Impact of Indian almond leaves on aquarium water quality

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Abstract. There are very few studies done to understand the impact of Indian almond leaves (IAL) on aquarium water quality. Therefore, in this study, Indian almond leaves (IAL) were used to investigate the impacts on water quality in an aquarium with Betta fish as they are hardy fish, tolerant to changes in water quality, comparatively cheaper and more readily available in tropical countries. For this study, the powdered form of IAL was used for faster dilution or mixing. As for the aquarium tank, smaller tanks with a capacity of 4.5 litres are used since Betta fish is small in size and it is easy to be accommodated in the aquarium. The various dosage of IAL in power form (0-200 mg/L) was used to determine physical, chemical and biological parameters of aquarium water quality in the presence of Betta fish. The parameters were Biochemical Oxygen Demand (BOD₅), Dissolved Oxygen (DO), turbidity, pH, chlorine and survivability test and tests were conducted on day 1, 3, 7, 14, 21 and 28. All experiments had an increasing value of DO until Day 21 and gradually decreased, while BOD₅ increased with increased IAL dosage. Ammonia concentration increased between day 5-10 and then started to decline at day 21 and pH values were decreasing with increased IAL dosage. The total coliform test has the highest total coliform count on day 28 for IAL dosage. The study revealed that Betta fish could survive for 28 days without any application of de-chlorinator. The optimum dosage of 50 mg/L of IAL can eliminate any requirement for de-chlorination for Betta fish.

1. Introduction
Fishkeeping in an aquarium is getting popular and many fish breeders and hobbyist are spending more time and giving a lot more efforts on keeping a variety of freshwater fish. The familiar freshwater fish people prefer to stay in the aquarium are Tetras, Betta, and Cichlids [1]. It is always essential to monitor aquarium water quality as poor water quality can quickly kill fish. The water parameters such as pH, DO, BODs, turbidity, temperature, ammonia is usually assessed for aquatic life. Most of the aquarium hobbyist or fish breeders prefer to use water conditioners to stabilize the water quality parameters. The various types of water conditioners can be available from fish or pet shop. However, few prefer to use Indian Almond Leaves (IAL) as an alternative to water conditioner. The IAL leaves act as “the poor man’s water conditioner” and mostly used on Betta and tetras fish for breeding [2-5].
However, there are very few scientific studies to prove the beneficial impacts of IAL on aquarium water quality.

Indian almond or *Terminalia catappa* is a tall (25 m) deciduous tree with broad leaves usually grown in the coastal, warm tropical areas. The leaves of IAL is rich in phytochemical components, mostly consist of tannins, flavonoids and triterpenoids [6]. Tannins possess antimicrobial and antioxidant properties [7-8] has many positive impacts on treating various diseases and wounds [9-10]. IAL can be used for creating a suitable environment for fish breeding.

In this study, IAL is used because it is readily available in tropical countries like India, Sri Lanka, Thailand, Malaysia, Indonesia and Brunei Darussalam. In our study, Betta fish is used as they are cheaper and readily available in the local market in many ASEAN and South Asian countries. Besides, Betta fish are known as hardy fish and more tolerant to water quality changes requires feeding only once a day. Their striking, colourful appearance is an excellent physical indicator. Betta fish are small in size (25-40 cm) and can be kept even in a small tank of 4.5 litres and those are the underlying cases for selection of Betta fish for this study. Gold, Angel or Tetra fish, although could be selected for this study, requires more space and less hardy in comparison to Betta fish. There are very few studies done to understand the impact of IAL on aquarium water quality, and therefore it is quite significant as all potential water quality parameters related to fish health are taken into due consideration in this study. The powdered form of IAL is used in this study for faster dilution or mixing. An aquarium or tank with a capacity of 4.5 litres was used as the fish can be accommodated in the aquarium due to its small size. The various dosage of IAL in powder form is used to identify aquarium water quality parameters in the presence of Betta fish. Therefore, the study was conducted to investigate the potential consequences of the IAL on aquarium water quality. The objectives were to analyze and compare the various dosage of IAL on water quality through (i) physical parameters such as DO and turbidity, pH, BODs, (ii) chemical parameters such as ammonia and total phosphorus and chlorine.

### 2. Methodology

All the relevant experiments were conducted between August 2017 and May 2018 at Universiti Teknologi Brunei (UTB) and Department of Drainage and Sewerage (DDS), Public Works Department (JKR).

#### 2.1. Samples collections

IAL, which became brown or yellowish were collected from the ground for many days located at Kg Kiarong in Brunei Muara District, Brunei Darussalam, as shown in Fig. 1.

![Figure 1. Almond trees and leaves.](image)

#### 2.2. Preparation of IAL

An industrial blender (Environmental Engineering Lab, UTB) was used to turn the leaves into finely powdered form. Mechanical sieves with mesh size 150 µm and 63 µm was used (Geotechnics Lab, UTB) and leaves retained in the powdered form on 63 µm size was used this study. The leaves retained on 63 µm sieve having 15 gm in weight was used for the study as it could dissolve much easily without any suspended particles.
2.3. Design of experiment
There were ten equivalent sized tanks (aquariums), and each container was filled with 4.5 litres of water. The experiments were designed for five different tests having different IAL concentrations, including control test which included no IAL. Each test had two tanks with a single Betta fish. In our experiment, two sets of containers were used as in case of any Betta fish died during the experiment could be done with another one. Table 1 shows the dosage of IAL used for each test.

| No. of experiment | Dosage (mg/L) for each tank | Total amount (mg/L) required for 2 tanks |
|------------------|-----------------------------|----------------------------------------|
| 1                | 0 (control)                 | 0                                      |
| 2                | 200                         | 400                                    |
| 3                | 300                         | 600                                    |
| 4                | 400                         | 800                                    |
| 5                | 500                         | 1000                                   |
| Total amount     | 2800 = 2.8 g/L             |                                        |
| The overall total amount for 4.5 litres of water | 12600 mg = 12.6 g |

A trial experiment was conducted in order to get familiar with the equipment and procedure. The water samples were collected and tested at day 1, 3, 7, 14, 21 and 28. Betta fish confined in a plastic bag was submerged and left into the water tank for 5-10 minutes so that the water temperature in the plastic bag with Betta fish can adjust with the new environment in the water tank. This process is known as acclimation. The fish was monitored on a regular basis to observe any sign of stress. Each fish was given the same amount of food pallets daily. Nest bubbles produced by fish were an indicator of healthiness and happiness.

2.4. Experimental procedure
Physical, chemical and biological tests were performed for aquarium water quality. Parameters tested were DO, BOD₅, ammonia, chlorine, turbidity, total phosphorus. At first, the tank water was tested for chlorine and then de-chlorinated before adding Betta fish in the tank. The tests were done on day 1, 3, 7, 14, 21 and 28.

2.5. Analysis of water quality
The water quality analysis for DO (10360 Direct Measurement, LDO Probe, EPA), BOD₅ (Method 10360) and Turbidity (Standard Methods 2130B) was conducted at Department of Drainage and Sewerage (DDS), Public Works Department (JKR) and ammonia (10001 Direct Measurement ISE Method), total phosphorus (Method Method 365.3 EPA), and pH (8156 pH Meter Electrode Method) test were conducted at Environmental Engineering Laboratory, UTB. The fishes were monitored continuously for any changes to physical appearances, stress and activate movement.

3. Results and Discussions
At first, the ammonia test was conducted as a trial test (Table 2). Ammonia is the most important parameters as the high presence of ammonia can even kill fish.

1st trial
The fishes looked active and healthy for the experiments E1 and E2. However, for experiments E3, E4 and E5, the fish developed stress and became inactive with cloudy slime coat falling from their bodies. There was no concern for experiments with a lower concentration of 50–200 mg/L; hence for experiments, E3, E4 and E5 tap water was added by 30% - 40% to dilute and lower the concentration. This resulted in fishes back to be active and healthy like other fishes the next day. The survival rate was 100% at day 1. Two fishes from E1 (control) were found dead on day 2. The death was caused due to the presence of chlorine (0.07 mg/L). Betta fish cannot tolerate chlorine’s presence even at
concentrations less than 0.05 mg/L and the fish may die [11]. Therefore, de-chlorination should be done to obtain free chlorine for control (E1) experiment. De-chlorination can be done by Aging (let the chlorine gas evaporate naturally for 24 hours) or adding De-chloramines. De-chlorination (< 0.01 mg/L) was preferred as ageing requires a higher temperature to dissipate chlorine gas. The experiment is conducted under the control environment with a constant temperature range of 25–27°C. Furthermore, other fishes from experiments: E2, E3, E4 and E5 were healthy with no signs of stress or inactiveness. This demonstrates that IAL can improve the survival rate of Betta fish. The survival rate was 80%.

**Table 2. Trial test for Ammonia.**

| Experiment | Control (E1) | 100 mg/L (E2) | 300 mg/L (E3) | 500 mg/L (E4) | 700 mg/L (E5) |
|------------|--------------|--------------|--------------|---------------|---------------|
| Values     | 0.17         | 1.07         | 5.7          | OR            | OR            |
| Mortality % (Day 1) | 0            | 0            | 0            | 0             | 0             |
| Mortality % (Day 2) | 20           | 0            | 0            | 0             | 0             |

*OR – Out of Range

2nd trial (Revised)

The experiments were revised by applying more doses of anti-chlorine and reducing the concentration of IAL to a maximum of 200 mg/L (Table 3).

**Table 3. Trial (Revised).**

| Trial 1 | Experiment | 50 mg/L | 100 mg/L | 150 mg/L | 200 mg/L |
|---------|------------|---------|----------|----------|----------|
| Control | E1         | E2      | E3       | E4       | E5       |
| 100 mg/L| E2         | E3      | E4       | E5       |          |
| 300 mg/L| E3         | E4      | E5       |          |          |
| 500 mg/L| E4         | E5      |          |          |          |
| 700 mg/L| E5         |          |          |          |          |

The increase in anti-chlorine doses, Control (E1) has been successful with no mortality like all other experiments using a dilution of IAL. This is supported by other researchers [11-12] as chlorine presence in water can kill the fish.

3rd trial

Tests were further carried out to identify how IAL can provide tolerance to Betta fish against the presence of chlorine by using IAL dosage of 50, 100, 150 and 200 mg/L without de-chlorination as shown in Table 4. The result shows that for the dosage of IAL from 50 – 200 mg/L, the fishes could adapt throughout the experiment up to week 4 without any de-chlorination. Thus the experiment demonstrates that optimum dosage of 50 mg/L of IAL can eliminate any requirement for de-chlorination for Betta fish.

**Table 4. Experiment on IAL without de-chlorination.**

| Experiment (Dosage of IAL in mg/L) | Remarks |
|-----------------------------------|---------|
| 50                                | Requires no de-chlorination until week 4 |
| 100                               |         |
3.1. Dissolved Oxygen
The values of DO increased to peak within a range of 8.14-8.44 mg/L for the various dosage of IAL at day 21 and then started to decrease with a value ranging from 5.40 to 7.10 mg/L at day 35 (Fig. 2). Particularly, a sample from E3 (100 mg/L) has shown a drastic drop in oxygen deficient. The decreasing trend of DO is because of the presence of bacteria that feeds on fish excreta and requires oxygen. Besides, the accumulation of left out food and excreting from fishes develops into toxic ammonia. It is therefore recommended to add fresh water about 25 per cent of tank’s volume every three weeks. This could prevent the build-up of toxic ammonia and increase the DO.

Experiments with a higher concentration of IAL has shown lower DO. For instance, E5 has the highest IAL concentration obtained through the lowest DO, followed by E4, E3, E2 and E1 (control). This shows that the application of IAL brought impact to water parameter, such as dropping the oxygen level. DO is decreasing when the concentration of IAL is increasing. This can have a relationship with the turbidity. The higher the turbidity, will have better chances of light absorption making the water in the tank warmer and also particles were allowing less water to interact with air at the water contact surface. Therefore, as the temperature got higher, the DO became lower. All experiments experienced increasing DO from day 1 until day 21.

3.2. Turbidity
The turbidity of E1 (control) experiment demonstrated clearer visibility of water even after day 28 as no IAL was added in E1. However, we observed a gradual decrease in turbidity from 2.28 to 1.39 NTU (Fig. 3). The turbidity starts to increase after 20 days as degradation process slow down due to decrease in DO.

Higher the dosage of IAL, higher the turbidity making the water darker and resembling natural habitats such as muddy ponds and streams. However, the higher dosage of IAL may lead to the problem of sediment deposition in the tank and cause gill damage to fish. The sediments at the bottom of the container might contain organic matter resulting in oxygen depletion. Besides, increased turbidity will decrease the DO of the tank. Among all the experiments carried out, the dosage of E2 (50 mg/L) would probably the most suitable the fish was clearly visible (Fig. 4).
3.3. **pH (Potential of Hydrogen)**
pH level under IAL concentrations from 50 to 200 mg/L experiment was mostly constant from 0-14 day, whereas the pH level was rapidly decreasing from 15-28 day (Fig. 5). The decrease in pH level is due to increase in ammonia. It is worthy to note that despite having an acidic condition, all fish survived. However, for E3 experiment fish became motionless and almost died at day 35 having pH 3.2.

![Figure 5. pH Vs Day with respect to various IAL concentrations.](image)

3.4. **Ammonia**
Ammonia test was conducted for one sample for every experiment for Day 1, 7, 14, 21 and 28, as shown in Fig. 6. The toxicity of ammonia depends on the pH and temperature as ammonia with a pH value of 7 or lower will be non-toxic while with the higher value it will be toxic. It is observed from the experiment E1, E2, and E4 that ammonia reaches a peak value of 0.015 to 0.035 mg/L between 7-12 days and then gradually decrease until day 21 and start increasing. While for Control (E1) and E5 experiment it shows a gradual increase reaching the value of 0.018 and 0.024 respective at day 28. However, for all experiments, ammonia is within the acceptable range of 0.05 mg/L and non-toxic. All the fishes looked healthy and active during the experiments.

3.5. **Total Phosphorus (TP)**
All tests had zero (0) mg/L of TP from day 1 – 7, as shown in Fig. 7. TP was detected after day 14 to all tests, and the concentration was 0.75 mg/L which was higher than desirable limit but lower than plankton/shrimp production level (1.0 mg/L). The significant of TP is that excess amount of it can lead to eutrophication and reduce DO. The optimum and productive TP level for fish culture is 0.05 – 0.07 mg/L[13]. No stress was observed among the fishes. This shows that Betta fish can tolerate a higher range of TP than the standard requirement for aquatic life E2 (50 mg/L) has indicated the most active presence of TP concentration from day 14 - 28. The reason for high TP could be from the left out fish food and faeces. To control the accumulation of TP, it is recommended to have regular water changes.
Algal blooming is not significantly shown in the aquarium even though E2 has shown the highest concentration of 1.5 mg/L of TP. Total phosphorus tests were done by using Test Kit for day 1, 3, 7, 14, 21 and 28.

Figure 6. Effect of IAL on Ammonia with respect to time.

Figure 7. Effect of IAL on Total Phosphorus with respect to time.

3.6. Biological Oxygen Demand (BOD₅)

BOD₅ for all the dosage of IAL at day 1 is at highest, as shown in Fig. 8. This could be due to the presence of bacteria on day 1.

Figure 8. BOD₅ for all the dosage of IAL.

The highest BOD₅ value of 10.86 mg/L under control experiment is obtained at day 1 and then keeps decreasing to 0.50 mg/L at day 10 and became constant (0.75 mg/L) up to day 28. E2 experiment demonstrated that BOD₅ values increased slowly from 6.9 mg/L to 7.2 mg/L from day 1 to 7 and then gradually declined to 2.97 mg/L. For experiment E2, E3, E4 (100, 150 and 200 mg/L), the values were fluctuating from day 1 to 28. This could be due to inconsistent amount of DO available and addition of distilled water to keep the water level in the fish tank same. From the analysis of BOD₅, it can be observed that at the end of day 28, BOD₅ value varies between 0.75 mg/L and 5.84 mg/L.

4. Conclusion and Recommendations

IAL has a potential impact on aquarium water quality as indicated through physical, chemical and biological tests. The study suggests that when the dosage of IAL increases, the pH decreases, while BOD₅ and total coliform increase. The study suggests that the application of IAL for Betta fish increases its survivability significantly. The optimum dosage of 50 mg/L of IAL can eliminate any requirement for de-chlorination for Betta fish. It is recommended to carry out tests whether IAL
concentrations have any impact on cultured bacteria under a controlled environment. Further tests are recommended to see if there is any correlation with salinity and IAL on aquarium water quality.

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