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Effect of different doses of saponins and salinity on giant tiger prawn *Penaeus monodon* and Nile tilapia *Oreochromis niloticus*

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Abstract. The Nile Tilapia *Oreochromis niloticus* is considering as a pest in shrimp farming ponds, as it is a shrimp competitor and predator of benthic organisms. This study aims to determine the effective and efficient saponins doses to eradicate tilapia without causing shrimp mortality. The study used two different saponins doses (200 kg/ha and 100 kg/ha) with seven different levels of salinity (5, 10, 15, 20, 25, 30, 35 ppt). The saponins doses used in this study had no effect on shrimp mortality, but had a significant effect on mortality of nile tilapia. The mortality of nile tilapia was 100% for all treatments. The death time of nile tilapia decreased significantly with increasing salinity. Longest nile tilapia death time was found at 10 ppt water salinity; this salinity is close to that of body fluids (14 ppt). The greater the difference between the ion content of body fluids and water ions in the test media, the faster the time of death. The effects of bleeding on the operculum, pectoral and caudal fins are more severe at high salinity. It is advisable not to increase the saponins doses, use the same saponins doses at any salinity, because predatory and competitive fish will die at any salinity.

1. Introduction

Various efforts have been made to increase the productivity of intensive and super intensive shrimp farming in a sustainable manner [1-7], including reducing the use of chemicals to eradicate competitor or predator fish, such as nile tilapia *Oreochromis niloticus* [8], and the use of natural pesticides [9]. One source of natural pesticides that are often used in ponds is saponins. *Camellia sinensis* is a plant whose leaves and shoots are used to make tea, while the waste, such as the remaining roots and leaves after being extracted, is made tea seed cake containing 5.2-7.2% saponins. Saponins extracted from *C. sinensis* roots are in the form of triterpenoid saponins [10], while those extracted from *C. sinensis* leaves are in the form of glucuronide saponin [11], and sapogenin glycosides [12].

Saponins are often used by fish farmers to eradicate predatory fish and competitors in shrimp culture [13]. Saponins from tea seed cake are able to interact with the membrane of erythrocyte cause the membrane disintegrated and the cell will be damage [14]. Saponins can also reduce the surface tension between water and gills, thus inducing hemolysis, which will inhibit oxygen absorption by the gills. Eventually the fish die slowly due to lack of oxygen [15].

Previous studies reported that the effective saponins doses used to eradicate predatory fish depends on the proportion of body weight and the salinity of pond [15]. These studies suspect that a decrease in the salinity of pond will cause a decrease in the toxicity of the saponins, so that the saponin dose used is adjusted to the salinity [16, 17].

The saponin dose that is commonly used to eradicate competitor fish and predatory fish in ponds is 200 kg/ha for shrimp ponds and 200-250 kg/ha for freshwater lobster cultivation [15]. This dose is lower than the actual dose because at the time of giving the saponins, the water level in the pond or pond is reduced to a third so that the actual dose is three times greater, so it can reach 600 kg/ha for ponds and 600-750 kg/ha for freshwater lobster cultivation.

In general, the saponin dose used varies widely, from 150 to 300 kg/ha [18, 19], and some even reach 555.6 kg/ha [17]. Although saponins are natural pesticides, the use of high doses can be dangerous because the active ingredients can last 10-12 days [15]. This can kill organisms that live on the surface and at the bottom of the brackish water ponds, causing the bottom of the pond to become arid. The condition is thought to be one of the triggers for brackish water pond cultivation in Indonesia, because saponins are listed as chemicals and biological products that are harmful to pond cultivation [12].

The pond fertility from the biological aspects of the organisms that live on the pond bottom has not been reported, so the impact of giving excessive saponins on pond fertility has not been observed. Almost all previous research on pond fertility has only focused on the physical and chemical aspects of pond water [19-22] or plankton that live in pond water [19]. Previous research has reported the results of the saponins toxicity test at different doses [23, 24], but until now there has been no reported toxicity test results for the same saponin dose at different salinity. Therefore, it is necessary to study the toxicity of saponin at different salinity so that it can be used as a reference for rational use of saponin. This study aims to assess the toxicity of saponin at different doses and salinity.

2. Materials and Methods

In this study, the toxicity test of saponin was carried out at two doses (200 and 100 kg / ha). Each dose was applied at seven levels of salinity, namely 5 ppt, 10, ppt, 15 ppt, 20 ppt, 25 ppt, 30 ppt and 35 ppt, and each treatment with three replications. The study used a round tub with a diameter of 55 cm and a height of 45 cm. Tub filled with water as high as 40 cm. Each tub was filled with three giant tiger prawn *Penaeus monodon* and three nile tilapia *Oreochromis niloticus*. Each treatment was given an aerator

The parameter measured was the time taken from the start of the treatment until the nile tilapia died. Observation was stopped when all of the nile tilapia died. Water quality parameters measured are salinity, temperature, pH and dissolved oxygen.

The size distribution curve of fish and shrimp used as test animals is made based on the frequency distribution of weight and length with reference to previous similar research methods [25]. Correlation curves of death time (minutes) and salinity (ppm) were drawn using trendline polynomials, and paired mean tests [26] to compare death times for each treatment.

3. Results

3.1. Giant Tiger Prawn

The length range of giant tiger prawns used in this study was 4.5-8.8 cm (6.1 ± 0.9 cm), with a weight range of 0.52-4.22 g (1.46 ± 0.69 g) (Figure 1). The distribution of the length-weight relationship has a very strong regression coefficient (0.967) (Figure 2). The same shrimp is used for saponins doses of 200 kg / ha and 100 kg / ha.

3.2. Nile tilapia

The length range of nile tilapia used for dose of 200 kg/ha was 5.8-10.1 cm (8.0 ± 1.0 cm), with a weight range of 3.29-17.19 g (8.85 ± 3.30 g) (Figure 3). The length range of nile tilapia used for dose of 100 kg/ha was 6.1-11.5 cm (8.3 ± 1.3 cm), with a weight range of 3.54-24.94 g (9.65 ± 4.68 g) (Figure 4). The coefficients of the length-weight relation are relatively the same (Figure 5).

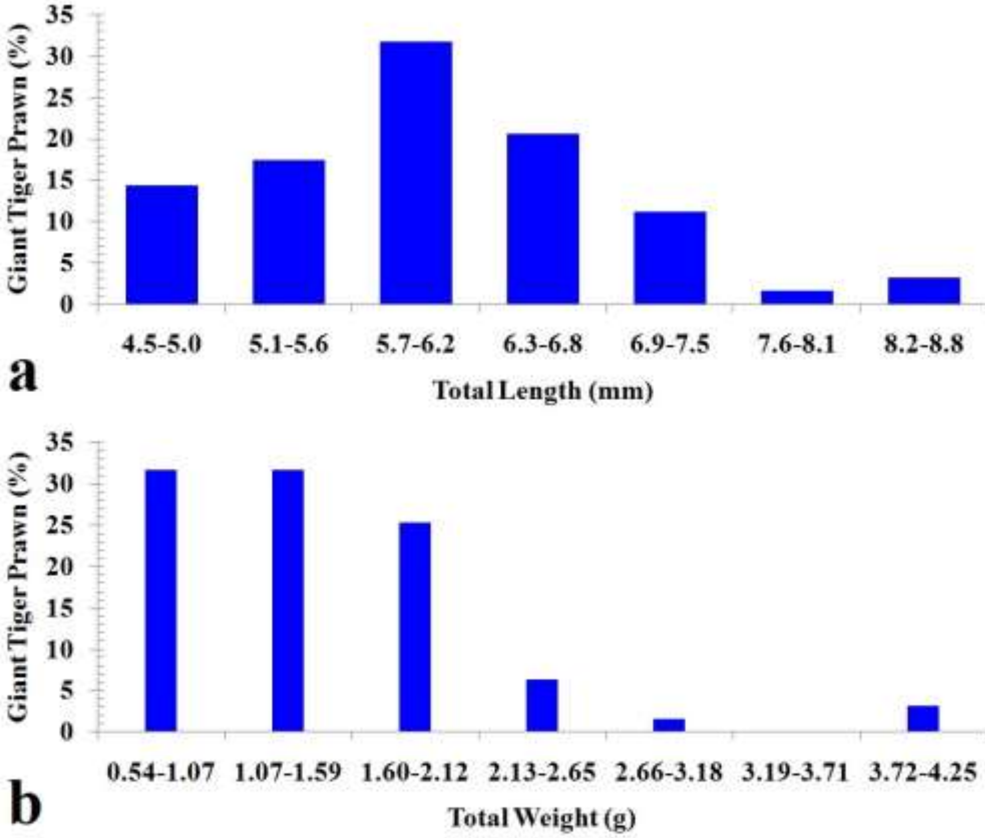

Figure 1. Distribution of length (a) and weight (b) of tiger prawns *Penaeus monodon* used in the experiment for doses of 200 kg/ha and 100 kg/ha.

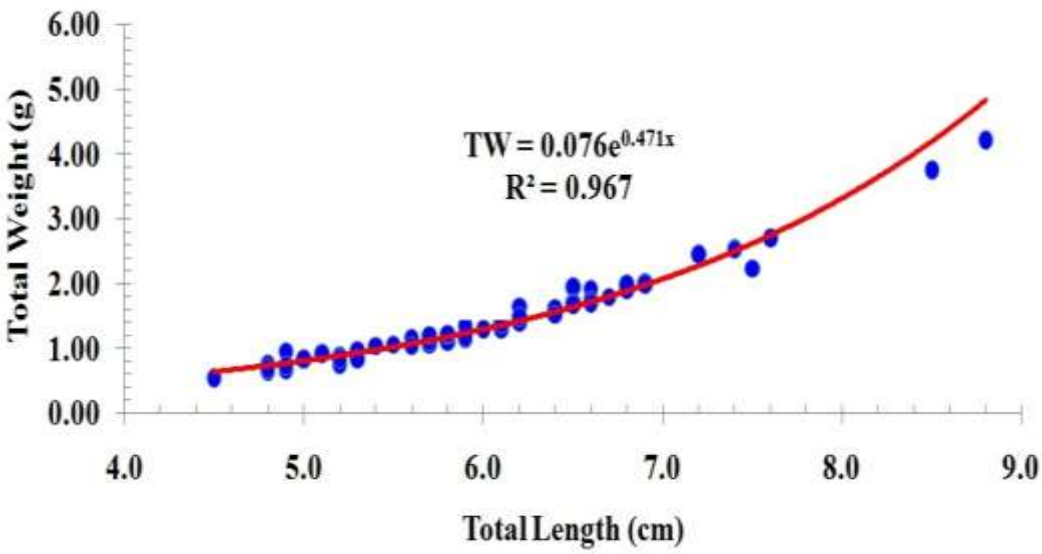

Figure 2. The relationship between length and weight of tiger prawn *Penaeus monodon* used as a test animal for the doses of 200 kg/ha and 100 kg/ha.

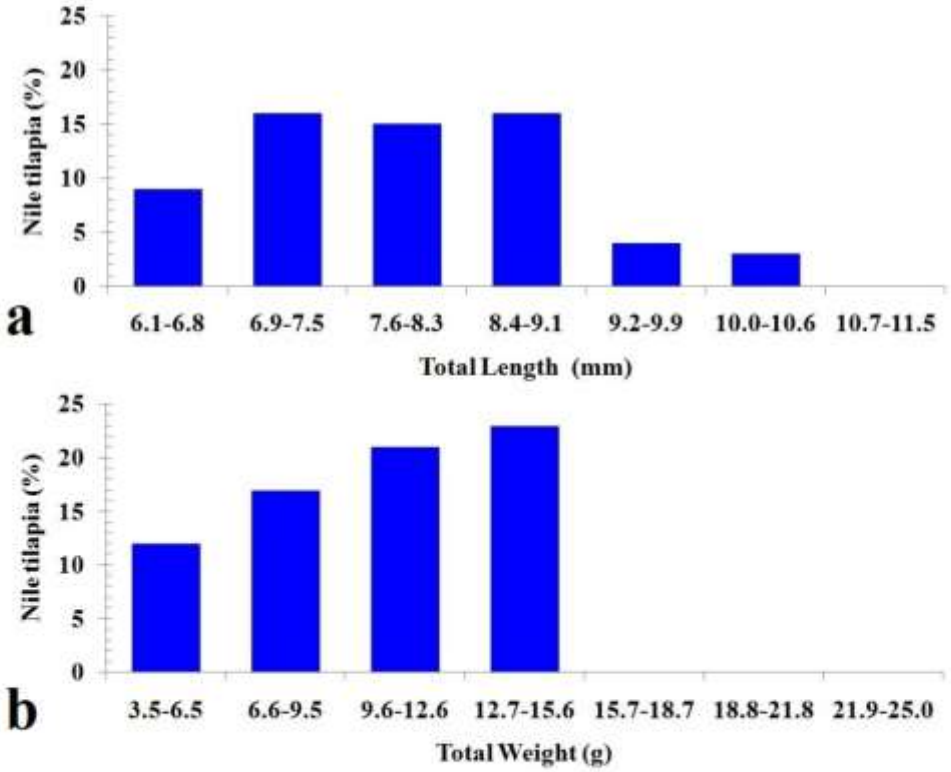

Figure 3. The length distribution (a) and weight (b) of nile tilapia *Oreochromis niloticus* used for saponin dose 200 kg/ha.

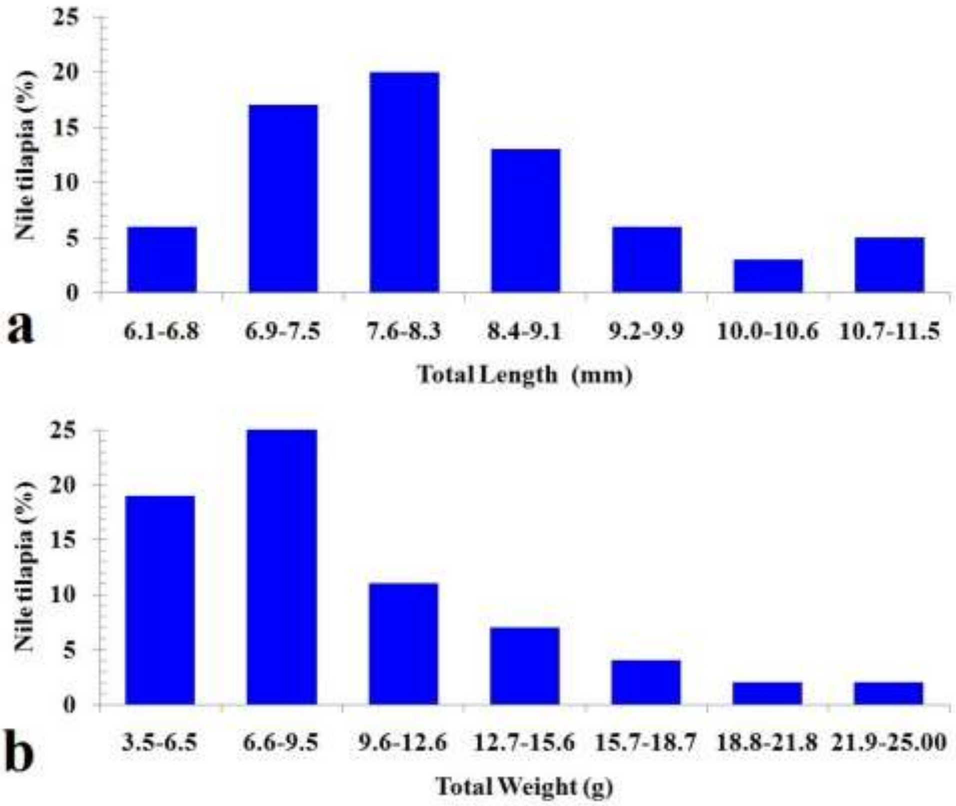

Figure 4. The length distribution (a) and weight (b) of nile tilapia Oreochromis niloticus used for saponin dose 100 kg/ha.

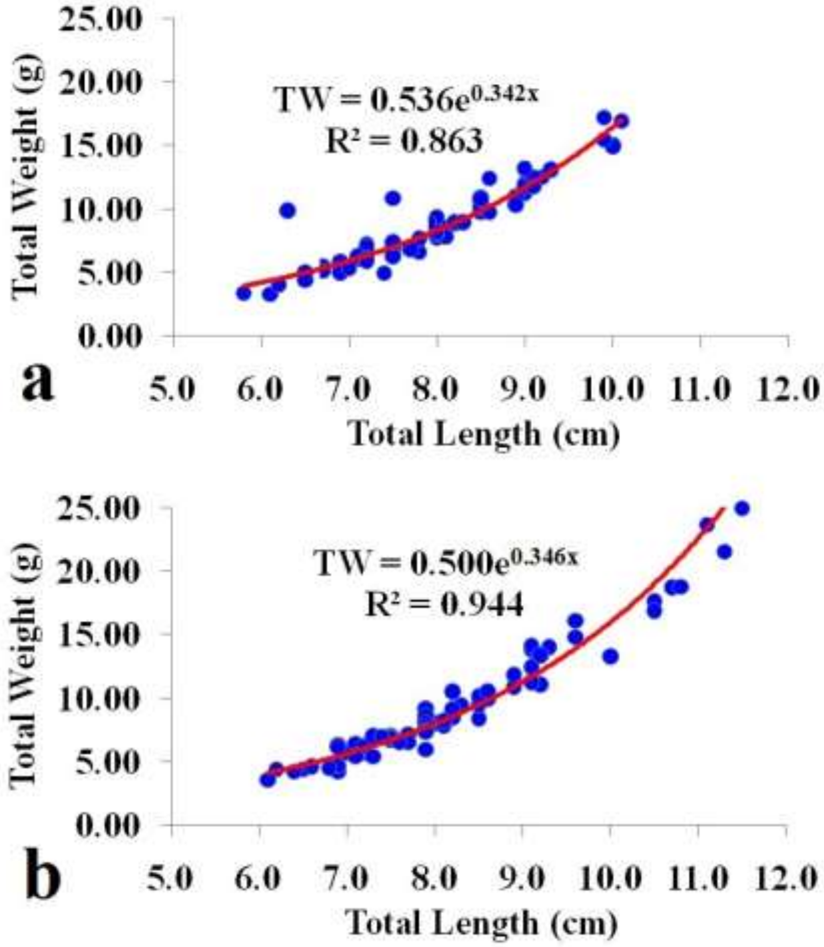

Figure 5. The relationship between length and weight of nile tilapia *Oreochromis niloticus* used as test animals for dose of 200 kg/ha (a) and 100 kg/ha (b)

3.3. Death Time

The tiger prawn survival rate is 100% for two doses of saponin and seven different salinity levels. While the survival rate for nile tilapia is 0%. Time of death at doses of 200 kg/ha dan 100 kg/ha of saponin varied widely at each salinity (Table 1). Nile tilapia die faster at a dose of 200 kg/ha (Figure 6).

Table 1. Time of death of nile tilapia *Oreochromis niloticus* at a saponin dose of 200 kg/ha and 100 kg/ha.

Salinity (ppt)	Death Time (Dose 200 kg/ha)		Death Time (Dose 100 kg/ha)	
	Range (minutes)	Mean±STD (minutes)	Range (minutes)	Mean±STD (minutes)
5	141-258	191±40	307-411	341±38
10	279-413	357±59	418-559	538±48
15	178-279	248±35	307-375	339±30
20	141-170	162±12	187-235	209±17
25	92-115	104±9	177-193	182±5
30	64-110	81±20	157-166	163±5
35	39-59	50±8	157-171	163±7

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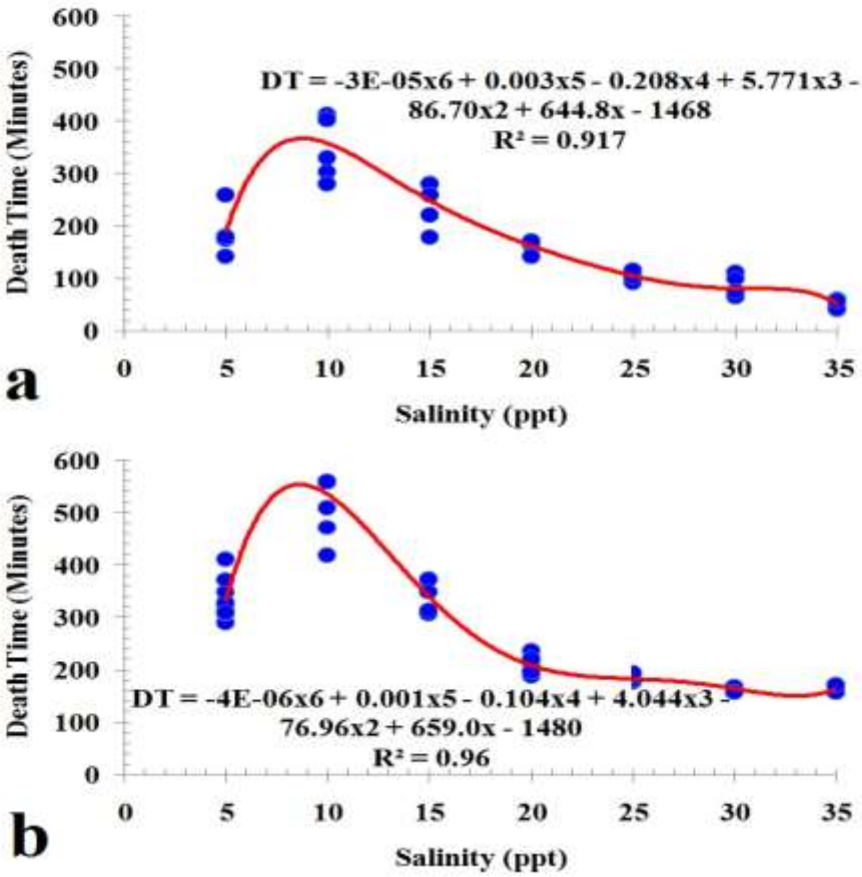

Figure 6. Death time curve of nile tilapia *Oreochromis niloticus* at a saponins dose of 200 kg/ha (a) and 100 kg/ha (b)

The time to death for nile tilapia given saponin at doses of 200 kg/ha with different salinity, all of which showed significant differences ($P<0.05$) (Table 2), while the one with saponin dose of 100kg/ha was also significantly different ($P <0.05$), except between salinity 5 and 15 ppt and between 30 and 35 ppt salinity (Table 3).

Table 2. The time of death time of nile tilapia *Oreochromis niloticus* at different salinity at a saponin dose of 200 kg/ha.

5							
10	s						
15	s	s					
20	s	s	s				
25	s	s	s	s			
30	s	s	s	s	s		
35	s	s	s	s	s	s	
Salinity (ppt)	5	10	15	20	25	30	35

Table 3. The time of death time of nile tilapia *Oreochromis niloticus* at different salinity at a saponin dose of 100 kg/ha

5							
10	s						
15	ns	s					
20	s	s	s				
25	s	s	s	s			
30	s	s	s	s	s		
35	s	s	s	s	s	ns	
salinity (ppt)	5	10	15	20	25	30	35

Bleeding from the gill caps, pectoral and caudal fins was more pronounced at high salinity for both doses of saponin used (Figure 7). The eyes of the fish treated with saponin turned white in the middle (Figure 7a, b, c), compared to the control without saponin (Figure 7d).

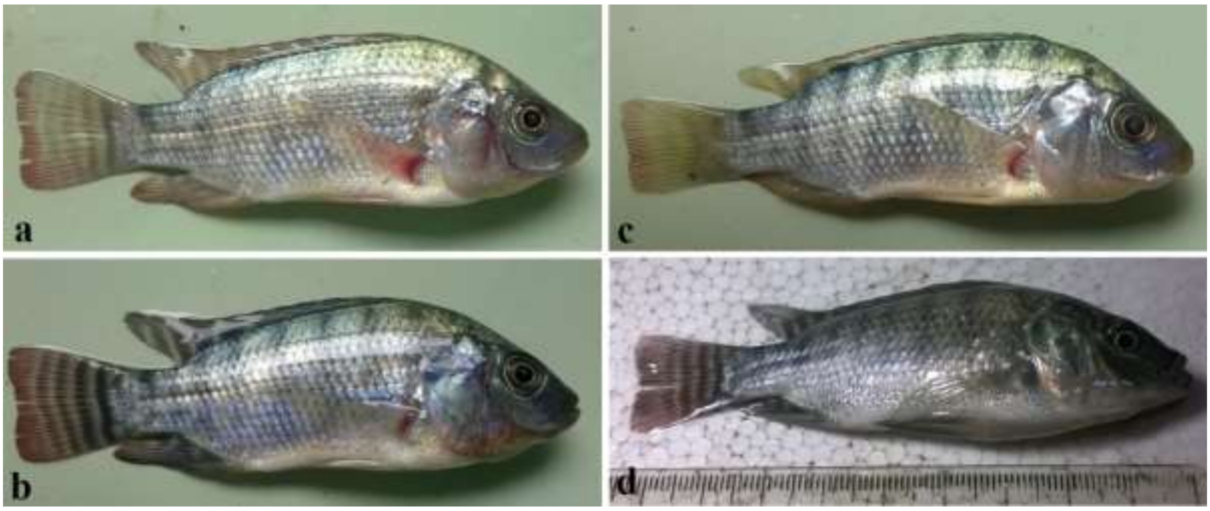

Figure 7. Bleeding conditions on gill covers, pectoral fins, tail fin of nile tilapia *Oreochromis niloticus* after treat saponins (doses of 200 and 100 kg/ha) at high salinity (30 and 35 ppt) (a), moderate (20 and 25 ppt) (b), low (5, 10 and 15 ppt) (c), and without saponin(d).

3.4. Water Quality

The water quality during the saponin treatment is ideal for the life of giant tiger prawns and nile tilapia. The temperature range is 27.5-29.0ºC with a mean of 28.5 ± 0.6; the pH range is 7.5-8.8 with a mean of 7.7 ± 0.1; and DO range is 6.8-7.3 ppm with a mean of 7.1 ± 0.2.

4. Discussion

Giant tiger prawns and nile tilapia used came from the same parent so they are genetically the same. The difference in size is caused by differences in growth that are common in one age group, some are growing faster, and some are slower. Therefore, the effect of the difference in size can be considered as not having a major effect on the results of the toxicity test.

Toxicity test results showed that giant tiger prawns were not affected by the saponins doses that given (200 kg/ha and 100 kg/ha) in all levels of salinity. This strengthens the results of previous studies which reported that the saponin dose of 200-250 kg/ha to eradicate wild fish is safe for giant tiger prawns [16].

Both doses of saponin can kill nile tilapia at all salinity levels tested, however the time of death for nile tilapia was significantly different at various levels of salinity. Nile tilapia dies faster at high salinity than at low salinity. The habit of farmers who tend to increase the saponin dose when the water salinity in the pond is low [16, 23], is actually does not needed because the active ingredient of saponin still can kill nile tilapia, even though it takes a longer time. This is not a problem because the active ingredients of saponin can last 10-12 days in water [15].

The toxicity of saponin is only effective in animals that have erythrocytes [15]. Shrimp do not have red blood cells (erythrocytes)' thus they are not affected by the active ingredients of the saponin (saponins). Therefore, the habit of pond farmers using low doses of saponin to stimulate shrimps molting [16] is not directly related to the effect of the active ingredient saponin on shrimp. Molting is thought to have occurred after the administration of low doses of saponin was related to the abundance of natural food in the form of fish carcasses. These natural foods ultimately lead to better shrimp growth, which then triggers molting in shrimp, not because of the direct effect of the active ingredient of saponin on shrimp.

This study showed that at very low salinity (5 ppt) fish died faster than at 10 ppt (Table 1). This is thought to have something to do with osmoregulation. At a salinity of 5 ppt, the ion content of fish body fluids is too far from the ion content of fish body fluids at ideal salinity (14 ppt) [27-29] so that the body's resistance of nile tilapia to saponin toxicity decreases. Faster death time at salinity above 15 ppt than death time at salinity of 10 ppt indicates that the greater the difference between ions in body fluids and in the ambient water, the faster nile tilapia dies.

More severely bleeding in the operculum, pectoral fin and caudal fin was found in fish reared in high salinity water. This bleeding is thought to be the cause of rapid death of nile tilapia maintained at high salinity. This reinforces the suspicion of previous studies which reported that saponins can destroy erythrocytes and reduce surface tension between water and gills. This condition induces hemolysis and inhibits the oxygen uptake in fish, as a result the fish will be deprived of oxygen, and eventually die [15]. The water quality parameters in this treatment are in ideal conditions, outdoor treatment makes the water quality parameters during treatment are better than the water quality parameters in indoor research [30].

5. Conclusion

The saponin dose used in this study had no effect on shrimp mortality, but had a significant effect on mortality of nile tilapia. The mortality of nile tilapia was 100% for all treatments. The death time of nile tilapia decreased significantly with increasing salinity. Longest nile tilapia death time was found at 10 ppt water salinity; this salinity is close to that of body fluids (14 ppt). The greater the difference between the ion content of body fluids and water ions in the test media, the faster the time of death. The effects of bleeding on the operculum, pectoral and caudal fins are more severe at high salinity. It is advisable not to increase the saponin dose, use the same saponin dose at any salinity, because predatory and competitive fish will die at any salinity.

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