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Administration of Different Doses of Basil Leaf Extract (Ocimum Sp.) as a Preventive Measure Against Disease in the Survival of 7-Day-Old Pearl Catfish (Clarias Gariepinus) Seeds

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ABSTRACT

This study aims to determine the ability of basil leaf extract as a disease prevention measure in pearl catfish seeds aged 7 days. The research was conducted using experimental methods. The experimental design used was a Completely Randomized Design (CRD) with one factor, namely the difference in the dose of basil leaf extract, which consisted of six treatments with four replicates. The treatment tested was the difference in the dosage of basil leaf extract, namely treatment A (0 mL), B (20 mL), C (30 mL), D (40 mL), E (50 mL), and F (60 mL), in 3 L of rearing water. The data were analyzed using ANOVA. The test animals used were pearl catfish (*Clarias gariepinus*) aged 7 days that were indicated to be sick. The results of the study showed that the administration of basil leaf extract had a significant effect as a disease prevention measure on the survival of pearl catfish seeds aged 7 days, with the highest survival rate reaching 82.5%. The optimal dose of basil leaf extract as a disease prevention measure was the administration of 30 mL/3 L of basil leaf extract (Treatment C). Water quality data were still supportive of the growth of catfish seeds, with pH values ranging from 6.08–7.08, dissolved oxygen ranging from 3.07–4.00 mg/L, and a temperature of around 27.7 °C, which is still within the water quality standard for fish rearing, except for ammonia levels ranging from 0.13–0.44 mg/L, which exceed the optimum water quality standard.

Keywords: catfish seeds; basil leaf extract; survival; preventive.

INTRODUCTION

Catfish hatcheries are one of the activities that are key to the success of catfish aquaculture operations (Suriyadin, 2023). One of the problems in current catfish cultivation activities is the larval maintenance process. The critical period of catfish occurs seven days after stocking (BBPPK, 2023). Pearl catfish (Peer Quality) is a leading strain developed through research by the Fish Breeding Research Institute (BRPI). The breeding process of pearl catfish has been carried out since 2015 through a series of trials. Pearl catfish are produced from the selective crossing of four African catfish strains in Indonesia, namely Egyptian catfish, Python catfish, Sangkuriang catfish, and Dumbo catfish. Pearl catfish also have relatively high resistance to diseases. This superior fish also has relatively high productivity at the grow-out stage, reaching 20–70 percent higher than other strain seeds (BPP-SDM-KP, 2021).

Efforts to prevent diseases in catfish seeds can be carried out through measures such as controlling aquaculture water quality or implementing preventive fish health management. Preventive measures in fish are a series of proactive efforts made to prevent disease, stress, deterioration, or death in fish before problems occur (Assefa et al. 2018). This approach focuses on proper aquaculture management practices to create a healthy environment and reduce potential risks. One preventive measure for fish diseases involves the use of natural ingredients

that contain antimicrobial compounds. The main advantage of using natural ingredients is their ability to reduce the risk of antimicrobial resistance, which is a major problem associated with the use of synthetic antibiotics (Telaumbanua et al., 2023). A natural ingredient that can be used as a preventive measure for fish diseases and is easy to obtain is basil leaves (*Ocimum sp.*). Farag et al. (2017) revealed that this oil can inhibit the growth of fish pathogenic bacteria, with 81% growth inhibition against *Aeromonas hydrophila* and 77% against *Edwardsiella tarda*. Basil leaf extract is effective in inhibiting the growth of *Aeromonas salmonicida* bacteria *in vitro*, with a very strong inhibition classification at a concentration of 100%, showing an inhibition zone of ±21.3 mm (Mahendra et al., 2021). Ballo et al. (2021) reported that a 70% ethanol extract of basil leaves (*Ocimum sanctum* L.) had antibacterial activity against *Staphylococcus aureus*, with Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) values of 80% and 100%, respectively. Batista et al. (2023) added that in their study, OEFOb, ES, and ES/β-CD showed significant antibacterial activity against *S. aureus* ATCC 25923 and *Escherichia coli* ATCC 25922 strains that are resistant to various drugs, in both *in vitro* and *in vivo* experiments. Basil leaves contain essential oils and flavonoids with antibacterial properties. Essential oils extracted from *Ocimum* using UEA (Ultrasound-Assisted Hydrodistillation) have been shown to be effective against a wide range of pathogenic microorganisms such as *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Salmonella enteritidis*, with OB (*Ocimum basilicum*) and OG (*Ocimum gratissimum*) showing very strong activity (Snebo et al., 2022). Basil leaf aqueous extract also shows strong growth inhibition against *Vibrio parahaemolyticus*, producing an inhibition zone diameter of 15 mm (Harlina et al., 2023).

The application of basil leaf extract has been proven to control diseases in aquaculture farming. Harlina (2022) reported that diseases in vanname shrimp can be prevented using the natural ingredient basil leaves (*Ocimum sp.*), which have been proven safe for use as a natural antibacterial source in the treatment of vanname shrimp diseases to increase aquaculture production. The results of egg soaking in basil extract at a concentration of 60 ppm showed the best results, with a fungal attack rate (prevalence) of 28.67%, egg hatchability of 71.33%, and survival of 84.13%. The optimal concentration of basil leaf extract to prevent *Saprolegnia* fungal attack and improve the hatchability of Dumbo catfish eggs is 66.5 ppm (Hasan et al., 2016). Rukka et al. (2022) also reported that the administration of basil leaf extract can reduce the prevalence of fungal infections in goldfish eggs, thereby affecting their hatchability. The best dose of basil leaf extract, which is 65 mg/L, can reduce the prevalence of fungal infection to 15%, resulting in carp egg hatchability of 85%. Putri et al. (2024) also reported that basil leaf extract at a concentration of 60 ppm produced the highest hatchability of carp (*Osphronemus goramy*) eggs at 86.67%. Basil leaf extract can also stimulate the immune system in fish and inhibit bacterial prevalence. Rahmania et al. (2023) reported that basil leaf juice at a dose of 25 mL/kg can enhance the immune response and inhibit *Aeromonas salmonicida* infection in Nilem fish (*Osteochilus hasselti*), with erythrocyte counts of 3.87×10^6 cells/mm³, hemoglobin (Hb) levels of 7.8 g/dL, hematocrit (HCT) values of 20.43%, platelets (PLT) of 37.67×10^3/mL, and leukocytes (WBC) of 53.61×10^3 cells/mm³.

Efforts to minimize mortality during the maintenance of fish seeds can utilize basil leaves (*Ocimum sp.*), which have been proven safe for cultivated organisms. Pearl catfish (*Clarias gariepinus*) is a type of catfish with relatively high productivity. Based on this, a study was conducted on the administration of basil leaf extract (*Ocimum sp.*) at different doses as a disease prevention strategy to improve the survival of 7-day-old pearl catfish (*Clarias gariepinus*) seeds.

The urgency of this research is underscored by the practical needs of small-scale catfish hatcheries, which form the backbone of rural aquaculture in many developing countries. These hatcheries often lack access to, or cannot afford, expensive synthetic drugs and therefore

require affordable, locally available solutions. Basil is easily cultivated and readily available, making its extract a highly accessible and low-cost input. Establishing a scientifically validated protocol for using basil leaf extract as a preventive bath treatment for catfish larvae would provide hatchery operators with a simple, affordable, and sustainable tool to reduce mortality, improve seed production, and enhance their livelihoods. This aligns with the broader goals of promoting sustainable aquaculture practices and reducing reliance on chemical inputs.

The novelty of this study lies in its specific focus on the application of basil leaf extract (*Ocimum sp.*) as a direct bath treatment for 7-day-old pearl catfish seeds, a critical and underexplored life stage for this strain. While previous research has established the antimicrobial properties of basil and its application in other contexts (egg treatment and feed additives for older fish), this study is the first to systematically evaluate a range of doses (0, 20, 30, 40, 50, and 60 mL per 3 liters of water) administered directly into the larval rearing water over a 30-day period. This approach directly targets the larval environment, potentially providing immediate protection against waterborne pathogens. By using pearl catfish (*Clarias gariepinus*), a commercially important improved strain, the research provides directly applicable knowledge for hatchery managers. The study not only assesses survival outcomes but also monitors critical water quality parameters (pH, dissolved oxygen, temperature, and ammonia) to ensure that any observed effects are attributable to the extract's biological activity rather than extract-induced deterioration of the rearing environment.

The primary purpose of this research is to investigate the efficacy of basil leaf extract (*Ocimum sp.*) administered as a bath treatment in improving the survival rate of 7-day-old pearl catfish (*Clarias gariepinus*) seeds. The specific objectives are: (1) to determine the effect of different doses of basil leaf extract on larval survival; (2) to identify the optimal dose that maximizes survival; and (3) to monitor key water quality parameters throughout the experiment to assess their potential influence on the outcomes. The contribution of this research is multifaceted. Academically, it fills a critical gap in the literature on larval health management in catfish aquaculture, particularly for improved strains. Practically, it provides evidence-based recommendations for hatchery operators, offering a simple, natural, and cost-effective method to enhance larval survival. By promoting the use of locally available natural resources, this study supports the development of more sustainable and resilient aquaculture systems, contributing to food security and rural livelihoods. The findings are expected to form the basis for developing standardized protocols for the preventive use of basil extract in catfish hatcheries, reducing mortality, increasing seed production, and minimizing the need for synthetic chemicals.

METHOD

This research was carried out on November 1-30, 2025 at the Fish Seed Center (BBI) of the Sidoarjo Fisheries Office, Wadung Asih Village RT 10 RW 03, Buduran District, Sidoarjo Regency. The test animals in the study were pearl catfish (*Clarias gariepinus*) 7 days old which was indicated to be unhealthy with the characteristic of catfish seeds being at the bottom of the pond and swimming passively when they were about to be taken, as well as being on the surface of the water and staying/standing. Another ingredient used is basil leaves (*Ocimum* sp.). The equipment used includes: pH meter (pH Pan-02), temperature thermometer (rod thermometer), DO meter (Brand AZ-8403), Spectrophotometer (Brand HACC – DR 3900), blender (Cosmos CB-190), maintenance tub, filter, syringe and other glassware.

The research was conducted using experimental methods. The experimental design used was the Complete Random Design (RAL) which consisted of six treatments and four replicates. The treatment tested was the administration of basil leaf extract with different doses, namely; treatment A (0 mL), B (20 mL), C (30 mL), D (40 mL), E (50 mL), and F (60 mL) treatment on 3 liters of water media.

Research Procedure

The first is the preparation of a maintenance tub filled with water media and basil leaf extraction. Extracting basil leaves is done by taking 1 kg of basil leaves plus 1 liter of water, then crushed with a blender at a speed of 10,000 – 11,000 rpm for 15 minutes. The basil leaf extract is then macerated at 50oC for 30 minutes, and further filtered to obtain a pure extract of basil leaves.

The second is to prepare the test animals. The 7-day-old pearl catfish seeds that will be used in this study are put into a maintenance container. Seed sowing is carried out in the morning at 08.00 – 09.00 WIB to avoid overheating due to sunlight.

The third is the implementation of research, starting with putting 20 7-day-old pearl catfish seeds into a maintenance tub that has been filled with 3 liters of water. The maintenance tub is placed in an *in-door* laboratory with *a* random experimental layout. The administration of basil leaf extract is carried out with a frequency of administration every week of 2 times according to the treatment dose. Catfish seeds are kept for 30 days. After 30 days of maintenance, the survival of pearl catfish seeds and water quality parameters of the maintenance media were then observed.

Survival Observation

Observation of the survival rate of catfish seeds was carried out by monitoring the number of surviving catfish seeds from the beginning of the treatment period to the end of treatment. To calculate survival or survival, the formula used by Rumondang (2023) can be used, as follows:

$$S = \frac{N_t}{N_o} X\ 100\%$$

Description:
S = Survival (%)
Nt = The number of biota at the time of harvest (tails)
Yes = Number of biota at the beginning of stocking (tail)

Water Quality Observation

As a support and control during the research, observations were made on water quality parameters which included measurements including temperature (bar thermometer), pH (pH Meter), dissolved oxygen (DO Meter) and ammonia (Spectrophotometry) at the beginning and end of the study.

Data Analysis

To determine the effect of treatment on the observed parameters, the data was analyzed using One Way Anova Fingerprint Analysis. If the results of the fingerprint analysis show that there is a difference between the treatments, then it is followed by the Duncan test with a test level of 95%.

RESULTS AND DISCUSSION

Survival of Pearl Catfish Seeds

The results of the one-way fingerprint analysis obtained a significance value of 0.0001 (< 0.05), which showed that there was a difference in the concentration of basil leaf extract on the percentage of survival of pearl catfish seeds. These results prove that the administration of basil leaf extract can increase the survival of unhealthy pearl catfish seeds compared to controls without the administration of basil leaf extract. Proper use of basil leaf extract (usually through soaking or mixed feed) can provide antibacterial protection, reduce stress, and increase the immune system of catfish seeds, which directly leads to increased survival. Basil leaves also have pharmacological properties due to the presence of polyphenols, phenolic acids, and flavonoids, which are antioxidant, antimicrobial, and anti-inflammatory (Romano *et. al*., 2022;

Noorbakhsh *et. al*., 2024). Basil leaves also contain nutrients that can help maintain the survival of pearl catfish seeds. As explained by Astawan (2011) that the nutritional content of basil includes provitamin A, vitamin C and rich in macrominerals namely calcium, phosphorus, iron and magnesium. The results of the average percentage of survival of pearl catfish seeds are presented in Figure 1.

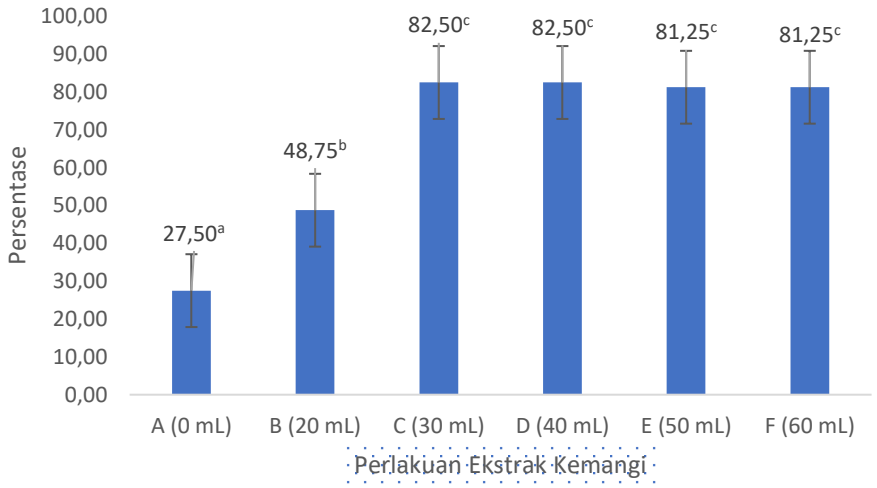

Figure 1. Average survival rate of 7-year-old catfish during 30 days of rearing (Note: different notations behind the mean number indicate a difference between treatment and the 95% test level Duncan test)

Figure 1 shows the lowest survival rate in treatment A (basil leaf extract 0 mL), which is 27.50% with a mortality rate of 73.50%. This is due to the fact that treatment A does not contain antimicrobial compounds, so pearl catfish that are unhealthy as test animals cannot survive. Treatment B (basil leaf extract 20 mL) with a survival percentage of 48.75% was significantly different from treatment A. The survival of pearl catfish seeds in Treatment C (30 mL basil leaf extract) increased with a survival percentage of 82.50% with a mortality rate of 17.50%. Furthermore, with the addition of the dose of basil leaf extract in Treatment D, E and F of 40, 50 and 60 mL, respectively, showed a different difference. Based on this, Treatment C (basil leaf extract 30 mL) has the maximum effect in its effect on the survival of pearl catfish seeds. This is due to the treatment of B, C, D, E and F already containing bioactive compounds that are antimicrobial that can kill disease microbes in pearl catfish seeds, both bacteria and fungi, so that they can increase the survival of pearl catfish seeds.

Several reports state that basil leaf extract can inhibit the growth of bacteria such as *A. salmonicida* (Farag *et. al*., 2017; Mahenda *et al*., 2021; and Rahmania *et. al*., 2023), *E. tarda* (Farag *et. al*., 2017), *S. aureus* (Ballo *et. al*., 2021; Snebo *et al*., 2022; dan Batista *et al*., 2023), *E. coli* (Snebo *et. al*., 2022; dan Batista *et. al*., 2023); *P. aeroginosa* and *S. enteritidis* (Snebo *et. al*., 2022), *V. parahaemoliticus* (Harlina *et. al*., 2023), *Streptococcus mutants* (Sari *et. al*., 2022) and *Yersinia nuckeri* (Noorbakhsh *et. al*., 2024). Basil leaf extract can also prevent fungal attacks thereby increasing the survival of fish seeds (Rukka *et. al*., 2022). Hasan *et. al.* (2016), reported that basil leaf extract can prevent Saprolegnia *fungal infestation* and increase the hatchability of dumbo catfish eggs. Increasing the survival of catfish seeds along with the addition of basil leaf extract can also be caused by basil leaf extract having an immune effect. As reported by Rahmania *et. al*. (2023), that basil leaf juice with a dose of 25 mL/kg has the ability to help the immune system and inhibit *A. salmonicida* bacterial infection in Nilem fish (*Osteochilus hasselti*). Noorbakhsh *et. al*. (2024) also reported that the addition of 2% basil

extract to rainbow trout fish feed rations can improve physiological function and growth outcomes and strengthen the immune system of fish from *Y. nuckeri* strain. Basil leaf extract can also serve as an anesthetic capable of recovering fainting fish and improving fish survival, as reported by Faqihudin et al. (2023), who proved that the administration of 20% basil extract was able to recover fish that fainted for 25.74 minutes, with a recovery time of 6.13 minutes, and catfish survival of 75%. Maryani *et al*. (2022) also reported that basil leaf oil (*Ocimum tenuiflorum*) can be anesthetic and increase the life expectancy of catfish during transportation and acclamation up to 98-100%.

The content of antimicrobial compounds in basil leaves can also be used as fish preservatives, as reported by Deviyanti *et. al.* (2015) that a 30% basil leaf concentration is able to suppress the growth of rotting bacteria up to 3.36 – 6.79 CFU/g so that it is able to maintain the quality of male mackerel (*Rastrelliger kanagurta*) until the 12th day during cold storage. Rahmiati *et. al.* (2023) also reports that

Basil leaf extract (*Ocimum sactum* L.) was able to maintain the freshness of mackerel (*Rastrelliger* sp.) for 8 hours at room temperature by reducing the TPC content in the control without basil leaf extract by 55.0 X 101 CFU/g to 19.7 X 101 CFU/g. The addition of basil leaf extract by 20% is the best treatment in the storage of dried salted mackerel with a microbial growth suppression of 9.2 X 105 CFU/g and a Mold Number Mahmir was 9.1 X 10^4 CFU/g during 30-day storage (Damopolli *et al*., 2024).

Water Quality Parameters

The results of the analysis of one-way fingerprint of water quality parameters of treatment maintenance media for pH values, dissolved oxygen (DO = dissolved oxygen), temperature, and ammonia obtained significance values of 0.956, 0.0001, 0.436 and 0.006, respectively. The significance value of pH and temperature showed greater than 0.05, which means that there was no effect of the difference in the concentration of basil extract on the pH and temperature values. The significance value of DO and ammonia levels was less than 0.05, which suggests that there is a difference in the concentration of basil extract on DO and ammonia levels. The results of the average water quality parameters of the maintenance media are presented in Table 1.

Table 1. Water quality parameters of maintenance media

Treatment	Average			
	pH value	DO (mg/L)	Temperature (^{oC})	Ammonia (mg/L)
A (basil leaf extract, 0%)	7.03a	3.07a	27.6a	0.16ab
B (basil leaf extract, 20%)	6.98a	4.00b	27.8a	0.13ab
C (basil leaf extract, 30%)	7.03a	4.03b	27.9a	0.29bc
D (basil leaf extract, 40%)	7.08a	3.98c	27.7a	0.44c
E (basil leaf extract, 50%)	7.05a	3.78c	27.6a	0.17ab
F (basil leaf extract, 60%)	7.03a	3.73c	27.7a	0.07a

Description: The different notations behind the mean number show that there is a difference between the treatment and the 95% test-level Duncan test.

The water medium for the maintenance of catfish seeds in this study uses media from the same source with homogeneous quality between maintenance tanks and has good media requirements for the maintenance of pearl catfish seeds. Maintenance media is maintained and monitored for 30 days during the research period so that there are no changes in the quality of maintenance media that can affect the results of the research. The observed water quality parameters include pH, DO, temperature and ammonia values. Cultivation intensification can be successful without slowing down the growth rate if four environmental factors are monitored, namely temperature, feed, oxygen supply, and metabolic waste (Abulias, 2014).

The pH value is an indicator of the level of acidity or alkaline in the maintenance medium which is assessed on a scale of 0 – 14 and is one of the environmental factors that affect fish growth (Andharani *et. al*., 2016). If the pH concentration value of a cultivation medium is low, it can affect the reduced oxygen content and increase respiratory activity, thus affecting the appetite of aquatic organisms. In addition, the low pH value of a water can spur the growth of pathogenic fungi and bacteria. Based on the results of the anova analysis that the pH showed a difference between treatments, it can be said that the quality of the maintenance media between treatments remained homogeneous, so that it did not affect the results of the monitoring of the survival of pearl catfish seeds. Observers of the pH value during the study ranged from the neutral pH, which was around 6.98 – 7.08. The nilia is at a decent value for catfish cultivation. This is in accordance with SNI 6484.4:2014 in catfish cultivation the optimum water pH value is 6.5-8 (BSN, 2014). Based on a study by the Bandung Regency Fisheries Office, the pH of the water of the edial catfish rearing media ranges from 6 – 9.3 (DP, 2018).

Temperature is a parameter of aquaculture water quality that greatly affects various chemical reactions in the water medium, including affecting the solubility of oxygen and the metabolism of the fish body, so that it will affect the growth of aquatic animals. Based on the results of the anova analysis that the temperature showed a difference between treatments, it can be said that the quality of the maintenance media between treatments remained homogeneous, so that it did not affect the results of the feeding of pearl catfish seeds. The temperature of the maintenance water medium is around 27 – 28oC, the temperature range is suitable for catfish farming, which is in accordance with the Indonesian National Standard (SNI) on the maintenance of catfish flies. Based on SNI number 6484.2:2014, the temperature requirements for maintaining catfish seeds range from 25 – 30oC (BSN, 2014). This is in line with the report of Aidil *et al*., (2016), which confirms that the temperature range between 25 to 31°C is considered optimal for the survival and growth of catfish. Based on a study by the Bandung Regency Fisheries Office, the water temperature of the edial catfish rearing media ranges from 22 – 32oC (DP, 2018).

Based on the results of the anova analysis, the DO content showed a real difference between treatments. The lowest value occurred in Treatment A, which was 3.07 mg/L and the highest occurred in Treatment C with a dissolved oxygen level of 4.03 mg/L. This means that the addition of basil leaf extract can maintain the dissolved oxygen content. Based on the results of dissolved oxygen concentrations ranging from 3.07 – 4.03 mg/L, which still meets the provisions of dissolved oxygen content for the maintenance of catfish fry. The good dissolved oxygen content for catfish cultivation based on SNI 6484.4:2014 is >3 mg/L (BSN, 2014). Based on a study by the Bandung Regency Fisheries Office, dissolved oxygen in catfish rearing media must be >1 mg/L (DP, 2018).

Waste that is very dangerous and toxic to fish is ammonia because it is very dangerous and can trigger toxins or diseases in fish. Based on the results of the anova analysis, the amniak level in the maintenance media showed unreal differences between treatments. This indicates that the quality of maintenance media between treatments remains homogeneous, so that it does not affect the results of the survival of pearl catfish seeds. The results of the measurement of ammonia levels range from 0.16 – 0.44 mg/L. This value needs to be watched out for because it exceeds the optimal value for catfish farming. Based on SNI 6484.4:2014, the optimum value of ammonia for catfish cultivation is <0.01 mg/L. The existence of ammonia, in addition to being influenced by the results of metabolic residues by the fish itself (Ramadhan, 2015), is also influenced by the availability of dissolved oxygen in water bodies. The presence of ammonia in a body of water can affect fish growth because it interferes with the osmoregulation process and results in physical damage to tissues (Pratama *et al*., 2017).

CONCLUSION

The administration of basil leaf extract (*Ocimum sp.*) had a significant effect as a disease prevention measure on the survival of 7-day-old pearl catfish (*Clarias gariepinus*) seeds, producing the highest survival rate of 82.5%. The optimal treatment was the administration of 30 mL of basil leaf extract per 3 L of water (Treatment C), which provided the most favorable survival outcome among the tested doses. Water quality parameters during the experiment generally supported the growth and survival of catfish seeds, with pH values ranging from 6.08–7.08, dissolved oxygen levels between 3.07–4.00 mg/L, and an average temperature of approximately 27.7°C, all of which are within acceptable standards for fish rearing. However, ammonia concentrations ranged from 0.13–0.44 mg/L, exceeding the optimal water quality threshold. Future research is recommended to further investigate the long-term effects of basil leaf extract on fish health and immune response, evaluate its efficacy under improved ammonia management conditions, and explore different extraction methods or application frequencies to optimize its use as a natural disease prevention strategy in catfish hatchery systems.

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