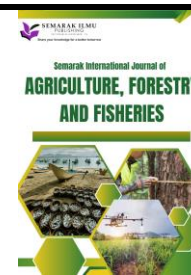




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Effect of *Terminalia catappa* Linn. Leaves Extract on Water Quality, Survival and Blood Profile of *Betta* sp.

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ABSTRACT

Betta sp., also known as Siamese fighting fish, is renowned in the ornamental fish trade for its captivating colours and unique shapes, particularly among male specimens. Despite extensive research, challenges persist in meeting market demand for this species due to high mortality rates during production. Therefore, this study focused on determining the water quality properties (pH, dissolved oxygen, temperature), survival rates and blood profile of the *Betta* sp. by applying different concentrations of *Terminalia catappa* leaves (TCL) extracts: 0 (Control treatment), 480 (Treatment 1), 960 (Treatment 2), 1440 (Treatment 3) and 1920 (Treatment 4) ppm on three months old *Betta splendens* for 30 days. The experiment employed a completely randomized design (CRD) with three replications for each treatment. Results indicated that temperature and dissolved oxygen remained unaffected by varying concentrations of TCL, while pH exhibited a significant decrease with increasing concentration, ranging from 7.07 to 5.88. Notably, treatment 2 (pH 6.73) demonstrated a significant improvement in survival rate, achieving 100 % survival compared to the control (pH 7.07) with 46.67 % survival. Moreover, treatment 2 exhibited a significantly higher specific growth rate (0.85 % per day) compared to the control (0.04 % per day). Red blood cell (RBC) content also exhibited significant differences, with the highest RBC content ($1.269 \times 10^6 \mu\text{L}$) observed at the highest TCL concentration (treatment 4, 1920 ppm). Therefore, the study concludes that the most optimal concentration of TCL, as determined from the research, was 960 ppm with a pH of 6.73. This concentration yielded the highest survival rate and growth performance for 3-month-old *Betta splendens*.

1. Introduction

Ornamental fish are commercialized globally until the present day, and the demand for ornamental fish has been rising over the years [1]. Out of over 5300 freshwater ornamental species traded, *Betta* sp. which is an altricial species [2], is one of the most famous ornamental fish due to its interesting and luring colour and shape, especially the male species [3]. The beautiful colour wrapping the whole body and its fin make it very highly demanded which can be highly produced with no doubt in the market trade [4].

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The species is widely distributed in northern and central Thailand, Cambodia, Vietnam and Malaysia [3-5]. Through the years, many experiments have been conducted to study and control all aspects. However, some problems still cannot be countered where the production of the species yet does not meet the requirement for the market demand due to the high mortality rate. Although many medicines have been produced to cure the diseases experienced by fish, their survivability cannot be only dependent on those medicines due to some of the medicines might be costly to purchase. The idea of prevention instead of treatment should be applied to ensure the good health of the fish. Commonly, *Terminalia catappa* leaves (TCL) extract is widely used to maintain the water quality parameter, especially in culturing *Betta splendens*.

Terminalia catappa Linn. or tropical almond tree is a native tree largely distributed in sub-tropical, tropical regions, and Asian countries [6], tropical zones of the Indian and Pacific Oceans [7]. According to Dianala [8], the tropical almond tree also known as “talisay” in Philippines, is a huge tropical tree in the Family Combretaceae (Leadwood tree) which is highly distributed in the sub-tropical. TCL contain naturally occurring antibacterial and antifungal compounds that protect against pathogens and maintain the health of the fish. According to Chen *et al.*, [9], the presence of tannin in the leaves is the main reason for the leaves becoming medicinal purposes. Instead, every part of the *T. catappa* tree can be utilized for medicinal purposes with different functions [10]. However, the only part that was used were the leaves due to the high amount of tannin component [11]. In some cases, the utilization of the TCL could also contribute to failure if the concentration of TCL in the water was too high. The extract also increased the ammonium ion level as the pH level decreased, indicating the ammonia increase in the water and was not good for the fish [12]. Therefore, the optimum concentration of TCL extract on *Betta splendens* is still being debated. Thus, an experiment should be conducted to discover the effectiveness and to determine the optimum concentration of TCL extract for the fish.

In addition, the utilization of *Terminalia catappa* has been used on different species of aquatic animals in recent years due to their ability to fight against various pathogenic microbes in the water culture [13]. In the study on the effect of TCL on the survival rate and growth performance of *Penaeus monodon* postlarvae [14], they stated that the water treated with TCL extraction gave better survival rates and growth performances to the postlarvae compared to the control experiment. Besides, the effect of the leaves on the *Carassius auratus* also had been studied by [15] to determine the concentration boundaries for toxicity of the leaf extract on *C. auratus*. In their study, all the samples experienced an early toxic effect at 1000 mg/mL which was the highest concentration of the experiment between 2 - 5 mins after being exposed to the concentration and was back to normal after being transferred into the freshwater. According to Chitmanat *et al.*, [16], the extracted leaf can be used in water to prevent the pathogen from attacking the Tilapia (*Oreochromis niloticus*) fish culture. As the results of the previous study showed, the survivability and growth performances of other species increased due to the extraction of the leaves at a certain concentration that improved the water quality for the species to live healthily. Thus, the objectives of this study were to determine the water quality, survivability, growth performances and blood profiles of *Betta splendens* in different concentrations of TCL extract.

2. Methodology

2.1 Experimental Site and Collection of Leaf Samples

The experiment was conducted at a wet laboratory at the Department of Aquaculture, Faculty of Agriculture, Universiti Putra Malaysia, 43400 Serdang, Selangor. The red and brown fallen leaves of TCL were collected. This was due to the fallen leaf of *T. catappa* being highly valuable in antibacterial

properties compared to the fresh green leaf [3]. TCL were collected at the Bukit Ekspo, Universiti Putra Malaysia and were brought back to the laboratory for experimental purposes. For this experiment, 75 samples of *Betta splendens* at the age of 3-month-old were supplied by a local breeder with an average weight (0.80 ± 0.08 g) and length (4.06 ± 0.30 cm).

2.2 Terminalia Catappa Leaves Extraction and Water Preparation

TCL were sterilized using an autoclave machine (Tomy SX 500 Lab Autoclave) at 121°C. TCL then was washed using distilled water to eliminate any extraneous matter from the leaves and immediately dried in the oven (Brand Shimaden, SRS 3) at 40°C for 36 hours to make sure the leaves were fully dried. The leaves were ground in small pieces and were sieved using an 8.5 mm opening passed sieve to get the powder of TCL. The powder then was immersed in 95 % ethanol (100g/L) for three days and was filtered to get the extract. By using a rotary evaporator, 95 % ethanol was removed from the extract to get the crude TCL extract. All the extraction was performed using methods by [6]. Tap water was seasoned and aerated three days before the experiment started. 1 L of seasoned water and the TCL extract were dropped in each of the aquarium tanks.

2.3 Experimental Design

The experiment was conducted using a completely randomize design (CRD) method with five different concentrations at 480 ppm, 960 ppm, 1440 ppm and 1920 ppm including 0 ppm of concentration as a control experiment. Each concentration was replicated into three replications in the aquarium's 1 L of 1.5 L capacity. For each tank, 5 samples of 3-month-old *Betta splendens* were chosen using a simple random sampling technique. All the fish were fed with pellets (Marubeni brand pellet, size no. 5) using a feed conversion ratio (FCR) below 1 % body weight in a day. The water treatment for each aquarium tank was changed every six days and all the waste was sucked out from the aquarium tank every day using a dropper. Temperature, pH and DO were monitored every six days and recorded using a 556 MPS YSI multiparameter until the end of the experiment. The survival rates and growth performance of the fish were observed and recorded every six days from day one until the end of the experiment. The experiment was conducted for 30 days.

2.4 Data Collection

Weight gain from the experiment was calculated using a specific growth rate (SGR) and length gain was calculated using an absolute growth rate (AGR) [17]. The survival data of the fish were recorded every six days. The blood profile of the 3-month-old *Betta splendens* was determined by calculating the red blood cell (RBC) content for each treatment where the blood was taken using a syringe and needle at the lateral line near the caudal part of the fish. MS-222 was applied to anaesthetize the fish before the process at a concentration of 200 mg/L [18]. All the data were presented in mean \pm standard deviation (SD). The effect of TCL extract on *Betta splendens* was analyzed using one-way analysis of variance (ANOVA) in SPSS, version 23. Significant differences (at the 95 % confidence level) were differentiated using multiple comparisons test group comparisons, Tukey Test. The formula for SGR, AGR and survival rate (SR) were as below in Eq. (1) – (3):

$$\text{SGR: } \frac{\log(\text{average final weight}) - \log(\text{average initial weight})}{\text{number of cultured days}} \times 100 \% \quad (1)$$

$$\text{AGR: } \frac{\text{average final length} - \text{average initial length}}{\text{number of cultured days}} \quad (2)$$

$$\text{SR: } \frac{\text{number of fish survived}}{\text{total of cultured samples}} \times 100 \quad (3)$$

3. Results and Discussion

3.1 Water Quality Parameter

Table 1 showed a significant difference ($p < 0.05$) in pH instead of DO and temperature ($p > 0.05$). The trend of pH (Figure 1) decreased when the concentration of TCL increased. The range of pH in this study was between 7.08 - 5.88. DO and temperature of the treatment were not affected by the TCL extract in the experiment. Results were expressed as mean \pm standard deviation (SD) of the temperature, DO and pH of the water quality for all the treatments.

Table 1
 Mean (\pm SD) of water quality parameter and SR in different concentrations of TCL extract

Variables/ Concentrations	Temperature	DO	pH	SR (%)
CT (0 ppm)	27.82 \pm 0.05 ^a	3.35 \pm 0.02 ^a	7.08 \pm 0.01 ^a	46.67 \pm 0.12 ^a
T1 (480 ppm)	27.63 \pm 0.11 ^a	3.33 \pm 0.04 ^a	6.91 \pm 0.09 ^a	60.00 \pm 0.2 ^b
T2 (960 ppm)	27.64 \pm 0.03 ^a	3.39 \pm 0.08 ^a	6.73 \pm 0.07 ^b	100.00 \pm 0.00 ^c
T3 (1440 ppm)	27.69 \pm 0.06 ^a	3.39 \pm 0.03 ^a	6.49 \pm 0.05 ^b	73.33 \pm 0.12 ^d
T4 (1920 ppm)	27.57 \pm 0.14 ^a	3.44 \pm 0.04 ^a	5.88 \pm 0.09 ^c	80.00 \pm 0.2 ^e

Note: Same superscript showed no significant difference between treatments

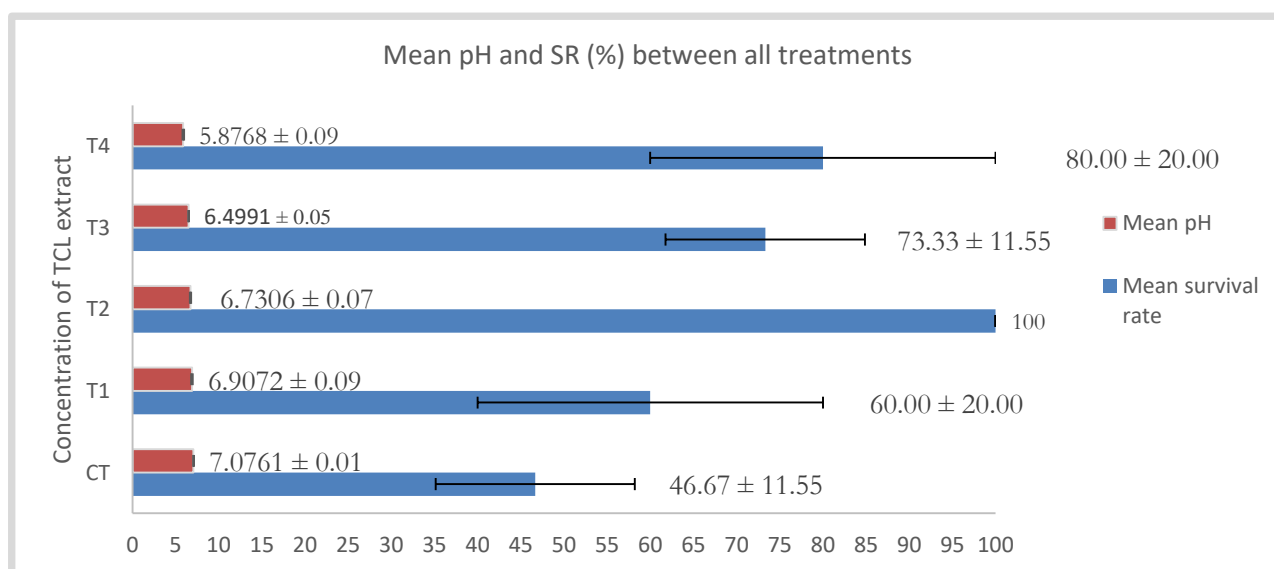


Fig. 1. Mean (\pm SD) pH and mean (\pm SD) SR of *Betta splendens* with different concentrations of TCL extracts

3.2 Survival Rate (SR)

Table 1 shows the result of the mean SR in the present study. There is a significant difference in T2 compared to CT and T1 ($p < 0.05$). The highest SR was in T2 at 100 % survival of the *Betta splendens*. The survival trend increased from control until the concentration of 960 ppm at T2 and decreased starting at the concentration of 1440 ppm until 1920 ppm (Figure 1). The line graph (Figure 2) showed the trend of the SR every six days until day 30. The control experiment showed the lowest survival rate compared to the other treatments.

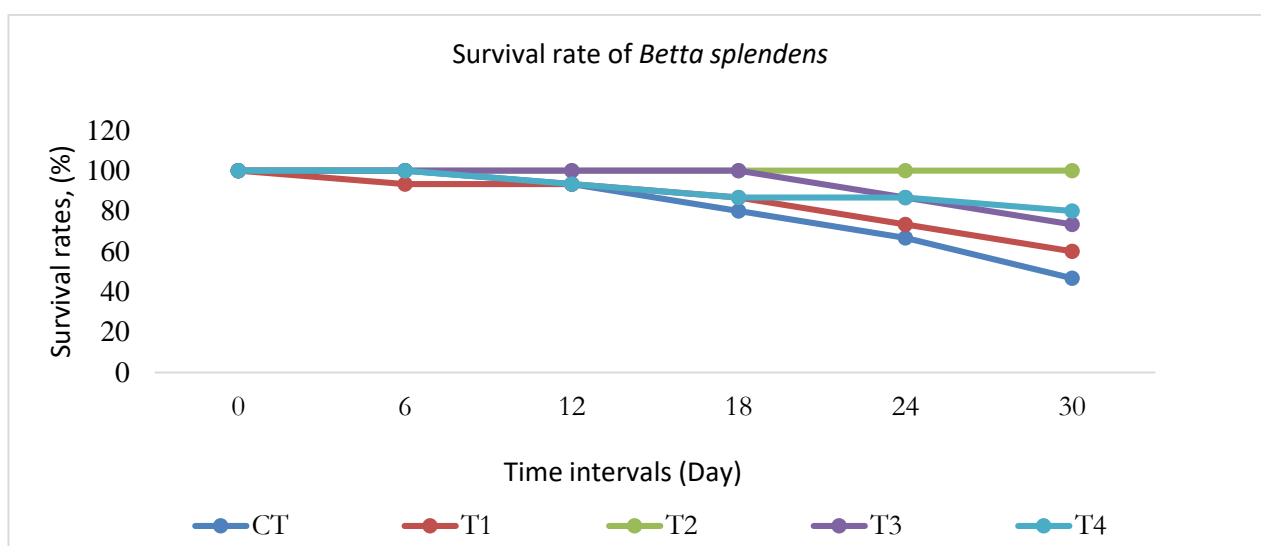


Fig. 2. Mean (\pm SD) SR of *Betta splendens* against time intervals with different concentration of TCL extracts

3.3 Absolute Growth Rate (AGR)

The result of the present study showed no significant difference ($p < 0.05$) in T3 compared to the control experiment as mentioned in Table 2. The other treatments showed no significant differences ($p > 0.05$) compared to the control. There was not much difference in total length gained by the fish in all treatments during the experiment. However, the highest AGR obtained was at T3 (0.28 m) with 1440 ppm concentration of TCL, and the lowest AGR was obtained at CT (0.1 mm) with no TCL extraction. The length gain of the fish started to decrease at T4 with 1920 ppm of TCL concentration.

Table 2
 Mean (\pm SD) of SGR, AGR and RBC content in different concentrations of TCL extract

Variables/ Concentrations	Specific growth rate (%)	Absolute growth rate (mm)	Red blood cell content (10^6 , μ L)
CT (0 ppm)	0.043 \pm 0.026 ^a	0.10 \pm 0.005 ^a	0.574 \pm 0.157 ^a
T1 (480 ppm)	0.582 \pm 0.368 ^{ab}	0.15 \pm 0.007 ^{ab}	0.521 \pm 0.078 ^a
T2 (960 ppm)	0.854 \pm 0.172 ^b	0.16 \pm 0.015 ^{ab}	0.971 \pm 0.309 ^{ab}
T3 (1440 ppm)	0.0795 \pm 0.033 ^a	0.28 \pm 0.006 ^b	0.934 \pm 0.161 ^{ab}
T4 (1920 ppm)	0.521 \pm 0.032 ^{ab}	0.17 \pm 0.005 ^a	1.269 \pm 0.268 ^b

Note: The same superscript showed no significant difference between treatments

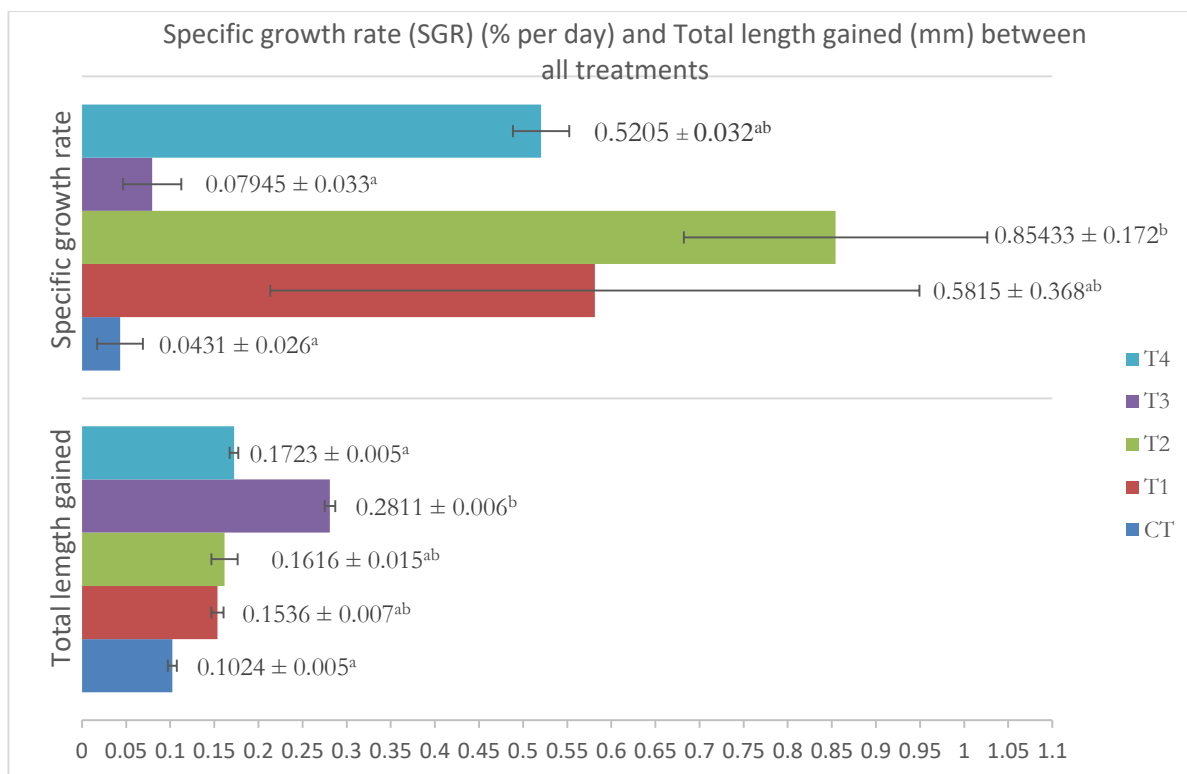


Fig. 3. Mean (\pm SD) SGR and mean (\pm SD) total length gained of *Betta splendens* with different concentration of TCL extracts. Different superscripts showed a significant difference between treatments

3.4 Specific Growth Rate (SGR)

Based on the present study as mentioned in Table 2, SGR (% per day) of *Betta splendens* showed significant differences between T2 and CT ($p < 0.05$). T1, T3 and T4 were not showing any significant differences with the CT ($p > 0.05$). T2 gave the highest SGR with 0.854 % per day whereas the CT showed the lowest SGR with 0.043 % per day between all the treatments.

3.5 Blood Profile

Based on the result (Table 2) of the present study showed a significant difference ($p < 0.05$) between T4 at 1920 ppm of TCL concentration with $1.296 \times 10^6 \mu\text{L}$ RBC content compared to CT with only $0.574 \times 10^6 \mu\text{L}$ RBC content. Other treatments showed no significant difference compared to the control experiment ($p > 0.05$). The highest RBC content was obtained at the highest concentration (T4), while the lowest RBC content was obtained at T1 with only $0.521 \times 10^6 \mu\text{L}$.

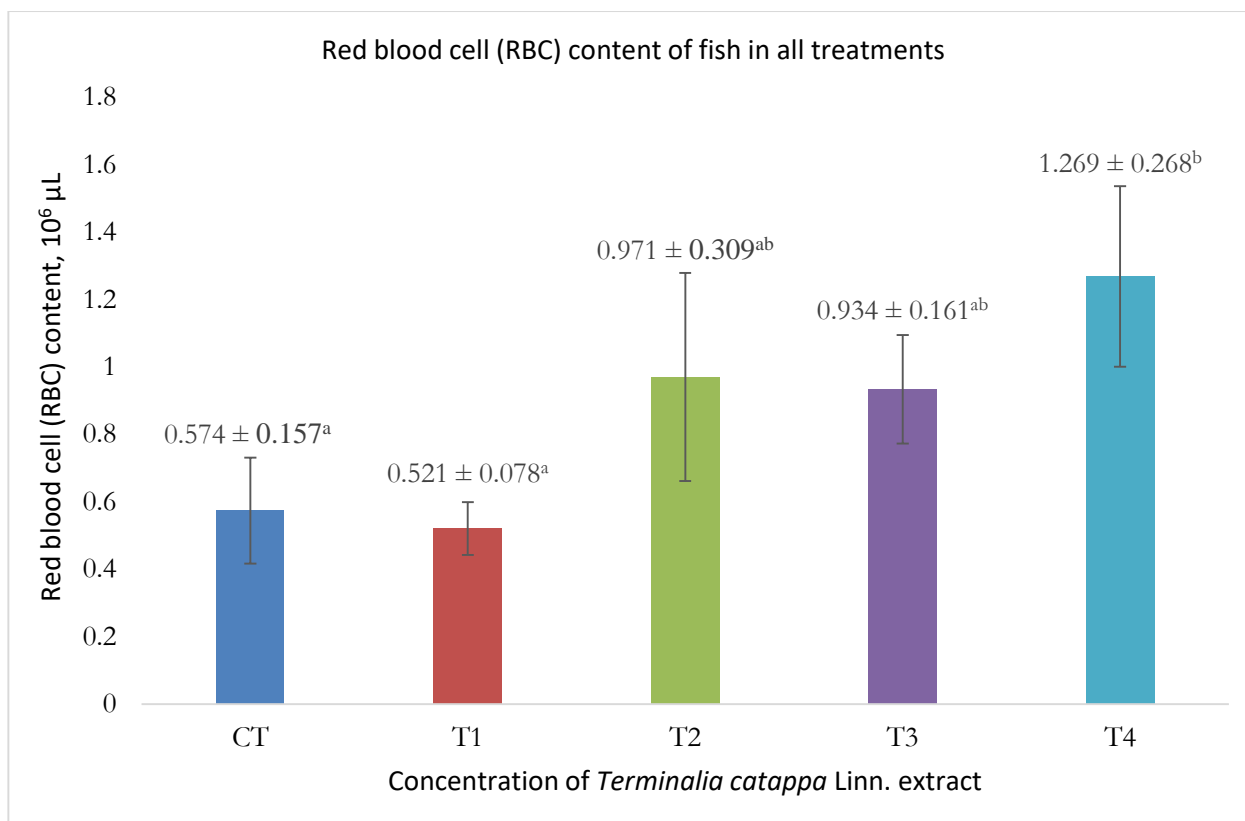


Fig. 4. Mean (\pm SD) RBC content of *Betta splendens* with different concentration of TCL extracts. Different superscripts showed a significant difference between treatments

Betta splendens showed a better response in the aquarium tank treated with TCL extract compared to the control experiment. According to Sharma, [19], a high level of expertise was needed for a better way of water quality management of the ornamental fish as those fish were more sensitive to poor water quality compared to the food fish. Based on the result of the present study, the water parameter varies according to the parameter that had been observed. High concentrations of TCL extract did not affect the temperature and DO in the experiment. The temperature and DO were not affected by any concentration of TCL immersion [6]. The pH level of other treatments in this present study was slightly lower compared to the control experiment, and the highest concentration showed the lowest pH. As the concentration increased, the pH of the water decreased [6,14,15].

The SR of the species of this present study was met in line with the previous study where the highest SR was obtained at T2 (960 ppm) with pH 6.73 compared to the other treatments including the CT. According to Sung and Abol-Munafi [23], the decreasing pH in the water was caused by TCL extract in addition to absorbing the harmful chemicals, inhibiting bacteria and creating better conditions for the fish. However, the SR of the fish decreased in T3 at 73.33 % and T4 at 80.00 %. The high concentration of TCL might cause death due to the adhered gills that were blocked from oxygen and irritated by the high concentration of tannins [12]. The rapid changes in pH over 0.2 due to the increased concentration of TCL extract would cause the ammonium ion level to increase, indicating the increase of ammonia and may cause mortality to the fish.

On the other hand, the AGR of the present study resulted in the highest AGR being obtained at T3 compared to the CT with the lowest AGR. However, T1 at 480 ppm of TCL extract showed a slightly lower AGR compared to the other treatments with TCL. Higher concentrations of TCL extract also caused the reduction of the growth of Amazon leaf fish larvae [20]. Nurhidayat *et al.*, [21] also

mentioned that the length of *Paracheirodon axelrodi* increased when treated with the TCL but slightly reduced at the highest concentration.

In this study, the highest growth rate was observed at T2 with the SGR, contrasting with the lowest growth rate indicated by the CT. The SGR results in this study align with those of previous research. Post larvae of *Penaeus monodon* gave a better growth performance in the treatment consisting of TCL extract [20]. The TCL also increases the weight gained by *Paracheirodon axelrodi* [21]. Ramos *et al.*, [20], stated that pH 6.5 - 6.0 is acidic to most aquatic animals. The high concentration of TCL extract might be a possible chance that caused the reduction in the growth of *P. monodon* postlarvae.

Based on the result obtained, the RBC content of the Betta fish increased as the concentration of TCL extract increased. T4 at 1920 ppm showed the highest RBC content compared to other treatments. It might be due to the phytochemical inside the TCL extract that caused the RBC content of the fish to increase. As highlighted by [6], although there was not enough evidence to prove that TCL extract can stimulate the haematological indices of *Betta* sp, the flavonoid content in the TCL can be a bio-catalysator to synthesize leukocytes and boost them as nonspecific cellular immunity. According to Soranzo *et al.*, [22] the immune response has a connection with haematology which was used to examine and measure the performance of the fish by referring to the haematology parameters such as total RBC, white blood cells, concentration of hemoglobin and total protein inside the blood. In fact, the increases in RBC content in *Betta splendens* in this study showed similar findings to the previous study by [6].

4. Conclusion

The study concluded that incorporating TCL extract into the water significantly improved the survival rate, growth performance and RBC content of *Betta splendens*. It demonstrated that *Betta splendens* exhibited better survival rates and growth performance when exposed to TCL extract concentrations. The chemical components particularly tannins, present in TCL extract played a crucial role in enhancing the culture of Betta fish. Remarkably, the study found that a concentration of 960 ppm of TCL extract at pH 6.7 resulted in the highest survival rate for *Betta splendens* and improved growth performance compared to other concentrations. However, concentrations of 1440 ppm and 1920 ppm showed a decrease in both survival rate and growth performance compared to the optimal 960 ppm concentration. These findings serve as practical guidance for farmers seeking to enhance *Betta splendens* production by maintaining optimal water conditions for the fish's health and growth.

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