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The Effect of the Tuba Root (*Derris elliptica*) Extract on BOD, COD, pH, and Microbial Population in Brackish Pond Water

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ABSTRACT

The application of tuba plant roots (*Derris elliptica*) as a piscicide has implications for water quality and microbial population within aquatic environments. This study aimed to evaluate the influence of rotenone of the tuba root extract on BOD, COD, pH, and microbial population in brackish pond water. The methodology involved the extraction of tuba roots using chloroform, followed by the quantification of rotenone concentration through GC-MS analysis. The experiment included using brackish pond water treated with different concentrations of tuba root extract: T1 at 10.000ppm, T2 at 50,000ppm, T3 at 100.000ppm, T4 at 500.000ppm, T5 at 1000.000ppm, and T0 as the control. The extraction process yielded 18.68965 grams of tuba root extract from 1000 grams of tuba root powder, with a rotenone concentration of 114,387.5535ppm, equating to 22.22878%. Observations of water quality parameters indicated that treatment T1 did not alter the biochemical oxygen demand (BOD), while treatments T2 and T3 resulted in an increase in BOD values. Conversely, treatments T4 and T5 led to a decrease in BOD. Additionally, chemical oxygen demand (COD) measurements revealed a positive correlation between the concentration of tuba root extract and COD values, as well as pH levels. Total microbial population assessments showed that treatment T1 of 5100 CFU/ml had no significant effect on microbial populations, whereas treatments T2 of 2900 CFU/ml and T3 of 2750 CFU/ml enhanced microbial population. In contrast, treatments T4 of 1060 CFU/ml and T5 of 1060 CFU/ml inhibited microbial populations, moreover the control treatment T0 exhibited a total microbial population of 3100 CFU/ml.

INTRODUCTION

Biological resources available in Indonesia can potentially be used to meet the needs of the community. For example, they can be used as sources of efficacious chemical compounds for pesticides, medicines, industrial crops, food crops, and fruit plants **(Rustam *et al.*, 2021; Basri *et al.*, 2024)**. One of the plants well-known among the

Indonesian population is the tuber root plant (*Derris elliptica*), traditionally used as fish poison. This plant belongs to the Leguminosae family **(Phairoh *et al.*, 2021)**.

The tuber root plant is a toxic plant well-known among traditional communities, found growing throughout almost all regions of Indonesia. This plant has many uses; its roots are commonly used as a biopesticide to poison fish. Poisoning with the roots of this plant causes fish to become intoxicated and float, making them easy to catch. Thus, the tuber root plant is used by locals to catch fish, facilitating the capture, especially in freshwater bodies such as rivers and lakes **(Usman *et al.*, 2023)**. For its use in fish poisoning, traditionally, the roots are crushed and mashed and allowed to release a white liquid in water, or the active ingredient is extracted from the roots, ground, and then formulated as a powder **(Roberston *et al.*, 2008; Dalu *et al.*, 2015; Puspito *et al.*, 2023)**.

The tuber root plant can kill fish quickly since it contains a toxic compound called rotenone. The presence of rotenone in aquatic environments can have an ecological impact on the balance of microbial populations within these ecosystems. This is related to the rotenone possesses inhibitory characteristics that affect the NADH dehydrogenase enzyme, which is essential for ATP production, thereby inhibiting the growth of microbial populations. **(Melaas *et al.*, 2001; Lee & Kim, 2020)**. The excessive or unregulated application of rotenone derived from the tuba roots can lead to a marked decline in water quality, resulting in increased biochemical oxygen demand (BOD) and chemical oxygen demand (COD), alongside a reduction in dissolved oxygen levels. Such conditions pose a threat to the survival of various aquatic organisms **(Mischke *et al.*, 2022)**. Research conducted by **Antoni (2019)** indicates that the application of tuba root extract at varying concentrations—specifically 240, 360, and 480.000ppm—exerts a notable influence on the biochemical oxygen demand (BOD) and chemical oxygen demand (COD) values, while the pH level remains relatively constant. This observation aligns with **Prariska *et al*. (2017)** assertion that the acidity level (pH) of the water used in the study remains stable when tuba root extract is administered at different dosages, maintaining an approximate pH of 7. Furthermore, findings from **Yosmidar (2017)** reveal that a concentration of 0.001ml/ L of tuba root extract resulted in elevated BOD and COD values, ultimately leading to the mortality of tilapia fish within 48 hours of treatment.

Furthermore, the presence of rotenone in tuba roots can alter the water's pH, affecting the metabolism of aquatic life and jeopardizing the integrity of the entire aquatic ecosystem **(Zixia *et al.*, 2022)**. Excessive use of rotenone may disrupt the life cycles of organisms within the ecosystem and disturb ecological balance **(Christina *et al.*, 2001; Zixia *et al.*, 2022)**. Consequently, it is imperative that the utilization of rotenone derived from tuba root extract is conducted with caution and in compliance with environmental regulations to mitigate adverse effects. The release of this compound into the environment, particularly in aquatic systems such as rivers and lakes, can have significant implications for water quality **(Lin *et al.*, 2022; Usman *et al.*, 2023)**.

The widespread use of rotenone in the environment raises concerns as it can enter the food chain and affect water quality. In freshwater ecosystems, the reduction in biodiversity is a significant concern because these environments are considered some of the most threatened systems, with invasions contributing to their degradation **(Rayner & Creese, 2006)**. The use of rotenone as a fish poison can disrupt the balance of total microbial life in the water, particularly microbial that plays a crucial role in decomposing organic pollutants in aquatic environments, such as bacteria involved in the breakdown of ammonia pollution in water **(Mischke *et al.*, 2022; Rupiwardani & Irfany, 2023; Fahruddin *et al*., 2025)**.

Rotenone exerts an inhibitory influence on microbial, leading to a reduction in their populations. This inhibition hampers the effective decomposition of organic materials present in aquatic environments, resulting in the necessity for alternative microbial to undertake this decomposition process. Consequently, this shift leads to an increased demand for oxygen **(Ling, 2003; Scherer *et al.*, 2014)**. A research conducted by **Brown and Davis (2018)** indicates that the introduction of 1ppm of rotenone into river water significantly elevates COD values, attributed to the chemical oxidation of organic matter by microbial affected by rotenone.

Accordingly, it is recognized that the use of rotenone derived from tuba roots has the potential to deteriorate water quality, and exacerbate oxygen depletion in aquatic environments. The consequences affect water quality parameters including BOD, COD, and pH, as well as impacting the total microbial population.

MATERIALS AND METHODS

1. Sampling

Tuber root samples were collected from wild-growing plants located in Maros Regency, South Sulawesi Province. The collected tuber roots were then cut into pieces and ground using a blender. Brackish pond water samples were taken from fish ponds located in Maros Regency, South Sulawesi Province.

2. Tuber root extraction

One kilogram of tuber root was weighed and then blended. It was subsequently extracted by dissolving it in 1 liter of chloroform and allowed to stand for 24 hours. After that, it was filtered, and the filtrate was collected in a beaker. The residue was added to chloroform and allowed to stand again for 24 hours before being filtered. The filtrate obtained was combined with the first filtration. The chloroform in the extraction result was evaporated using an evaporator to obtain the tuber root extract. Tuba root extract preparation includes root pieces and powder (Fig. 1).

Fig. 1. **A.** Tuber root plant, **B.** Tuber root cut into pieces, and **C.** tuba root powder

3. Determination of rotenone concentration

The concentration of rotenone in the tuber root extract was determined by first dissolving it in chloroform. The solution was then identified using gas chromatography-mass spectrometry (GC-MS) and the concentration of rotenone was determined using a calibration curve of a rotenone standard.

4. Treatment with tuber root extract

The experiments were conducted in a plastic container filled with 10 liters of brackish pond water. Each treatment group received varying concentrations of tuba root extract, specifically: T1 at 10.000ppm, T2 at 50.000ppm, T3 at 100.000ppm, T4 at 500.000ppm, T5 at 1000.000ppm, while T0 served as the control with no tuba root extract added. The research lasted for a period of 24 hours, with observations conducted following 24 hours of treatment to assess the effect of the different concentrations of tuba root extract on biochemical oxygen demand (BOD), chemical oxygen demand (COD), pH levels, and total microbial.

5. Measurement of BOD

A 300mL water sample was divided into two portions: one designated for the initial analysis and the other for post-incubation assessment. The latter was stored in a sealed container at 20°C for a duration of five days to inhibit the exchange of oxygen with the environment. The concentration of dissolved oxygen in the initial sample was determined using the titration-iodometric method with a 0.025N sodium thiosulfate solution, and the same procedure was applied to the incubated sample. By comparing the dissolved oxygen levels before and after the incubation period, the resulting difference provides the BOD value for the water sample.

6. Measurement of COD

A volume of 100ml of the water sample intended for treatment was collected. Potassium dichromate reagent ($K_2Cr_2O_7$) and concentrated sulfuric acid (H_2SO_4) were subsequently introduced to facilitate the oxidation of organic materials present in the sample. Mercury sulfate ($HgSO_4$) was incorporated to mitigate the potential interference from chloride ions. The sample was then subjected to heating in a test tube at approximately 150°C for a duration of two hours. Following the cooling phase, titration was performed using ferrous ammonium sulfate (FAS) until a color transition from green to reddish-brown was observed. The COD of the water sample was determined by calculating the difference in the volume of potassium dichromate utilized before and after the titration process.

7. Measurement of pH

The pH measurement was performed utilizing a pH meter that had been previously calibrated with buffer solutions at pH 4 and pH 7, followed by a stabilization period of 15 minutes. Subsequently, the electrode of the pH meter was placed into the water sample collected from the brackish pond water, and after a brief interval, the pH value was recorded from the scale of the pH meter **(Fahruddin *et al.,* 2020)**.

8. Enumeration of microbial population

The enumeration of total microbial plate count is a technique employed to quantify the total microbial population. In this procedure, water from a brackish pond water was subjected to serial dilutions ranging from 10^{-1} to 10^{-4} using a 0.9% physiological solution of NaCl. Subsequently, the diluted samples were inoculated onto petri dishes containing nutrient agar medium. The samples were then incubated at room temperature for 48 hours at 37°C. Following the incubation period, the colonies that developed were counted, and the colony numbers were assessed based on macroscopic observations **(Fahruddin *et al.,* 2019)**.

RESULTS AND DISCUSSION

1. Extraction of tuber root

The extraction using chloroform yielded 37.3793 grams of extract, according to the Gas Chromatography and Mass Spectrometry (GC-MS) analysis, 1000 grams of tuber root powder produces 18.68965 grams of tuber root extract containing 114.387.5535ppm of rotenone, equivalent to 22.22878%. Thus, 1000 grams of tuber root powder contains 4.26515 grams of rotenone or 0.4265% rotenone content. This finding is consistent with previous research, which stated that the rotenone content in tuber roots from the *Derris elliptica* plant ranges between 0.3 and 12% **(Nurjayanti *et al.,* 2022)**.

2. BOD in treatments

The observation of BOD values in the treatment with tuba root extract in brackish pond water shows that treatment T1 has a BOD value of 3mg/ L. Both treatments T2 and T3 exhibit the same BOD value of 4mg/ L. Similarly, treatments T4 and T5 share a BOD value of 2mg/ L, while treatment T0, serving as the control, has a BOD value of 3mg/ L, which is identical to the BOD value of treatment T1 (Fig. 2).

Based on these observations, the addition of 10.000ppm of tuba root extract is insufficient to alter the amount of organic material in the water, as the concentration of organic material in the root extract is relatively low and does not significantly affect the activity of microbial involved in the decomposition of organic matter. Consequently, the BOD does not differ from the treatment without the addition of the root extract, namely in the treatment T0 as control. At low concentrations, the root extract allows microbial in the water to effectively decompose the existing organic material, leading to a more efficient decomposition process. As a result, the BOD values will not exhibit significant differences between treatments T1 and T0 as control. The findings further indicate that in the T0 treatment, which served as a control and lacked the inclusion of tuba root extract as an organic material source, there was no occurrence of biological decomposition. Consequently, this control treatment did not lead to an increase in the BOD value **(Abdullahi *et al.*, 2021; Luyani *et al.*, 2022)**.

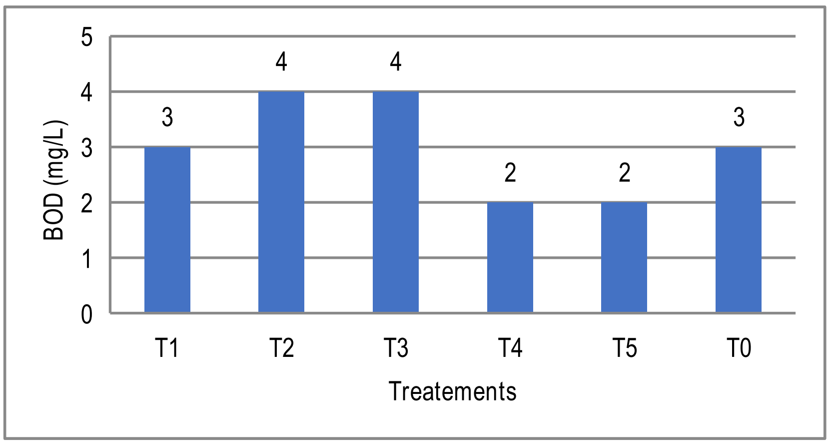

Fig. 2. The BOD values of brackish pond water treated with varying concentrations of tuba root extract are as follows T1= 10.000ppm, T2= 50.000ppm T3= 100.000ppm, T4= 500.000ppm, T5= 1000.000ppm and T0 = as control

In treatments T2 and T3, the BOD values were also identical at 4mg/ L. This finding is consistent with the research conducted by **Tanjung *et al.* (2019)**, which indicates that the organic load introduced into the water does not significantly alter the BOD values, despite the variations in the amount of extract used. According to **Shahid *et al.* (2023)**, microbial can adapt to various concentrations of tuba root extract, resulting in consistent oxygen consumption rates. Chemically, the presence of certain phytochemicals in the root extract may also influence microbial activity, leading to stabilization of oxygen demand **(Fahruddin *et al.*, 2019)**.

In treatments T4 and T5, the BOD values were identical and lower at 2mg/ L. This phenomenon is associated with the high concentration of tuba root extract, which can inhibit the growth of microbial population and lead to the accumulation of toxic byproducts that impede metabolic processes in these organisms **(Othman *et al.*, 2016; Nugraha & Wibowo, 2020)**. This inhibition may result in a decrease in overall biodegradation processes, leading to lower oxygen consumption and thereby reducing the BOD **(Tangahu & Putri, 2017; Supardi *et al.*, 2022)**.

This is consistent with the findings of **Kurniawan (2019)**, who examined the application of rotenone in river water. The study indicated that in the first week, the BOD value reached 6.2mg/ L; however, from the second to the fourth week, there was a significant decrease in the BOD value to 3.2mg/ L. This decline occurred due to the increased organic load that needed to be degraded by microbial, disrupting the microbial ecosystem balance. According to **Sihombing and Parulian (2021)**, the increase in BOD serves as evidence that the root extract contributes additional organic matter and chemicals to the river water.

3. COD in treatments

The determination of COD values in the treatments revealed that treatment T1 had a COD value of 15.5mg/ L, treatment T2 had a COD value of 19.5mg/ L, treatment T3 had a COD value of 25.5mg/ L, treatment T4 had a COD value of 31mg/ L, treatment T5 had a COD value of 38mg/ L, and treatment T0, serving as the control, had a COD value of 12mg/ L (Fig. 3). This indicates that as the amount of tuba root extract added increases, the COD values also rise. This phenomenon can be attributed to the chemical and biological properties of the root extract, which contains rotenone and other organic compounds. These components contribute to the overall organic load in the water, thereby influencing the observed increase in COD values **(Othman *et al.*, 2016; Tanjung *et al.*, 2019)**.

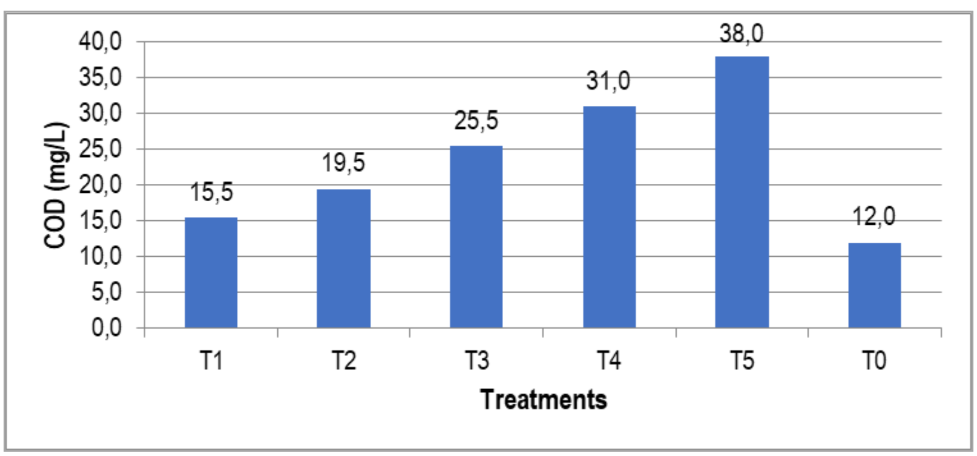

Fig. 3. The COD values of brackish pond water treated with varying concentrations of tuba root extract are as follows: T1= 10.000ppm, T2= 50.000ppm T3= 100.000ppm, T4= 500.000ppm, T5= 1000.000ppm and T0 = as control

The observations indicate that even with the application of tuba root extract at a low concentration of 10.000ppm in treatment T1, there was still an increase in COD. This suggests that at lower doses of the root extract, there remains an influence on the elevation of organic matter levels in the water, although this effect is less pronounced compared to higher concentrations **(Tanjung *et al.*, 2019; Sharma & Singh, 2020)**. The organic matter contained in the tuba root extract necessitates significant chemical decomposition, which requires oxygen; thus, the COD values will increase in proportion to the amount of available organic matter. This is related to the amount of oxygen required to oxidize the total organic matter present in the water **(Pasaru *et al.*, 2022)**.

This is congruent with the findings of **Sharma and Singh (2020)**, who demonstrated a significant increase in COD values following the application of rotenone from the tuba root extract in river water. The COD value rose from 15.5mg/ L in the first week to 26.5mg/ L by the fourth week. This increase in COD indicates a heightened oxidation process in the decomposition of organic matter from rotenone. In the study by **Sihombing and Parulian, (2021)**, water exposed to rotenone at a concentration of 1ppm showed a significant increase in COD. The COD value increased from 15.5mg/ L in the first week to 35.2mg/ L by the fourth week. This sharp rise in COD illustrates that the application of rotenone leads to enhanced oxidation of organic matter in the water. The control exhibited the lowest COD value, recorded at 12.0, in comparison to all other treatments. This outcome can be attributed to the absence of additional tuba root extract as an organic material for oxidation during the chemical decomposition process in T0, which served as the control. Consequently, this lack of organic material in the T0 treatment resulted in a low COD value **(Kurniawan, 2019)**.

4. pH in treatments

The pH observations for the treatments with tuba root extract revealed that treatment T1 had a pH value of 6.85, treatment T2 had a pH of 7.02, treatment T3 had a pH of 7.13, treatment T4 had a pH of 7.15, treatment T5 had a pH of 7.17, and the control treatment T0 had a pH of 6.69 (Fig. 4). The observations indicate that as the concentration of tuba root extract increases, the pH value also rises. This phenomenon is related to the chemical properties of the tuba root extract, specifically its alkaline compounds, which increase the concentration of hydroxide ions (OH^-) in the water, thereby raising the pH value **(Monica *et al.*, 2020)**. Additionally, the root extract affects the levels of dissolved carbon dioxide (CO_2) in the water; as the concentration of carbonic acid (H_2CO_3) decreases, the pH tends to increase. Thus, it can be stated that the greater the amount of root extract added, the more CO_2 is dispersed or neutralized **(Charles *et al.*, 2010; Brown & Davis, 2018)**.

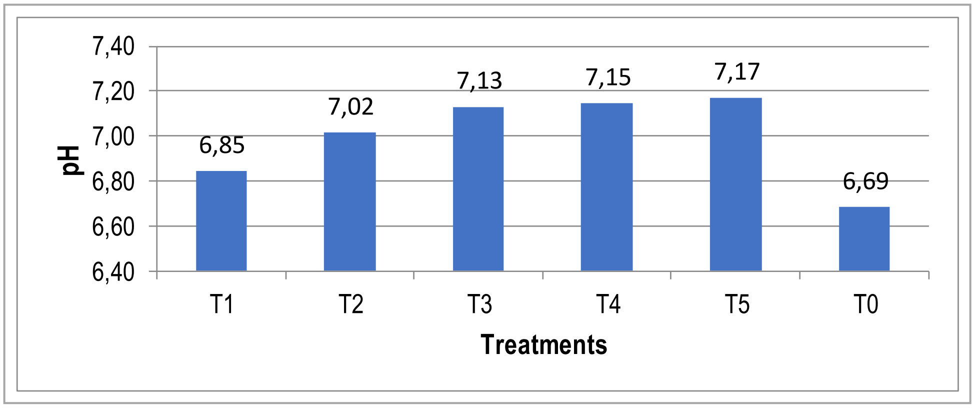

Fig. 4. The pH values of brackish pond water treated with varying concentrations of tuba root extract are as follows: T1= 10.000ppm, T2= 50.000ppm T3= 100.000ppm, T4= 500.000ppm, T5= 1000.000ppm and T0 = as control

Furthermore, the organic content in a plant, following decomposition, can release functional groups such as hydroxyl (OH) and carboxylate (COOH). The increase in hydroxyl and carboxylate functional groups will adsorb the H^+ ions present in the brackish pond water, ultimately resulting in a rise in pH. The pH value at T0 is maintained at 6.69 as a control due to the absence of organic material derived from tuba root extract. This extract typically contains functional groups, such as hydroxyl and carboxylate, which are known to elevate the pH value **(Christina, *et al.*, 2001; Pitaloka *et al.*, 2023)**.

However, the measured values of water quality parameters from the treatments with *Derris elliptica* root extract remain within normal criteria. The pH measurements of the water ranged approximately from 6 to 7, indicating a neutral condition. **Boyd (1986)** states that the optimal pH range for the growth and development of aquatic organisms is around 6.5 to 9.0, as these pH levels do not disrupt the metabolism of organisms. Accordingly, **Nugraha and Wibowo, (2020)** posited that the optimal pH values for fish transportation generally range between 7 and 8.5, which are considered very ideal for fish farming. According to **Nanda *et al.* (2018)**, the pH values in each treatment fall within the range of 7 to 8, with dissolved oxygen levels exceeding 5mg/ L, thus supporting the life of aquatic organisms in general.

5. Total microbial population

The quantity of microbial population colonies is represented in Colony Forming Units per milliliter (CFU/ml) of the water sample at the 24-hour observation of total microbial population for each treatment in tuba root extraction. Treatment T1 had a total microbial of 5100 CFU/ml, treatment T2 had 2900 CFU/ml, treatment T3 had 2750 CFU/ml, treatment T4 had 1350 CFU/ml, and treatment T5 had 1060 CFU/ml. The control treatment T0 exhibited a total microbial of 3100 CFU/ml (Fig. 5) and the appearance of total colony growth of the microbial population (Fig. 6).

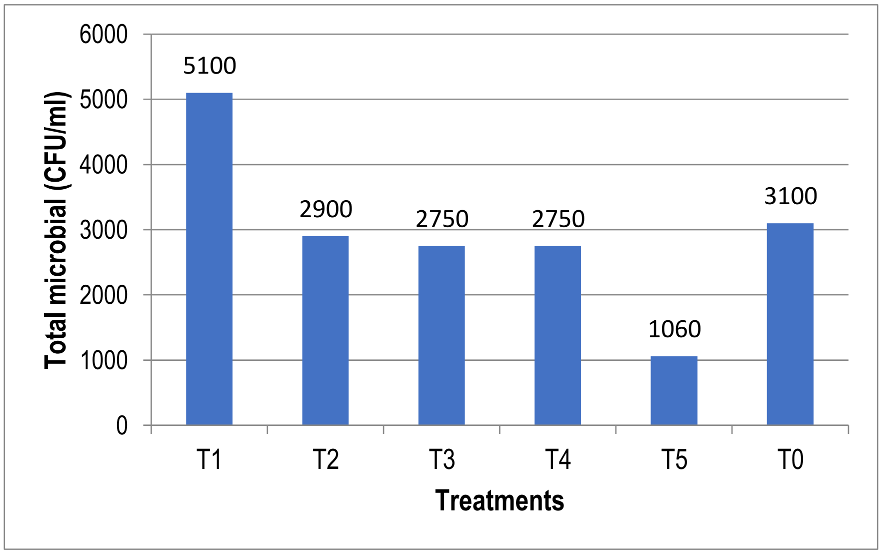

Fig. 5. The quantity of microbial colonies (CFU/ml) of brackish pond water treated with varying concentrations of tuba root extract are as follows: T1= 10.000ppm, T2= 50.000ppm T3= 100.000ppm, T4= 500.000ppm, T5= 1000.000ppm and T0 = as control

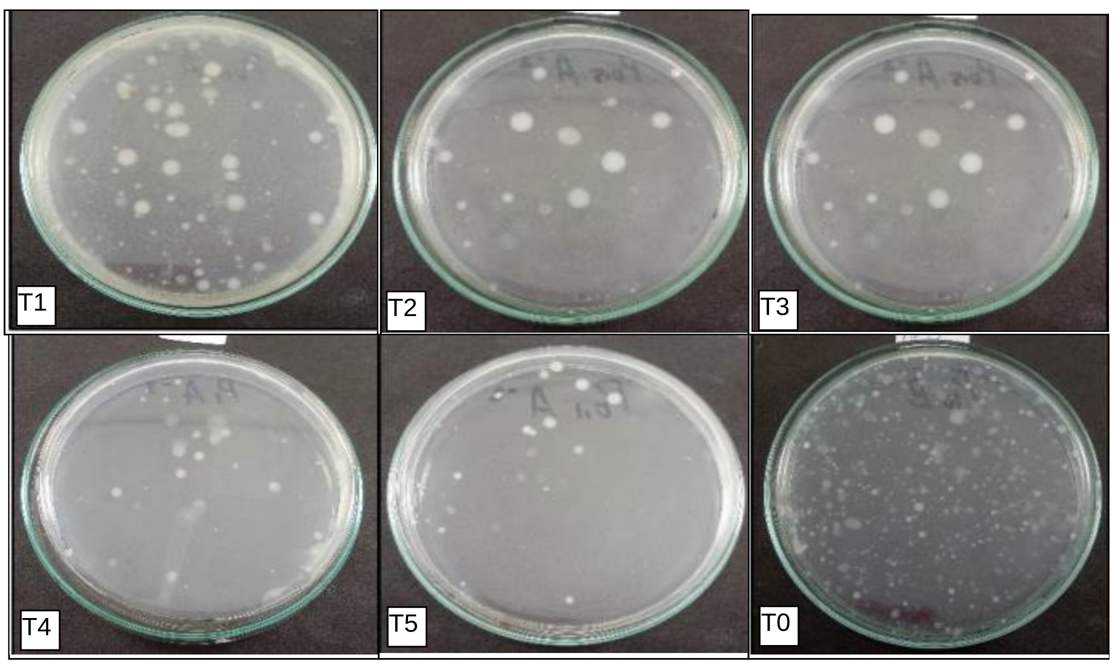

Fig 6. The appearance of total bacterial colony growth on nutrient agar media at a dilution of 10^{-3} is representative of each treatment: T1= 10.000ppm, T2= 50.000ppm T3= 100.000ppm, T4= 500.000ppm, T5= 1000.000ppm and T0 = as control

Based on these observations, the addition of 10.000ppm of tuba root extract (T1) does not appear to affect microbial activity, whereas the addition of 50.000ppm (T2) and 100.000ppm (T3) stimulates microbial population growth, indicating that the microbial are degrading the organic compounds present in the root extract. In T0, serving as a control, the total microbial populations exhibited stability from the outset, as it did not undergo treatment with tuba root extract. Consequently, the microbial population in T0 did not face any inhibition. In contrast, when evaluated against the T1 treatment, the total microbial populations were found to be greater, as the rotenone treatment provided a nutrient source. Meanwhile, the control group did not receive any supplementary organic material during the 24-hour incubation period, which would have served as an additional nutrient source **(Das & Kaushik, 2018)**.

In contrast, treatments with the addition of 500.000ppm (T4) and 1000.000ppm (T5) demonstrate an inhibition of microbial population. According to **Das and Kaushik (2018)**, rotenone belongs to the class of alkaloids that exhibit antimicrobial properties at higher concentrations. The study of **Pereira *et al.* (2023)** further indicates that the rotenone compound in the root extract disrupts enzymes involved in microbial respiration, rendering the microbial unable to effectively degrade organic matter.

According to **Othman *et al.* (2016)**, the active compound rotenone from the tuba root exhibits antimicrobial properties. However, certain types of microbial are not inhibited and may continue to proliferate, as observed in treatment T1. **Das and Kaushik (2018)** noted that some components within the tuba root extract serve as substrates for microbial, facilitating their growth. The presence of specific organic materials in the extract can enhance microbial activity, leading to population increases. Furthermore, **Mischke *et al.* (2022)** stated that the application of tuba root extract can alter environmental conditions, such as pH and dissolved oxygen content, creating a more favorable environment for the growth of certain microbial species.

CONCLUSION

The utilization of rotenone derived from tuba root extract (*Derris elliptica*) in brackish pond water and its impact on various water quality parameters, including biochemical oxygen demand (BOD), chemical oxygen demand (COD), pH, and the microbial population. The findings indicate that these parameters remained within the limits established by the Indonesian National Standard (SNI 8037.1:2014) for brackish pond water quality, specifically BOD values ranging from 5 to 20mg/ L, COD values between 10mg/ L and 50mg/ L, and pH levels maintained between 6.5 and 8.5. Furthermore, these values did not significantly affect the microbial population. Consequently, the implications of employing tuba root extract for fish capture in brackish water aquaculture suggest that environmental parameters, including BOD, COD, pH, and total microbial population, remain stable and do not present adverse conditions, provided that the extract is applied at concentrations below 1.000.000ppm.

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