure was done following the method described by Chansue & Assawawongkasem (2008). The extracts were prepared at concentration 0.2 mg mL^{-1}, 0.8 mg mL^{-1}, 1.4 mg mL^{-1}, 2.0 mg mL^{-1}, 2.6 mg mL^{-1}, 4.0 mg mL^{-1}, 6.0 mg mL^{-1}, 8.0 mg mL^{-1} and 10.0 mg mL^{-1}.

To yield 0.2 mg mL^{-1} concentration, 0.2 g of dried leaves was soaked in 1 L of sea water for 72 hours with continuous aeration. To prepare for the other concentration of 0.8 mg mL^{-1}, 1.4 mg mL^{-1}, 2.0 mg mL^{-1}, 2.6 mg mL^{-1}, 4.0 mg mL^{-1}, 6.0 mg mL^{-1}, 8.0 mg mL^{-1} and 10.0 mg mL^{-1}, 0.8 g, 1.4 g, 2.0 g, 2.6 g, 4.0 g, 6.0 g, 8.0 g, and 10.0 g of dried

leaves were also soaked in the same volume of seawater and duration of soaking time, respectively. After that, the leaves were removed from the aquarium.

Toxicity test of the extract – fifty percent lethal concentration (LC_{50}). All the prepared concentration of 0.2 mg mL^{-1}, 0.8 mg mL^{-1}, 1.4 mg mL^{-1}, 2.0 mg mL^{-1}, 2.6 mg mL^{-1} 4.0 mg mL^{-1}, 6.0 mg mL^{-1}, 8.0 mg mL^{-1} and 10 mg mL^{-1} were tested on 200 PL in 10 L glass aquarium for 96 hours with continuous aeration without water exchange. This procedure was done as acute toxicity to determine the 50% lethal concentration (LC_{50}) of the extract shrimp PL.

Experimental design. After the LC_{50} was determined, the next experiment was done with four different concentrations at 1.0 mg mL^{-1}, 2.0 mg mL^{-1}, 3.0 mg mL^{-1} and 4.0 mg mL^{-1} for 18 days from PL 3 till PL 20 stages. A control was prepared without addition of the extract. The experiment was done in three replicates for each treatment concentration in 10 L larvae rearing aquarium for survival data. Additional one replicate group was prepared for growth data. The aquariums were painted in black colour and filled with 10 L of seawater at salinity 30 ppt and temperature 28-30°C with the capacity of 20 PL per litre. The aquariums were covered with dark netting to avoid fluctuation in water temperature and also to reduce light intensity. The PL were fed with newly hatched *Artemia* (PL3-PL11) and formulated pellet feed (PL8-PL20) every 6 hours for 4 times daily. At the end of the experiment, the survival and growth rates were determined and recorded. Specific Growth Rate (SGR) was calculated based on formula given:

$$SGR = \frac{100\ [\ln \text{average final weight} - \ln \text{initial weight}]}{\text{number of culture days}}$$

Data analysis. A measure of the lethal toxicity of the *T. catappa* extract on PL was determined as the LC_{50} and the data were processed using probit analyses to derive its value. Statistical analysis will be done on the effect of *T. catappa* extract at different concentration on growth and survival rate using one-way analysis of variance (ANOVA). Significantly differences (at the 95% confidence level) were distinguished using multiple comparisons test using group comparisons, Tukey Test (Zar 1984).

Results

Toxicity test. One hundred percent mortality was observed in larvae treated in 10.0 mg mL^{-1} (Table 1) where no survived shrimp PL were observed in the aquarium after one day. The probit analysis showed that the LC_{50} of *T. catappa* extract on PL after 96 hours was at the concentration 4.7 mg mL^{-1} (Figure 1).

Table 1

Toxicity test of 96 hours on *P. monodon* post-larvae (PL) exposed to different concentrations of *T. catappa* extract (n = 200 PL for each concentration)

T. catappa concentration (mg mL^{-1})	*No. of dead PL*	*Percentage of mortality*
0.2	8	4.0
0.8	14	7.0
1.4	20	10.0
2.0	33	16.5
2.6	39	19.5
4.0	35	17.5
6.0	149	74.5
8.0	183	91.5
10.0	200	100.0

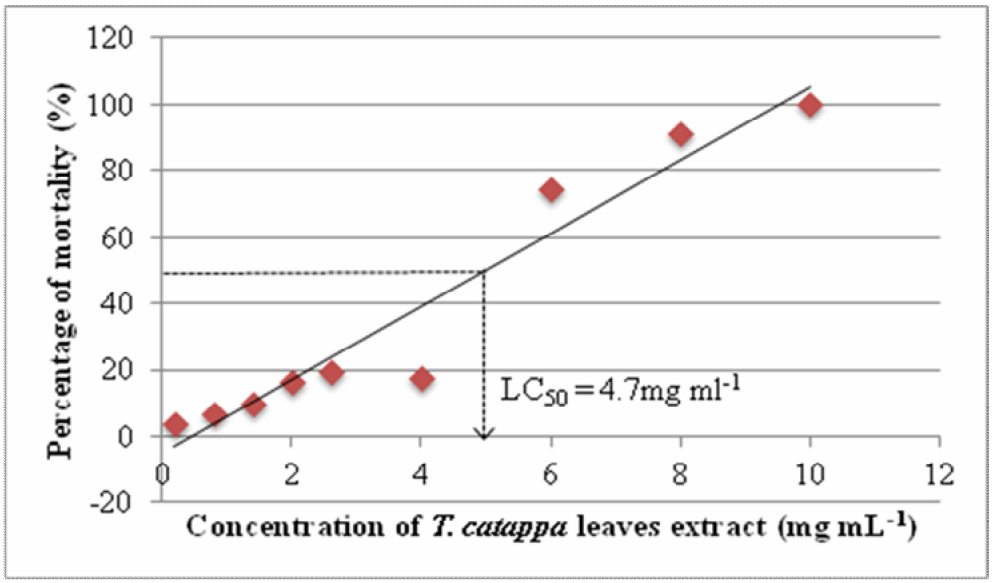

Figure. 1. Probit analysis of determining the LC_{50} of *T. catappa* leaves extract on *P. monodon* PL.

The mean values for water quality parameters are shown in Table 2. High ammonia concentration was obtained at 6.0 mg mL^{-1}, 8.0 mg mL^{-1} and 10.0 mg mL^{-1} of extract concentration.

Table 2

Water quality parameters of different concentrations of *T. catappa* leaves for toxicity test on *P. monodon* post-larvae

T. catappa concentration (mg mL^{-1})	*pH*	*Ammonia (ppm)*	*Dissolved Oxygen (ppm)*
control	7.58	0.00	4.25
0.2	7.57	0.00	4.31
0.8	7.52	0.10	4.77
1.4	7.50	0.10	3.98
2.0	7.47	0.15	3.48
2.6	7.43	0.15	3.83
4.0	6.74	0.15	4.81
6.0	6.49	0.20	5.06
8.0	6.19	0.20	4.64
10.0	5.89	0.20	4.39

Survival rate. The present study showed significant differences in mean survival rate between treated PL and control ($p < 0.005$). The highest survival rate was recorded at concentration of 3.0 mg mL^{-1} with 91.3% mean survival (Figure 2). There was no significant difference in survival between the concentrations 1.0 mg mL^{-1}, 2.0 mg mL^{-1} and 4.0 mg mL^{-1} ($p > 0.05$). The survival rate decreased at higher concentration (4.0 mg mL^{-1}) with 81.3% survival. Control treatment showed the lowest survival rate at 71.2%.

Growth performance. The present study showed significant differences in growth between PL treated with *T. catappa* as compared to control ($p < 0.05$). The highest growth rate was observed in PL treated with 2.0 mg mL^{-1} *T. catappa* extract with 14.09 mm ± 0.58 mean of the total length and control showed the lowest mean growth rate with 11.06 mm ±0.48 of the total length. There was no significant difference in mean growth among all treated PL ($p > 0.05$) (Figure 3). In terms of SGR, PL treated with 2.0 mg mL^{-1} *T. catappa* extract also showed the highest mean SGR with 82.83% ± 0.58 while control showed the lowest mean SGR with 65.05% ± 0.48 (Figure 4). The present

study also shows no significant differences among the treatments ($p > 0.05$), however significant difference was recorded between PL treatment with *T. catappa* and control ($p < 0.05$) for mean SGR (Figure 4).

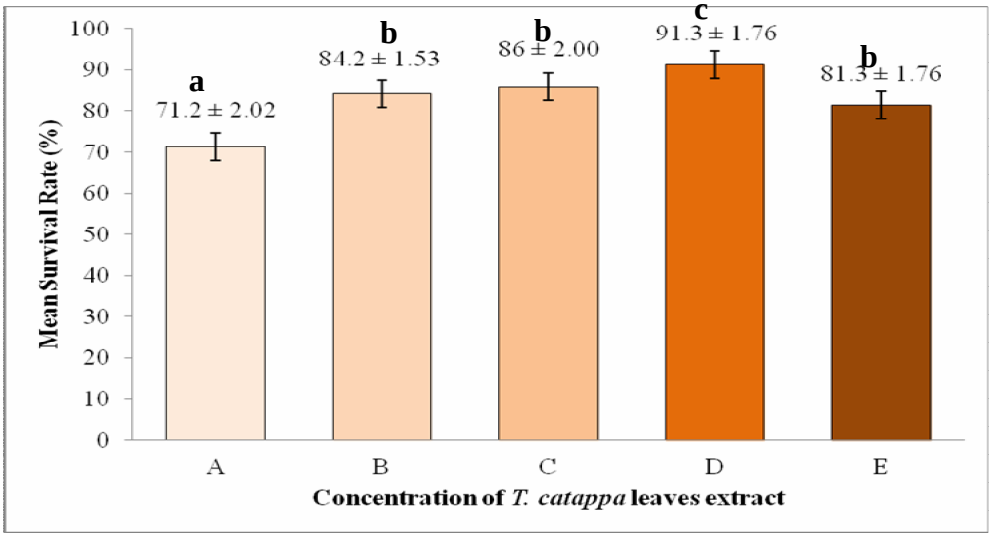

Figure 2. Mean survival rate of *P. monodon* PL treated with different concentrations of *T. catappa* leaves extract, 0.0 mg mL^{-1} (A - control), 1.0 mg mL^{-1} (B), 2.0 mg mL^{-1} (C), 3.0 mg mL^{-1} (D) and 4.0 mg mL^{-1} (E). Small alphabet shows no significant difference between the treatments.

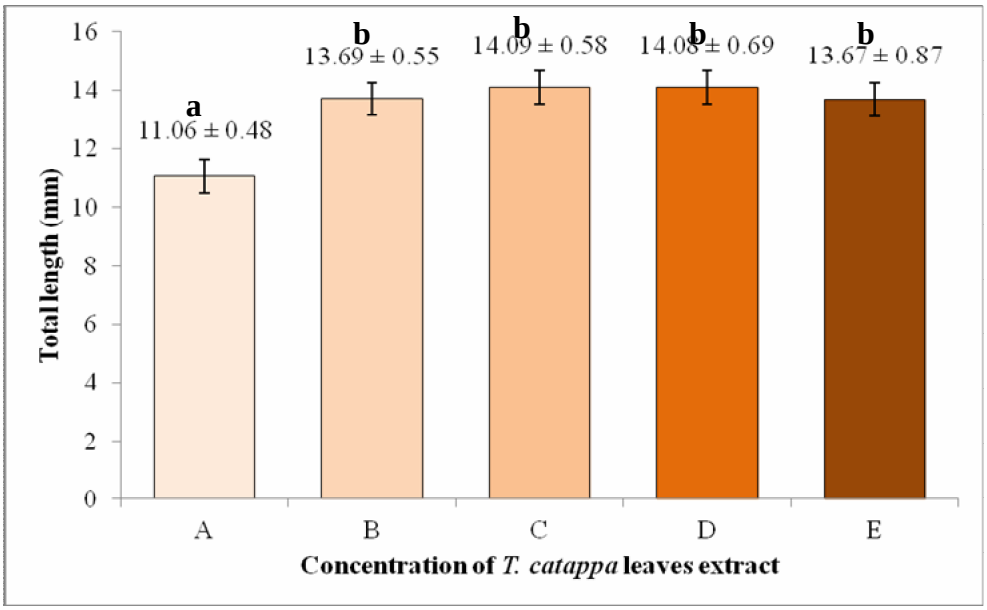

Figure 3. Mean growth (total length) increment of *P. monodon* PL treated with different concentrations of *T. catappa* leaves extract, 0.0 mg mL^{-1} (A - control), 1.0 mg mL^{-1} (B), 2.0 mg mL^{-1} (C), 3.0 mg mL^{-1} (D) and 4.0 mg mL^{-1} (E). Small alphabet shows no significant difference between treatments.

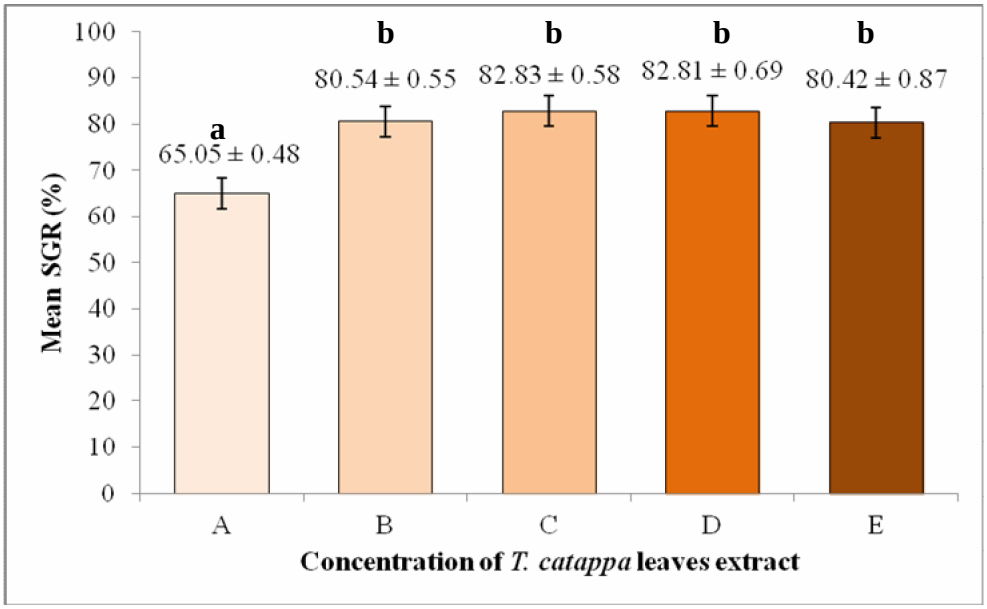

Figure 4. Mean Specific Growth Percentage (SGR) of *P. monodon* PL treated with different concentrations of *T. catappa* leaves extract, 0.0 mg mL^{-1} (A - control), 1.0 mg mL^{-1} (B), 2.0 mg mL^{-1} (C), 3.0 mg mL^{-1} (D) and 4.0 mg mL^{-1} (E). Small alphabet shows no significant difference between treatments.

Discussion. The *P. monodon* PL reared with *T. catappa* water extract treatments showed better survival rates and growth performances compared to control.

Chansue & Assawawongkasem (2008) reported rapid increment of tannin concentration in *T. catappa* extract within three days of extraction and it gradually increased after day three. This result explained that a higher concentration of tannin can be obtained at longer duration of extraction. Chansue & Assawawongkasem (2008) stated three days of extraction is the most appropriate duration because longer extraction time will cause microorganism contamination.

The toxicity test of *T. catappa* extract had been done on ornamental fishes including guppies (*Poecilia reticula*), fancy carp (*Cyprinus carpio*) and Siamese fighting fish (*Betta splendens*) by Chansue & Assawawongkasem (2008) in which the result showed high sensitivity in guppies towards the extract compared to fancy carp and Siamese fighting fish. In the present study, acidity was increased as the concentration of the extract increased. High mortality was observed in treatment with higher concentration, 10 mg mL^{-1} of *T. catappa* extract may be due to high acidity in the water.

High concentration of the *T. catappa* can affect on water quality which resulted in increased of ammonia level and decrease the pH level as in the present study. Wickins (1976), Chin & Chen (1987) and Jayasankar & Muthu (1983) observed that high concentration of ammonia reduced the growth of penaeid shrimp where the sensitive concentration level of ammonia is higher than 0.1 ppm. The pH value of various treatments i.e. 0.2–4.0 mg mL^{-1} were slightly lower (6.7–7.6) compared to control treatment. However at higher extract concentration (6.0-10.0 mg mL^{-1}), the water became more acidic (6.0–6.5). This result showed higher toxicity of *T. catappa* leaves extract LC_{50} 96 hours was found at 4.7 mg mL^{-1}. Thus, this result explained that concentration higher than 4.7 mg mL^{-1} (LC_{50}) could create an acidic condition which was toxic to the larvae. This result is in line with observations done by Chansue & Assawawongkasem (2008) who reported that the concentrations of 6.0–10.0 mg mL^{-1} were acidic to aquatic animals with pH value 6.5–6.0 and ammonia > 0.2 ppm. There may be a possible chance that high concentrations of *T. catappa* extract caused mutagenic effects towards the growth of the post larvae according to Mininel et al (2014).

Chansue & Assawawongkasem (2008) show that the Minimum Inhibitory Concentration (MICs) observed that the *T. catappa* extract at low concentration was able to eliminate *V. parahemolyticus* and Chitmanat et al (2005) reported that the *T. catappa*

leaf extracts also showed antifungal activity towards fish ectoparasites, *Trichodina.* This may explain the better performance of the larvae treated with *T. catappa* leaves extract. Highest survival rate of *P. monodon* PL was achieved in 3.0 mg mL^{-1} of *T. catappa* leaves concentration. The survival rate of *P. monodon* PL appeared to be positively correlated to pH and ammonia, meaning that an increase in ammonia level and decrease of pH variable coincided with reduced survival. The suitable pH for marine shrimp larval hatching and rearing optimal growth ranged between 7.5 and 8.5 (Noor-Hamid et al 1994). However, the present study showed 91.06% survival rate at pH 7.16 in treatment concentration 3.0 mg mL^{-1}.

Harpaz & Schmalbach (1986) explained that the addition of *T. catappa* leaves in the feed increases the growth rate of giant freshwater prawn, *Macrobrachium rosenbergii* which proven to have improved the nutritional status in the feed. This can be further concluded that the *T. catappa* leaves extract possessed potential source of antimicrobial properties against vibriosis in aquaculture (Nadirah et al 2013). Harpaz & Schmalbach (1986) also reported that the leaves of *Ailanthus altissima* in the larvae rearing tank of giant freshwater prawn (*Macrobrachium rosenbergii*), the result showed increase in average weight and reduce in intermolt intervals confirmed that plant extracts can increase the growth rate of this prawn PL. However, increasing concentrations of *T. catappa* water extract did not change the mean growth increment of the *P. monodon* PL where, the increment percentage were similar for all treatments in the present study.

Conclusions. The present study showed an noticeable observation that *P. monodon* PL survived and grew better in *T. catappa* leaves extraction. The best concentration promoting the highest survival rate and best growth performance was obtained at 3.0 mg mL^{-1} concentration. The water extract of *T. catappa* leaves have potentials to substitute chemicals, antibiotics and probiotics which may result in chemical residue and antibiotic resistance in shrimp PL rearing. Further study may be necessary to investigate its effectiveness in improving shrimp PL health and production in a long term treatment.

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