TREATING MOTIL AEROMONAS SEPTICEMIA DISEASE WITH *Terminalia catappa* (L) LEAF FLOUR EXTRACT ON *Cyprinus carpio (*L) LARVAE

ABSTRACT

This study aimed to evaluate the effectiveness of *Terminalia catappa* (L) leaf flour extract in controlling Motile Aeromonas Septicemia (MAS) disease caused by *A. hydrophila* in common carp larvae. The research method used in this study is an experimental method using a complete randomized design (RAL). This research consists of 2 stages; in vitro and in vivo tests using *T. catappa* leaf extract. In the in vitro test, *T. catappa* leaf extract consists of 5 different doses;.The treatments are as follows: A. using 20 g/L, B. using 40 g/L *T. catappa* leaf flour extract, C. using 60 g/L *T. catappa* leaf flour extract, D. using 80 g/L *T. catappa* leaf extract, E. using 100 g/L *T. catappa* leaf flour extract, F. using Stroptomycinsulphate 0.005 g, G. Untreated sample (without *T. catappa* leaf extract.). In the in vivo test, 3 best doses were selected from the in vitro test results; 60, 80, and 100 g/L, with positive (using Stroptomycinsulphate 0.005 g and untreated sample (without *T. catappa* leaf flour extract), each with 3 replicates. The results showed that using *T. catappa* leaf flour extract can significantly increase the immune response, resistance, and survival of goldfish. Duncan's further test showed that the 100 g/L dose treatment showed an increase in immune response, resistance, and survival compared to the F and G treatments.

Keywords: Erythrocytes, Leukocytes, Hemoglobin, Hematocrit, Phagocytosis Index, Survival

Introduction

Common carp (*Cyprinus carpio* L.) is a very valuable aquaculture commodity. In addition to the high protein content of the fish, the price is also reasonable to most communities, making it quite popular and in high demand on the local market. Disease attacks on fish are frequently problematic in fish aquaculture. Disease attacks can result in economic losses and even harvest failures. Diseases onset can be caused by excessive fish density during rearing, larvae transportation and handling, and poor water quality (Thanikachalam et al., 2010).

Bacterial disease is one of the most common diseases affecting cultured fish. Bacterial infections in fish, particularly those caused by *A. hydrophila*, have been reported in Indonesia since 1980 (Lukistyowati and Kurniasih, 2011; 2012). *Aeromonas* sp. bacteria consist of various species that can cause bacterial diseases in fish including *A. caviae, A. hydrophila, A. salmonicida,* and *A. sobria* (Austin and Austin, 2007). *A. hydrophila* is still often endemic in Southeast Asia and attacks freshwater fish, both ornamental and commercial food fish, and can cause up to 100% mortality. Clinical symptoms are in the form of wounds on the body of fish and this bacterium attacks all ages and almost all fishery commodities in Indonesia (Kamiso and Triyanto, 1993).

Fish farmers often use antibiotics (Li et al., 2020) or other chemicals to treat *A. hydrophila* infections. Long-term use of antibiotics can have negative effects, including the fear of developing strains of bacteria resistant to these antibiotics. Finally, it damages the microecology (fish) and harms the environment and the health of consumers (Tan et al. 2019; Wang et al., 2021). Phytopharmacology can be used to prevent and treat diseases (Galina et al., 2009). One type of phytobiotic plant is *Terminalia catappa* leaves. *T. catappa* leaves contain chemicals such as tannins and flavonoids that can damage the peptidoglycan in bacterial cells and thus prevent bacterial growth.

One of the natural ingredients with potential as an antibacterial ingredient is *T. catappa* leaves (Hardhiko et al., 2004; Heyne, 1987). *T. catappa* leaves are generally known to effectively maintain water quality in aquaculture operations. *T. catappa* may contain medicinal compounds such as flavonoids (Lin et al., 2000), triterpenoids (Gao et al., 2004), tannins (Ahmed et al., 2005), alkaloids (Mandasari 2006), steroids (Babayi et al., 2004) and fatty acids (Jaziroh 2008), chebulagic acid, and corilagin (Kinoshita et al., 2007).

Materials and Methods

Experimental Set-Up

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The research was conducted from January to March 2023, at the Fish Health Laboratory, Department of Aquaculture, Pangkep State Polytechnic of Agriculture. Fiber tubes, Petri dishes, Erlemeyer, bunsen, aluminum foil, digital scales, sieves, tweezers, measuring cups, autoclave, and Whatman No 42 paper are important materials. Common carp with a length of 2 cm as experimental fish, *T. catappa* leaves, *A. hydrophylla*, distilled water, PL 500 commercial feed, TSA media.

Experimental Design

The research method used in this study is an experimental method using a completely randomized design (RAL). This study consists of tests using *T. catappa* leaf extract in vitro and in vivo. *T. catappa* leaf extract is treated with five treatment doses and two controls in the in vitro test, the best three doses are chosen from the in vitro test results with positive and negative controls, each with three replications, in the in vivo test. The doses of *T. catappa* leaves in the in vitro test are as follows:

A. 20 g L^{-1} *T. catappa* leaf flour extract
B. 40 g L^{-1} *T. catappa* leaf flour extract
C. 60 g L^{-1} *T. catappa* leaf flour extract
D. 80 g L^{-1} *T. catappa* leaf flour extract
E. 100 g L^{-1} *T. catappa* leaf flour extract
F. Control+ (Stroptomycinsulphate antibiotic 0.005 g)
G. Control- (without T. catappa leaf flour extract)

While in vivo test, the doses of *T. catappa* leaves are as follows:

T1. 60 g L^{-1} *T. catappa* leaf flour extract
T2. 80 g L^{-1} *T. catappa* leaf flour extract
T3. 100 g L^{-1} *T. catappa* leaf flour extract
T4. Control+ (Stroptomycinsulphate antibiotic 0.005 g)
T5. Control- (without *T. catappa* leaf flour extract)

Preparation of *Terminalia catappa* leaf flour extract

T. catappa leaves are washed with clean water, drained at room temperature, and then dried in sunlight until the leaves are easily broken. After drying, the leaves are mashed and sieved until a fine flour extract is obtained. The process of dissolving *T. catappa* leaf flour was done by dissolving a few grams of *T. catappa* leaf flour in sterile distilled water. A mixture of *T. catappa* leaf flour and sterile distilled water is then brewed at 50°C for 15 min. The brewing results are filtered with Whatman No.42 paper and the extract is obtained in liquid form.

Preparation of *A. hydrophila* bacteria

Isolates of *A. hydrophila* bacteria were collected from the Fish Health Laboratory of Pangkep State Polytechnic of Agriculture, South Sulawesi Province of Indonesia. The collected bacteria were cultured on TSA media.

In vitro Test

In vitro tests were performed using the Kirby-Bauer method (Lay, 1994) or disc paper to detect the antibacterial activity of *T. catappa* leaf flour extract against *A. hydrophila* bacteria. The test provides an optimal dose of *T. catappa* leaf flour extract that has effectively inhibited the growth of *A. hydrophila* bacteria, which is then used as a standard dose in the in vivo test. The results of the antibacterial activity test using the disc paper method show a clear zone around the disc paper.

In vivo test

Preparation of containers

The containers used in the in vivo test were 20-liter plastic buckets filled with 15 liters of water. The containers were first washed with detergent and allowed to dry for 24-h before use.

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Experimental Fish

The fish used was carp (*C. carpio*) obtained from the Ompo Fish Larvae Center, Soppeng Regency, South Sulawesi, Indonesia. The fish used in this study were 30 days old after hatching with a body length of 2 cm.

Fish Acclimatization

Acclimatization is the process of adjusting fish from their previous environment to a new environment. Acclimatization of common carp larvae aimed to prevent stress when transferred to a new and different environment**.** Acclimatization was carried out by floating the larvae bag in a 250-liter container for 25-30 min, after which the bag was opened, allowing water to enter slowly, thus the common carp larvae came out on their own.

Soaking with *T. catappa* leaf extract

To soak with *T. catappa* leaf extract, a bucket was filled with 15-L of fresh water. When the *T. catappa* leaf extract solution is homogeneous, 2 cm common carp larvae are placed in the container, up to 30 fish per treatment unit, and soaked in water containing *T. catappa* leaf extract for 25 min**.**

Challenge Test

A challenge test was carried out to determine the resistance of common carp larvae to *A. hydrophila* after being soaked with *T. catappa* leaf flour extract. The challenge test was carried out on the 30th day by infecting *A. hydrophila* intramuscularly at a dose of 0.1 ml/ind. With a bacterial density of 10^5 CFU/ml. During the challenge period, the experimental fish were still feeding. Siphoning of feces and uneaten feed was carried out after feeding. After the challenge test, rearing of the experimental fish was continued for 10 days to determine the fish's survival and immune response.

Observed parameters

In vitro test parameters

Antibacterial activity of *T. catappa* leaf extract against *A. hydrophila* was observed. The zone of inhibition formed is a measure of the strength of the antimicrobial substance against *A. hydrophila*. The resistance around the disc depends on the absorption of the active ingredient used. If the antimicrobial agent is inhibitory or deadly. Bacterial growth stops around the disc, this is visible as a clear circle that is not covered by bacteria after 24 h of incubation, and this clear circle indicates the effect of bacteria. Haryadi (2012) and Bernatová et al. (2013) stated that if an antibacterial agent is only bacteriostatic or can only prevent, then after prolonged incubation bacteria have the opportunity to grow slowly, or bacteriostatic antibacterial substance work by preventing the multiplication of bacterial populations and not killing them, but if the substances contained in it is bacteriocidal or kills bacteria, then bacterial growth stops.

In vivo test parameters

The parameters observed in this study were the prevalence of *A. hydrophilla* bacterial attack, Phagocytosis Activity, Total Leuocytes, and carp survival.

Results and Discussions

In vitro test

Figure 1 indicated that the use of *T. catappa* leaf flour extract can inhibit the growth of *A. hydrophila* with a larger zone of inhibition compared to 20 g L^{-1}, 40 g L^{-1}, the positive and negative control. The doses of *T. catappa* leaf extract tested at 60, 80, and 100 g L^{-1} had the highest zone of inhibition. Therefore, in the following study (in vivo test), the doses of *T. catappa* leaf extract used were 60, 80, and 100 g L^{-1}**.**

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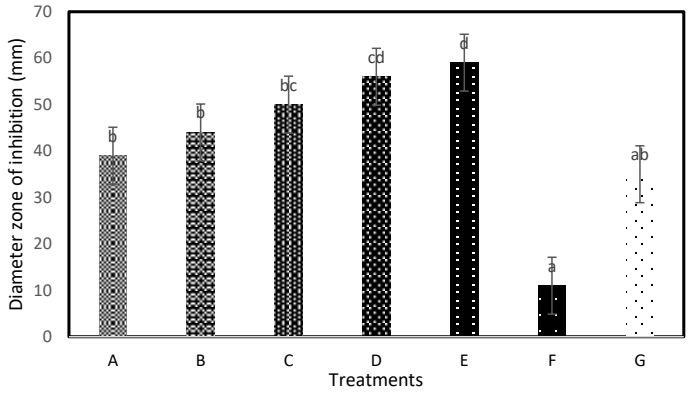

Figure 1. Dose and zone of inhibition of T. catappa leaf flour extract extract against the growth of *A. hydrophylla* .

The results of the Invitro test showed that the use of T. catappa leaf flour extract can inhibit the growth of *A. hydrophila* with a larger zone of inhibition compared to the control+ and control-. The doses of *T. catappa* leaf extract tested at 60, 80, and 100 g L^{-1} had the highest zone of inhibition. Therefore, in the following study, *T. catappa* leaf flour extract was used in doses of 60, 80, and 100 g L^{-1}.

These findings support the comparatively high inhibitory action of *T. catappa* leaf flour extract against *A hydrophila*. The antibacterial capabilities of *T. catappa* leaf flour extract allow it to suppress the growth of *A. hydrophila*. Nursal (1998) highlighted that the antibacterial capacity and zone of inhibition increase with increasing extract concentration. This agrees with the remark made by Hasim (2003) that an inhibitory zone diameter of 20 mm or more is considered to be extremely strong, 10-20 mm is considered to be strong, and 5–10 mm is considered to be moderate. Conversely, a zone with a diameter of less than 5 mm is considered weak. The presence of active chemicals with antibacterial capabilities in the *T. catappa* leaf flour extract can limit the growth of bacteria, as evidenced by the development of an inhibitory zone during the in vitro test. According to Lay (1994), the development of an inhibitory zone surrounding paper discs demonstrated the activity of antibacterial compounds. Alkaloids and flavonoids are compounds found in *T. catappa* leaf flour extract. The antibacterial properties of *T. catappa* leaf flour extract include tannins, flavonoids, and saponins, as previously reported by Wahjuningrum et al. (2008). *T. catappa* leaf flour extract exhibits superior antibacterial activity than commercial antibiotics, as reported by Manzur et al. (2011). It can kill 70% of Gram-positive bacteria and 63% of Gram-negative bacteriaIn vivo test.

In vivo test

Common carp (*C. carpio*) larvae exposed to *T. catappa* leaf flour extract may exhibit changes in their immune system and ability to survive the *A. hydrophila* challenge test. The characteristics of total leukocytes (white blood cells), total erythrocytes (red blood cells), phagocytic activity, hemoglobin, and hematocrit all influence the immunological response.

Total Erythrocytes of Common carp.

Total erythrocyte counts of common carp were measured at the end of rearing (day 30) and in the post-challenge test using *A. hydrophila*.

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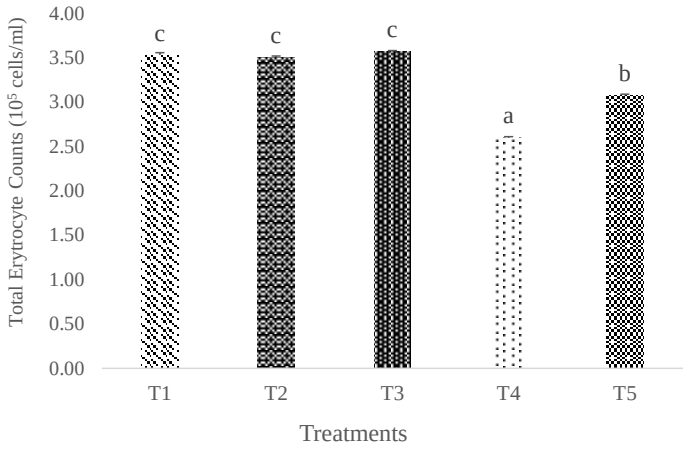

Figure 2. Observation of Total Erythrocytes Count of Common Carp Larvae

The statistical analysis results showed that the administration of *T. catappa* leaf flour extract had a significant effect (P <0.05) on the total number of erythrocytes in common carp larvae. Based on the above data, the highest total number of common carp erythrocytes was obtained after 30 days of the study period in T3 (100 g/l) with a concentration of 3567 cells/ml and the lowest in T4 (control + / Streptomycine) 2600 cells/ml (Figure 2). In 30-day-old carp, the range of the mean total erythrocyte count was 3067 to 3567 × 105 cells/mm3. A total of 3567 x 105 cells/mm3 was found in treatment T3, the highest total erythrocyte count, and 3670 x 105 cells/mm^3 in treatment T4. For the duration of the trial, the total erythrocyte count was within normal limits. Common carp's total erythrocyte count ranged from 1,026 to 2,140 × 106 cells/mm3, according to Syawal et al. (2011).

Because *T. catappa* leaf extract can enhance the number of erythrocytes in fish blood, the total number of erythrocytes in the treatment with *T. catappa* leaf extract is higher than in the treatment with Streptomycin and without *T. catappa* leaf extract (T5). *T. catappa* leaf flour extract, according to Nugroho et al. (2013), functions as an immunostimulant, which can strengthen and improve fish immune systems. Antigen penetration into the body causes the body to produce an immunological response, which is evident in the total number of erythrocytes. When compared to T1 (60 g/L) and T2 (80 g/L), a dose of 100 g/L water (T3) produced greater total erythrocyte counts at lower doses. These results suggest that T1 and T2 dosages did not provide the strongest possible immune response. According to Bahar et al. (2017), there is a specific dose that can trigger the best possible immune response, and changing the amount of *T. catappa* leaf flour extract provided can alter the immunogenicity. The age, size, and feed composition of common carp all play a role in the proliferation of erythrocytes during the duration of the 30-day rearing period. The amount of erythrocytes is dependent on several factors, such as age, sex, size, nutrition, physical activity, and species (Emu, 2010). Erythrocyte cell counts in Jamba Siamese fish can rise by 1.89–2.32 × 10^6 cells/mm3 when *T. catappa* leaf flour extract is applied by dipping (Sinaga et al., 2019). According to Nugroho et al. (2016), fish treated with *T. catappa* leaf flour extract had greater hemoglobin, leukocyte, and erythrocyte levels than the control or other groups.

Total Leukocytes

The measurement of total leukocytes of common carp larvae was carried out at the end of rearing (day 21) and day 7 after the challenge test using *A. hydrophylla*.

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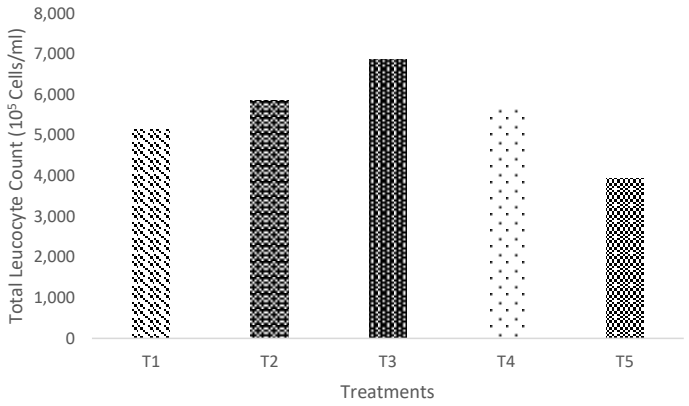

Figure 3. Observation of total leukocytes on common carp larvae.

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According to statistical analysis, the total number of leukocytes in common carp larvae was significantly (P <0.05) affected by the administration of *T. catappa* leaf flour extract. The results presented above indicate that after 30 days of culture, the total leukocyte count in common carp was 6867 cells/ml in T3 (100 g/l) and 5667 cells/ml in T5 (control) (Figure 3). Similarly, the administration of *T. catappa* leaf flour extract was shown to be able to raise the total leukocyte count following the A. hydrophylla challenge test. T3 (100 g/l) had the highest total leukocyte count, 6867 cells/ml, whereas T5 (control/non-*T. catappa* leaf flour extract) had the lowest total leukocyte count, 5667 cells/ml (P<0,05).

At day 30, common carp had mean total leukocyte counts ranging from 3933 to 6877 x 105 cells/mm^3. The highest total leukocyte value was observed in the T3 treatment (6877 x 105 cells/mm^3) and the lowest in the T5 treatment (3933 x 105 cells/mm^3). The total leukocyte count was within the normal range throughout the study. The normal leukocyte count for common carp varies between 16,240 and 24,620 x 10^5 cells/mm^3 (Anonymous, 2013). In addition, Salasia et al. (2001) and Nuryat et al. (2008) reported that common carp blood leukocyte counts ranged from 3390 to 14200 cells/mm^3. The total leukocyte value in the treatment with *T. catappa* leaf flour extract is higher than in the treatment without *T. catappa* leaf flour extract (P0). This is because *T. catappa* leaf flour extract can increase the number of leukocyte cells in fish blood. Nugroho et al. (2013), *T. catappa* leaf flour extract acts as an immunostimulant, which can enhance the immune system of fish. A dose of 100 g L^{-1} water (T3) produced higher total leukocyte counts at lower doses compared to T1 (60 g L^{-1}) and T2 (80 g L^{-1}). This indicates that doses of T1 and T2 did not elicit a maximal immune response. Bahar et al. (2017), increasing the dose of administered *T. catappa* leaf flour extract can change the immunogenicity and certain doses can induce an optimal immune response.

A dose of 100 g L^{-1} water (T3) produced a higher total leukocyte count than T1 (60 g L^{-1}) and T2 (80 g L^{-1}) at lower doses. This indicates that doses of T1 and T2 did not elicit a maximal immune response. Bahar et al. (2017), increasing the dose of administered *T. catappa* leaf flour extract can change the immunogenicity and certain doses can induce an optimal immune response. The increase in the total leukocyte count of common carp during a 30-day rearing period is due to several factors; the age, size of the fish, and the diet of the feed. Factors affecting leukocyte count include species, sex, age, feed intake, size, physical activity, and age (Emu, 2010). Nugroho et al. (2016), the group of fish treated with *T. catappa* leaf extract had higher values of erythrocytes, leukocytes, and hemoglobin compared to other groups. Some researchers like Nair et al. (2002) and Lyu and Park (2005) suggested that plant flavonoids can promote cellular immunity by modulating Th-1 cytokines such as IL-2 (interleukin 2) and INFγ (interferon). Flavonoids can also act as biocatalysts to produce leukocytes and stimulate leukocytes as non-specific cellular immunity.

Haemoglobin Level

The results of hemoglobin (g/L) counts of common carp larvae were conducted at the end of rearing (day 21) and day 7 post-challenge test using *A. hydrophylla*.

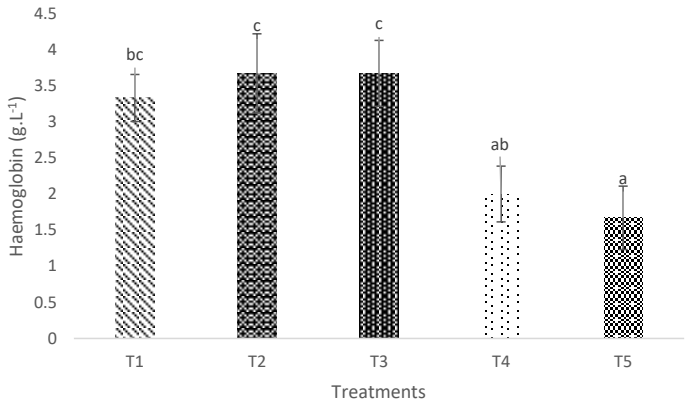

Figure 4. Observation of Haemoglobin Level in common carp Larvae

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The statistical analysis revealed that the common carp's hemoglobin level was significantly ($P<0.05$) affected by the administration of *T. catappa* leaf flour extract. According to the data above, common carp had the highest hemoglobin levels at P3 (100 g/l) at the end of the 30-day study period, and the lowest at P0 (control -), at 1670 g/l.

The hemoglobin content was found to be increased by feeding a flour of *T. catappa* leaf extract on day 7 following the challenge test (day 30 of rearing) with A. hydrophylla. P3 (100 g L-1) had the greatest hemoglobin level at 3670 g L-1, while P0 (control group not fed *T. catappa* leaf extract) had the lowest level at 1670 g L-1 ($P<0.05$). *T. catappa* leaf extract's most likely active components were *T. catappa* leaf extract is said to include tannins, alkaloids, flavonoids, and saponins (Mandasari, 2006). Rinawati (2011) stated that the way flavonoids work is through protein-bound anti-inflammatory hydrogen bonds, which cause disruption to protein structure and disrupt the stability of the cell wall and plasma membrane. This exposes the bacteria to proximity Alkaloid chemicals are among those that have antibacterial properties because they can harm bacterial cell walls, which stops bacteria from proliferating (Saifudin, 2006). Tannins have an antibacterial effect because they precipitate proteins. The antibacterial action of tannins usually involves reactions with cell membranes, deactivation of enzymes, and deactivation of the functions of the genetic material of bacteria. Robinson (1995) explains that saponins can clean and act as an antiseptic agent that kills or prevents the growth of microorganisms in a wound so that the wound does not develop a serious infection.

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Hematocrit Level

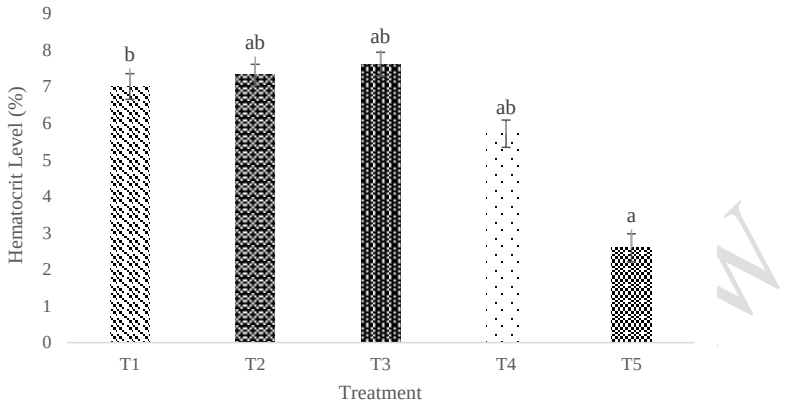

Figure 5. Hematocrit Level Observation in Common carp Larvae

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The hematocrit of common carp larvae was significantly affected (P <0.05) by the administration of *T. catappa* leaf flour extract, according to the statistical analysis results. According to the aforementioned data, the greatest common carp hematocrit after 30 days of rearing in P3 (100 g/l) was found to be 9.607%, while the lowest value was 2.593% in P0 (control -).

On the 7th day of rearing, the carp's average hematocrit ranged from 6.66 to 1.58%. The P1 treatment period had the highest hematocrit level (6.56%), however on day 14 of rearing, the average carp hematocrit level ranged from 9.67% to 2.59%. The P1 therapy group had the highest hematocrit level (9.67%), albeit it was still within the normal range. The carp's increased hematocrit suggests that the leaf flour extract of *T. catappa* can trigger the hematocrit-increasing reaction.

According to Santika et al. (2009), giving fish leaf flour extract from *T. catappa* tends to cause a reaction that manifests as an increase in hematocrit. This rise in these blood cell components therefore causes a quicker rise in particular defense responses (antibodies). Carpal hematocrit is reported by Shabirah et al. (2019) to range from 21 to 44%, however, Soltanian et al. (2018) reported that carp typically have a hematocrit of 40%.

Phagocytotic activities

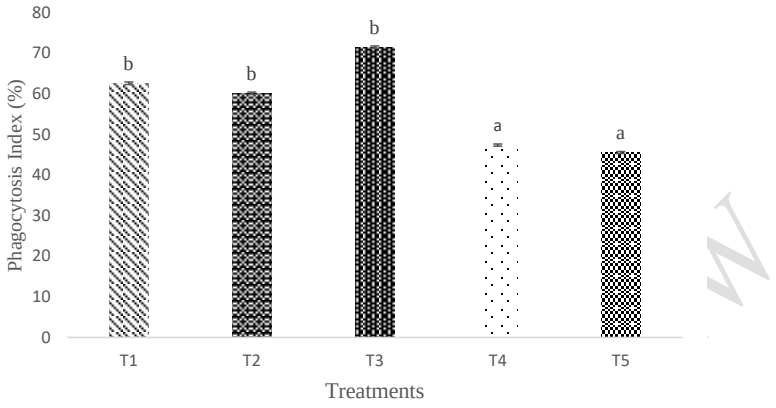

Figure 6. Observation of Phagocytosis Index of Common carp Larvae

The statistical analysis revealed that the phagocytotic activity of common carp larvae was significantly affected (P <0.05) by the administration of *T. catappa* leaf flour extract. According to the statistics above, after 30 days of raising, the maximum common carp phagocytotic activity was 3670% in P3 (100 g/L), while the lowest was 1670% in P0 (control).

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Following the A. hydrophylla challenge test, the phagocytosis index demonstrated that feeding *T. catappa* leaf flour extract may raise the index. At P3 (100 g L-1), the phagocytosis index was 3670%, the highest, while at P0 (control), it was 1670% ($P < 0.05$).

On the 7th day of rearing, the average phagocytosis index of common carp varied between 60.10 and 42.69%. Phagocytosis index values were highest (65.72%) in the P3 treatment and lowest (42.69%) in the P0 treatment. This demonstrates that compared to other treatments, the administration of 100 g/l (P3) of *T. catappa* leaf flour extract can result in better organism resistance.

On the 14th day of treatment, the average phagocytosis index of common carp ranged from 62.57 to 45.52 g/dl. Phagocytosis index values were highest (71.48%) in the P3 treatment and lowest (42.52%) in the P0 treatment. This demonstrates that administering 1000 g/L (P3) of *T. catappa* leaf flour extract can increase the organism's resistance more than other treatments.

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Survival Rate

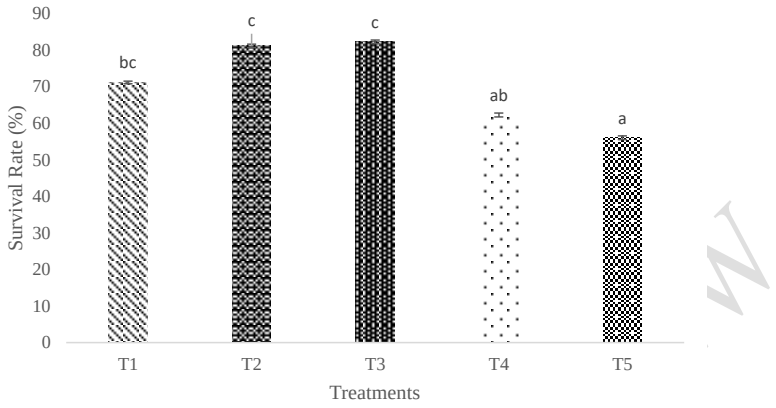

Figure 7. Survival of Common carp Larvae (*C. carpio* L.)

Tilapia were raised for 21 days, and the survival rate revealed variation between treatments. Treatment P3 (100 g/L) had the highest percentage of survivorship (82.22%), followed by P2 (800 g/L) at 81.11%, P1 at 71.11%, control at 62.22%, and lowest control at 56.67%. Still, statistical analyses revealed no statistically significant difference in the effects of *T. catappa* leaf extract administration treatments (P>0.05).

The average survival rate for common carp during the trial was 97%. Based on observations, the fish died during the study because the fish were still adjusting to their new surroundings in the rearing media when the study began. Since good survival rates are also correlated with ideal water quality, it is believed that the quality of the water used as a rearing medium affects survival rates. The impact of *T. catappa* leaf extract on the survival of betta fish (*Betta* sp.) in an experiment with *A. hydrophila* was reported by Nugroho et al. (2017) and Nugroho et al. (2016a). According to research, the percentage of Betta fish that survived after being exposed to *A. hydrophila* ranged from 60% to 100%.

Water quality

The water temperature fluctuates between 25 and 30, which is classified as the normal limit for common carp growth. Based on the requirements of SNI (2016), the optimal temperature for the common carp growth is between 25-30 °C, where the water temperature of that water affects the appetite of the fish, while based on the measurement results, the pH is between 7.50-30. °C. 8.52. According to Kordi (2009), the optimum pH is 6.5-8.5. Water quality indicators are still normal and in good condition during cultivation.

Table 1. Water quality parameters during the rearing period of common carp (*C. carpio* L)

Parameters	Measurement Results					Optimum Range
	P1	P2	P3	P4	P5	
Temperature (^{OC})	25,8 - 26,6	25,4 - 26,5	25,1 - 27,0	24,9 - 26,3	25,7 - 26,7	25-3 (SNI 8296.4:2016)
pH (-log H^+)	7,81 - 7,99	7,81 - 8,03	7,86 - 7,75	7,84 - 7,85	7,88 - 7,87	6,5-8,5 (SNI 8296.4:2016)

Commented [AT29]: The standard deviation of the data in this figure needs to be corrected.

Commented [AT30R29]: The increase of several parameters of larval immunity also plays an important role in its ability to survive A. hydrophila bacterial infection. You can relate it to previous data.

According to the above table, fish survival, growth, and reproduction are impacted by water temperatures in this range because they have an impact on metabolic processes (Effendie, 2003). Higher temperatures can decrease dissolved oxygen and fish appetite, but lower temperatures slow down meal digestion and increase it (Aliyas et al., 2016). (Kelabora, 2010). The acidity of pond water can be determined by looking at the pH value. Temperature is one of the many factors that greatly affect this pH level. A digital pH meter was used for the daily pH measurements in this investigation. The measurement findings indicated that the pH range was 7.50 - 8.52. The ideal pH range, according to Kordi (2009), is 6.5 to 8.5. Based on the literature study results, the water quality indicators (pH 6.5–8.5 and temperature 25–30 °C) are still normal and in good condition during the rearing period (SNI, 2016).

Conclusions

Adding *T. catappa* leaf flour extract can increase the immune response of common carp larvae. *T. catappa* leaf flour extract applied through soaking can increase the resistance and survival of common carp (*C. carpio L*) against *A. hydrophila.* The concentration of *T. catappa* leaf flour extract that is effective for controlling MAS disease in common carp larvae (C. carpio L) at a dose of P3 (1000 g L^{-1}).

Based on the results of the above research, suggestions can be given from this research: to produce maximum results to increase the immune response in common carp larvae by using fish sizes > 2cm.

Reference

Commented [AT31]: It is best to use the most recent references, at least the last 10 years.

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