

Evaluating the Impact of Timsen (N-Alkyl Dimethyl Benzyl Ammonium Chloride) on a Spectrum of Water Quality Parameters

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Abstract: The experiment sought to evaluate the effect of Timsen on numerous water quality metrics on the rooftop of the fisheries faculty building from 10 March to 25 March 2023. The trial had four treatments (T₁, T₂, T₃, and T₄), each with three replications, with doses of 0, 1, 2, and 3 g of Timsen per ton of water, respectively. Twelve buckets, each with a capacity of 110 litres, were utilised for this experiment, and each bucket was filled with 100 litres of water. Water quality metrics were systematically documented during the study period. The application of Timsen resulted in elevated levels of pH, dissolved oxygen, nitrite, alkalinity, and hardness, while ammonia levels decreased, indicating its suitability for aquaculture. The maximum pH, dissolved oxygen, nitrite, alkalinity, and hardness were recorded in T₄, with average values of pH 7.96, dissolved oxygen 4.72 mg/L, nitrite 0.11 mg/L, alkalinity 186.6 mg/L, and hardness 195.6 mg/L. The lowest level of ammonia was measured in T₄, with an average reading of 0.048 mg/L. Overall, Timsen raised pH, dissolved oxygen, alkalinity and hardness while reducing ammonia, keeping all parameters within the optimal range for aquaculture and indicating its potential to support enhanced fish production.

Keywords: Aquaculture, Timsen, Water quality parameters.

Introduction

Bangladesh, known for its abundance of rivers and diverse fish and fisheries resources, relies heavily on the fish and fisheries sector for supplying animal protein, foreign exchange revenue, employment opportunities, food security and poverty reduction. Aquaculture has emerged as a crucial sector in the global food production system. In Bangladesh, the total fish production is 46.21 lac Metric Tons (MT), while pond aquaculture contributes 21.66 lac MT (DoF, 2023). Aquaculture accounts for 3.57% of our national GDP and contributes more than a quarter (26.5%) to the agricultural GDP (DoF, 2022). It results in a 2.1% rise in earnings in Bangladesh and lifts over eighteen million people out of poverty (Rashid et al., 2019). Fish and fishery products constitute about 60% of animal protein (Sayed *et al.*, 2018).

However, with the shift towards commercial fish culture, increasing stocking densities and the use of commercial feeds, managing water quality and maintaining a suitable culture environment has become challenging, resulting in heightened susceptibility to diseases. Aquaculture employs a range of substances beyond antibiotics, including growth promoters, hormones, zeolite, feed additives, and lime, to ensure optimal water quality and enhance fish health and productivity (Kawsar *et al.*, 2022). There are various commercial water quality treatment drugs available in the market. The most commonly employed drugs and chemical products are Timsen, Geofresh, Geoprime, Geotox, and Virex. The

excessive and indiscriminate utilization of aqua-drugs and chemicals can trigger various issues such as resistance to medicines, tissue debris, and negative impacts on biodiversity, and eventually affect the cultured species, humans, and the surroundings. As fish are cold-blooded organisms, their development, reproduction, maturation, survival, and productivity are predominantly influenced by the state of the water (Sorower *et al.*, 2020).

Timsen is a combination of 40% n-alkyl dimethyl benzyl ammonium chloride and 60% stabilized urea, an ideal disinfectant, which is used in aquaculture pond water to kill all types of microorganisms (Faruk *et al.*, 2008). It is used as a preventive measure to control all types of diseases. The 40% n-alkyl dimethyl benzyl ammonium chloride, a component of Timsen, also known as benzalkonium chloride. This compound serves as effective biocidal agents that act quickly and maintain their efficacy for a moderate duration. They exhibit activity against bacteria, certain viruses, fungi, and protozoa. Bacterial spores are regarded as highly resistant. Gram-positive bacteria tend to be more vulnerable compared to Gram-negative bacteria. Water quality parameters are essential in aquaculture, significantly affecting fish growth, spawning, maturity, survival, and production, particularly through the influence of water temperature. The quality of water, defined by its physico-chemical and biological traits, significantly influences plankton efficiency, the biology of propagated organisms, and ultimately, the yields. Water quality influences the species best suited for cultivation in various environments (Dhawan and Karu 2002). Good water quality imparts a favorable environment for fish growth while poor water quality will lead to fish diseases, slower growth and even a large number of deaths (Jino Ramson *et al.*, 2019). This study is focused on investigating the impact of Timsen on various water quality parameters in aquaculture.

Materials and Methods

Study area

The research was performed on the rooftop of the Faculty of Fisheries Building and at the Fish Pathology and Nutrition Laboratory inside the Department of Aquaculture at Patuakhali Science and Technology University. The chemical structure and key features of Timsen are displayed in Figure 1.

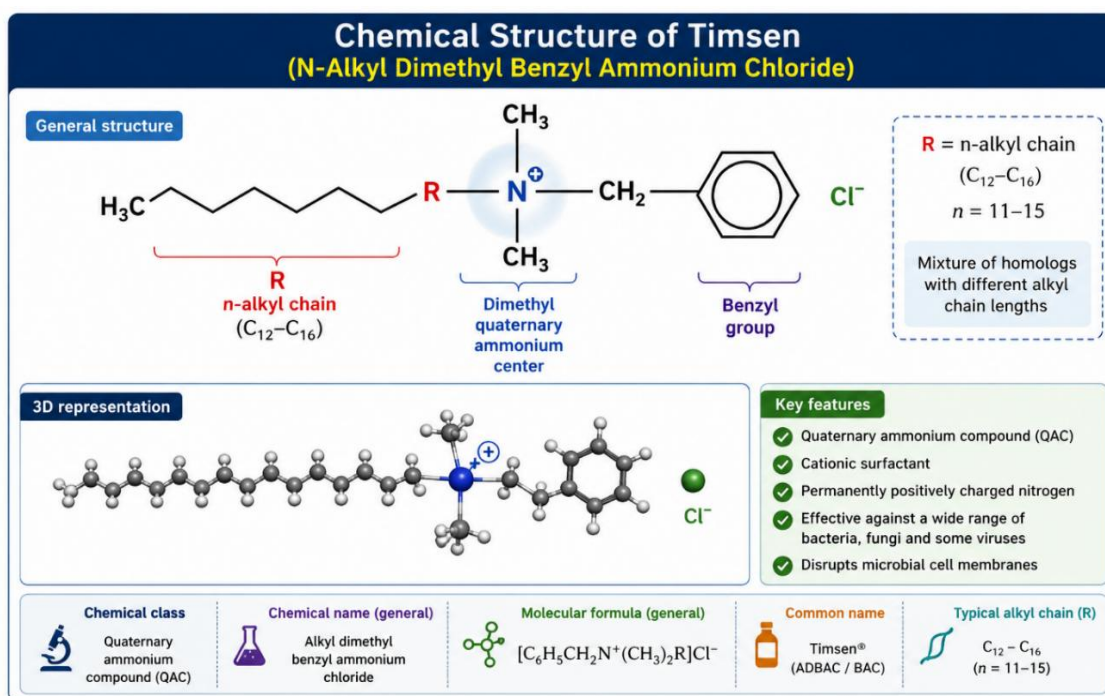


Figure 1. Chemical structure and features of Timsen.

Experimental design

The experiment was conducted with four treatments: 0 g, 1 g, 2 g, and 3 g of Timsen per ton of water. Each treatment consisted of three replications, making a total of twelve buckets for the experiment. Each bucket had a water-holding capacity of 110 litres and was filled with 100 litres of water. The experimental design is depicted in Figure 2.

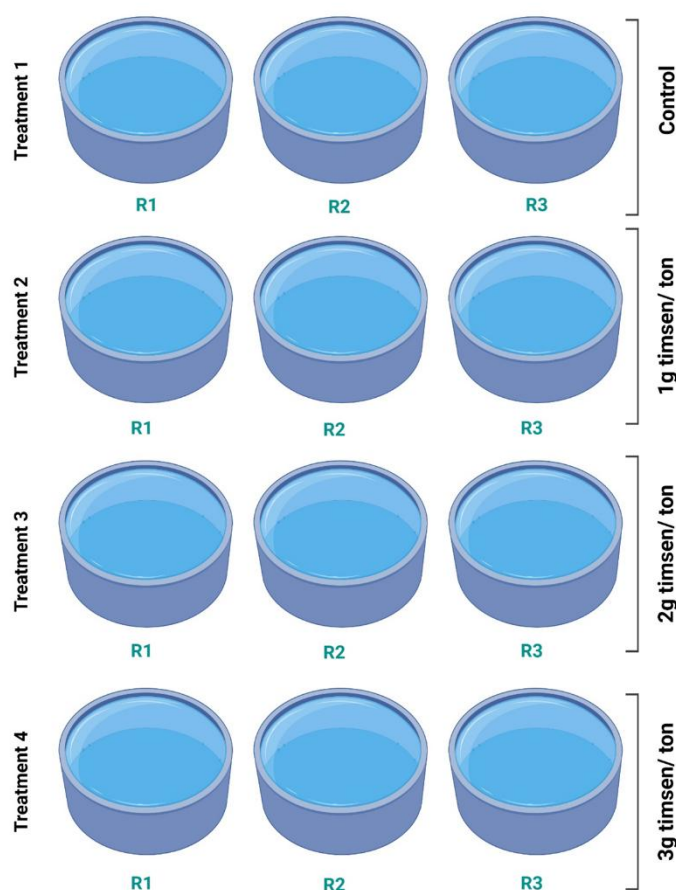


Figure 2. Schematic representation of the experimental design showing four treatments including Control, 1 g Timsen/ton, 2 g Timsen/ton, and 3 g Timsen/ton with three replicates (R1, R2, R3), arranged in a completely randomized design (CRD).

Duration of study

This research was conducted from 10 March to 25 March 2023 to examine the alterations in multiple water quality metrics resulting from the application of Timsen in different dosages.

Application of Timsen

The weighed of Timsen and the water quantities in each bucket have been estimated in accordance with the experimental design (Figure 2). Each bucket was filled with 100 litres of water, and weighed Timsen was added directly to the buckets and swirled gently until thoroughly combined.

Water quality parameters measurement

The parameters of water quality were determined every day at a certain time during the study period by using experimental instruments and reagents. Data were collected on a daily basis during the

experimental period, specifically from 4:00 to 5:00 pm. To measure the water temperature, a portable thermometer was used. Water pH was measured with a pH meter. Dissolved oxygen was measured using HANNA instruments and reagents provided in the kit (HI 3810 Dissolved Oxygen Test Kit). Ammonia was determined using HANNA instruments and reagents provided in the kit (HI 3824 Ammonia Test Kit), and nitrite was determined using HANNA instruments and reagents provided in the kit (HI 3873 Nitrite Test Kit). Alkalinity was determined using HANNA instruments and reagents provided in the kit (HI 3811 Alkalinity Test Kit), and hardness was also determined using HANNA instruments and reagents provided in the kit (HI 3812 Hardness Test Kit) according to the instruction manual (Acosta-Pérez *et al.*, 2022).

Data analysis procedure

The collected data were analyzed each time after measurement and compared with previous data to determine the changes in water quality parameters. All data were input into Microsoft Excel software to compare the mean differences. Ordinary one-way ANOVA analysis was done using GraphPad Prism v9.5.1 (Shafiujjaman *et al.*, 2024). We used the Pearson correlation approach to find out how temperature, pH, dissolved oxygen, nitrite, alkalinity, hardness, and ammonia (which are all water quality factors) are related to each other (Figure 3).

Results and Discussion

Effect of Timsen on water quality parameters

Throughout the experimental phase, water quality was regularly measured. This was done daily for each replication of every treatment at a specific time. The findings of the current experiment concerning the influence of Timsen on various water quality parameters, including temperature, pH, dissolved oxygen, ammonia, nitrite, hardness, and alkalinity, are illustrated in Table 1.

Table 1: Water quality parameters (mean $\pm$ SD) across treatments during the experimental period.

Parameters	Treatments (T)				p-value
	T ₁ (Control)	T ₂	T ₃	T ₄	
Temperature (°C)	28.27 $\pm$ 0.506 ^a	28.27 $\pm$ 0.506 ^a	28.27 $\pm$ 0.506 ^a	28.27 $\pm$ 0.506 ^a	1.0
pH	7.190 $\pm$ 0.088 ^c	7.460 $\pm$ 0.143 ^b	7.760 $\pm$ 0.143 ^a	7.960 $\pm$ 0.143 ^a	<0.001
DO (mg/L)	3.960 $\pm$ 0.143 ^c	4.180 $\pm$ 0.175 ^{bc}	4.450 $\pm$ 0.222 ^{ab}	4.720 $\pm$ 0.175 ^a	<0.001
NH ₃ (mg/L)	0.296 $\pm$ 0.005 ^a	0.088 $\pm$ 0.010 ^b	0.078 $\pm$ 0.010 ^b	0.048 $\pm$ 0.010 ^c	<0.001
Nitrite (mg/L)	0.000 $\pm$ 0.000	0.000 $\pm$ 0.000	0.040 $\pm$ 0.052 ^a	0.110 $\pm$ 0.088 ^a	<0.001
Alkalinity (mg/L)	151.7 $\pm$ 1.636 ^b	174.6 $\pm$ 8.099 ^a	180.6 $\pm$ 8.099 ^a	186.6 $\pm$ 8.099 ^a	<0.001
Hardness (mg/L)	170.1 $\pm$ 1.912 ^b	186.6 $\pm$ 8.099 ^a	192.6 $\pm$ 8.099 ^a	195.6 $\pm$ 8.099 ^a	<0.001

In each row, values with the same superscript indicate no significant difference ($P > 0.05$), and values with different superscripts indicate a significant difference ($P < 0.05$).

In Bangladesh, fish farmers employ various chemicals on their farms to enhance growth, improve water quality, and eradicate diseases. Commonly employed chemicals include antibiotics, growth promoters, disinfectants, lime, salt, insecticides, fungicides, microbe killers, and oxygen suppliers, among others. The application of chemicals has been on the rise alongside the growth of aquaculture in the realm of aquatic health management. The commonly utilised substances include lime, rotenone, various types of inorganic and organic fertilisers, phostoxin, salt, dipterex, antimicrobials, potassium permanganate, copper sulphate, formalin, sumithion, malathion, and others. The presence of these chemicals and drugs

influences the parameters of water quality. This study aimed to assess the effects of Timsen on various water quality parameters.

Temperature significantly influences the fish's essential physiological activities, including breathing, growth, feeding, migration, and reproduction. It is inversely related to dissolved oxygen (Ekubo and Abowei, 2011). Throughout the study period, the average temperature under the different treatments was $28.27 \pm 0.506^\circ\text{C}$, which was equal for each treatment (Table 1). There was no significant ($P > 0.01$) difference in water temperatures among the treatments. The recorded temperature was more or less similar to (Boyd and Pillai, 1985) for freshwater fish culture. (Sorower *et al.*, 2020) observed that Timsen maintained water temperature $28.25 \pm 3.38^\circ\text{C}$, which was suitable for fish culture. (Swann, 1997) described the suitable ranges of water temperature for warm water species as 23.9 to 32.22°C . The observed water temperature in the experiment showed that there was no effect of Timsen on water temperature in different treatments.

The average pH values observed in this study were 7.19 ± 0.14 , 7.46 ± 0.14 , 7.76 ± 0.14 , and 7.96 ± 0.14 for T_1 , T_2 , T_3 , and T_4 , respectively (Table 1). The lowest average pH was found 7.190 ± 0.088 in T_1 and the highest average pH was found 7.960 ± 0.143 in T_4 . The pH levels in T_2 , T_3 , and T_4 were significantly higher ($P < 0.01$) compared to T_1 . There was no significant difference ($P > 0.01$) between T_3 and T_4 . According to (Alam *et al.*, 2014; El-Shafei, 2016), the recorded pH was suitable for fish culture. It was observed that the pH increased in every treatment that used Timsen throughout the experimental period. The observed pH was higher than (Ezeanya *et al.*, 2015) and more or less similar to (Boyd and Pillai, 1985; Jena *et al.*, 2011; Sorower *et al.*, 2020). The evaluated pH showed that Timsen had a positive significant impact on water quality parameters. The DO level is an indicator of the degree of pollution in aquatic environments. The dissolved oxygen (mg/L) content under different treatments ranged from 3.960 ± 0.143 mg/L to 4.720 ± 0.175 mg/L (Table 1), which were more or less similar to (Bhatnagar and Devi, 2013). The significantly highest ($P < 0.01$) dissolved oxygen content was found 4.720 ± 0.175 mg/L in T_4 , whereas the minimum dissolved oxygen content was 3.960 ± 0.143 mg/L found in T_1 . The DO levels were significantly ($P < 0.01$) elevated in T_4 compared to T_1 and T_2 . However, no significant difference ($P > 0.01$) was observed between T_3 vs T_4 , T_2 vs T_3 , and T_1 vs T_2 . These findings agree with (Bhatnagar and Devi, 2013; Sorower *et al.*, 2020) because of using Timsen, and oxygen level of T_4 was found to be 4.720 ± 0.175 mg/L, which is suitable for fish culture. (Sayed *et al.*, 2018; Karnatak *et al.*, 2021) observed higher DO values, (Davies and Ansa, 2010; Munni *et al.*, 2015) detected lower DO values than our findings. The results showed that Timsen plays a significant role in increasing dissolved oxygen levels in the water bodies of the aquaculture pond.

The average ammonia (mg/L) content under different treatments varied from 0.048 ± 0.010 mg/L to 0.296 ± 0.005 mg/L (Table 1). The highest ammonia content (0.296 ± 0.005 mg/L) was found in T_1 , whereas the minimum ammonia content (0.048 ± 0.010 mg/L) was found in T_4 . The average ammonia values exhibited significant variation ($p < 0.01$) across the various treatments. The excretion quantities of ammonia and urea facilitate the assessment of fish nitrogen balance and serve as valuable indicators for analysing the impacts of environmental and dietary factors on the digestion of proteins (Fournier *et al.*, 2003; Uliano *et al.*, 2010). The resulting ammonia content of each Timsen-treated water treatment was under 0.1 mg/L, which was optimum for fish culture. (Hossain *et al.* 2000) observed that the NH_3 contained in different water bodies like beels and ponds in Natore was higher than our findings, more or less similar to (Boyd and Pillai, 1985; Begum *et al.*, 2014; Davidson *et al.*, 2019; Sorower *et al.*, 2020). The application of Timsen in water bodies significantly reduces the ammonia content from the water bodies.

The average nitrite (mg/L) content under different treatments was found to be 0 to 0.110 ± 0.088 mg/L. The nitrite contents of T₁ and T₂ were 0 throughout the experiment, while the average nitrite content was 0.040 ± 0.052 mg/L in T₃ and 0.110 ± 0.088 mg/L in T₄ (Table 1). (Swann, 1997) described that for suitable warm water species, culture nitrite would be 0.03 to 0.06 mg/L, which was similar to our findings. (Begum *et al.*, 2014; Opiyo *et al.*, 2014; El-Shafei, 2016) also observed similar results to our findings.

The average alkalinity (mg/L) under different treatments varied from 151.7 ± 8.099 to 186.6 ± 8.099 mg/L (Table 1). The highest alkalinity, 186.6 ± 8.099 mg/L, was found in T₄, whereas the minimum alkalinity, 151.7 ± 8.099 mg/L, was found in T₁. The average values of alkalinity under different treatments were more than control T₁ and varied significantly ($p < 0.01$) from each other. The optimum alkalinity for increased fish production would be 20 mg/L to 300 mg/L (Njoku, Agwa and Ibiene, 2015), which was similar to our findings. (Munni *et al.*, 2015; Tumwesigye *et al.*, 2022) detected lower alkalinity than our findings, where (Hossain *et al.*, 2000) observed higher alkalinity than our results. The observed alkalinity was suitable according to (Boyd and Pillai, 1985; Devi *et al.*, 2017).

The average hardness (mg/L) content under different treatments varied from 170.1 ± 1.912 to 195.6 ± 8.099 mg/L (Table 1). The highest hardness content (195.6 ± 8.099 mg/L) was found in T₄, whereas the minimum hardness content (170.1 ± 8.099 mg/L) was found in T₁. The average hardness values under various treatments exhibited significant differences ($p < 0.01$) from each other. Our findings fall within the acceptable range according to (Boyd and Pillai, 1985; Bhatnagar and Devi, 2013).

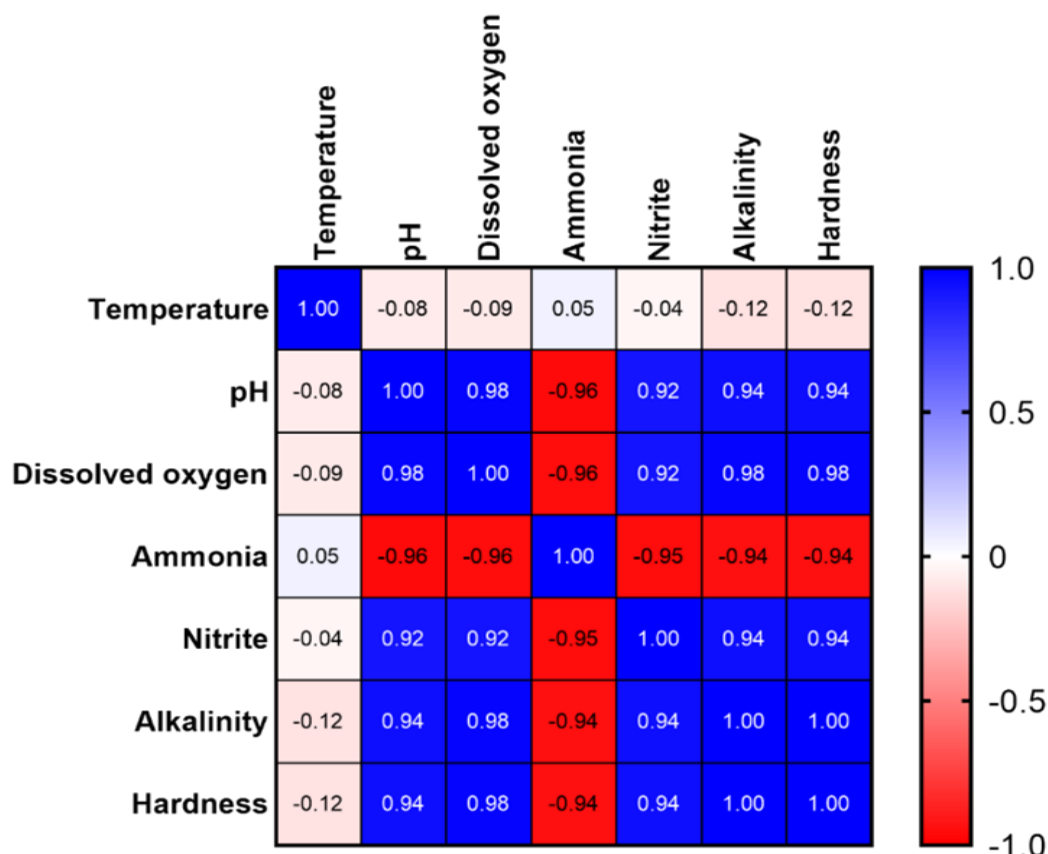


Figure 3. Pearson's correlation of water quality parameters in different treatment groups.

Pearson's correlation matrix reveals several strong associations among the study parameters (Figure 3). Most water quality parameters exhibit strong positive correlations with one another, with coefficients ranging from 0.92 to 1.00, indicating that these parameters tend to increase together. In particular, some

water quality parameter pairs show almost perfect positive relationships ($r = 0.98\text{--}1.00$), suggesting substantial similarity or possible multicollinearity. Conversely, ammonia demonstrates strong negative correlations with several other variables, with coefficients between -0.94 and -0.96 . This indicates an inverse relationship, meaning that increases in ammonia are associated with decreases in the positively correlated parameters. Temperature appears to have very weak negative correlations with the remaining water quality parameters (ranging from -0.12 to -0.04) except ammonia ($r=0.05$), suggesting relative independence and minimal linear association with the other parameters.

Conclusion

This experiment investigated the effect of Timsen on various water quality parameters along with dosing rates of Timsen, that are necessary to achieve optimum water quality parameters for fish culture. It compared different treatments to determine the appropriate quantity of Timsen required for water treatment to attain the desired water quality standards. The findings indicated that Timsen significantly influenced the pH, dissolved oxygen, hardness, ammonia, alkalinity, and nitrite levels in the water. The study revealed changes in pH across different Timsen dose treatments, with an overall increase in average pH. For effective fish culture, it is essential to maintain alkaline conditions, which are crucial for fish survival and enhanced production. Furthermore, it was noted that Timsen positively impacted water quality, resulting in increased dissolved oxygen levels and decreased ammonia concentrations. These findings highlight the necessity of proper management and regulation of Timsen usage to ensure the sustainability of water resources and safeguard fish health. Since Timsen not only supports fish health but also upholds optimum water quality parameters, it is recommended for use in aquaculture for disease control and to maintain these quality standards. Further research is needed to examine the effects of Timsen on the microbiomes of ponds with different doses.

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This study did not obtain any particular funding from public, commercial, or non-profit organizations.

Declaration of Conflict of Interest

The authors affirm that there are no conflicts of interest concerning the publication of this paper.

Ethical Considerations

This study does not involve any human or animal subjects. Consequently, there are no ethical dilemmas or necessities for unique authorizations.

Authorship contribution statement:

Md. Rafiqul Islam: Writing- original draft, reviewing & editing, methodology, data curation, and data analysis.

Farha Talukdar: Methodology, performed experiment, writing- original draft, and data curation.

Mohammad Lokman Ali: Conceptualization, validation, methodology, and funding.

Md. Hafijur Rahman: Reviewing and editing, data curation, and data visualization.

Sudipta Kumar Nag: Data curation, formal analysis, and graph preparation.

Abdullah -Al- Hasan: Reviewing & editing, and resources.

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