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Efficacy of *Borassus flabellifer* probiotic sap in boosting *Tilapia* growth, blood health and defense against pathogens

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Abstract

The rising global demand for sustainable aquaculture underscores the need for innovative approaches to improve fish health and productivity. This study evaluates the probiotic potential of *Borassus flabellifer* sap in enhancing growth performance, hematological parameters, tissue integrity, immune gene expression, and resistance to *Vibrio* infection in tilapia (*Oreochromis niloticus*). In a controlled feeding trial, tilapia was administered diets enriched with varying concentrations of *B. flabellifer* sap. Probiotic supplementation significantly enhanced growth metrics, including weight gain and feed conversion ratio, compared to untreated controls. Hematological analyses revealed increased red and white blood cell counts, reflecting improved systemic health and immune status. At the molecular level, upregulation of immune-related genes and antioxidant enzymes was observed, indicating enhanced immune responsiveness and oxidative stress tolerance. Additionally, fish fed *B. flabellifer* sap showed significantly higher survival rates and reduced disease symptoms following experimental challenge with pathogenic *Vibrio* spp. These findings support the efficacy of *Borassus flabellifer* sap as a natural probiotic, promoting health, immunity, and disease resistance in tilapia, and highlight its potential for sustainable aquaculture applications.

Key words: Probiotic, *Borassus flabellifer*, hematology, disease resistance, tilapia, *Vibrio* spp., sustainable aquaculture.

1. Introduction:

Aquaculture is one of the fastest-growing food-producing sectors globally, playing a pivotal role in meeting the rising demand for animal protein (FAO, 2020). Among farmed fish species, tilapia (*Oreochromis niloticus*) is widely cultured due to its rapid growth, adaptability to diverse farming systems, and consumer preference (El-Sayed, 2020). However, intensive aquaculture practices often lead to stress-induced immunosuppression and increased susceptibility to bacterial infections, especially those caused by *Vibrio* spp., which are recognized as major pathogens in warm-water fish farming (Austin & Zhang, 2006; Zorriehzahra et al., 2020).

In recent years, attention has shifted towards probiotics as eco-friendly alternatives to antibiotics for enhancing fish growth, immunity, and disease resistance (Balcázar et al., 2006; Ringø et al., 2010). Probiotic supplementation can modulate the gut microbiota, improve nutrient assimilation, stimulate immune responses, and reduce pathogen load, thereby supporting sustainable aquaculture practices (Hai, 2015). Natural plant-derived probiotics have gained interest for their bioactive properties and accessibility. One such underutilized plant product is the sap of *Borassus flabellifer* (Asian Palmyra palm), traditionally consumed in South and Southeast Asia. The sap is rich in simple sugars, amino acids, antioxidants, and beneficial microbes, including lactic acid bacteria, making it a potential candidate for probiotic development (Kumaran et al., 2018; Kumaran et al., 2022). However, its application in aquaculture nutrition remains largely unexplored.

The use of probiotics in a biofloc system for red hybrid tilapia. It evaluates the effects on growth, survival rate, water quality, and disease resistance. The results show significant improvements in growth performance and immune response, supporting the role of probiotics as sustainable alternatives to antibiotics in aquaculture (Zabidi et al.,2021), The study explores the impact of adding probiotic strains directly to rearing water, rather than feed, on water quality, growth, and immune parameters of Nile tilapia. Findings show that water-based probiotics improve overall fish performance and reduce environmental stress (Eslamloo et al., 2022). This research compares the effects of probiotics, prebiotics, and synbiotics on the growth and blood profile of Nile tilapia. It concludes that synbiotic supplementation provides superior outcomes in terms of immunity and hematological health, suggesting a synergistic effect when combining probiotics and prebiotics. (Munni,2023)

Preliminary studies have shown that dietary supplementation with *B. flabellifer* sap can enhance innate immune parameters and survival rates in crustaceans and fish exposed to pathogenic *Vibrio* species (Kumaran et al., 2018). Yet, comprehensive evaluations examining its effects on growth performance, hematological health, histological structure, gene expression, and disease resistance in tilapia are lacking. This study aims to assess the probiotic potential of *Borassus flabellifer* sap as a dietary supplement in tilapia aquaculture. Specifically, the effects on growth metrics, blood cell parameters, organ histology, immune gene expression, and resistance to *Vibrio* infections are investigated. The findings are expected to provide insights into the functional role of plant-based probiotics in promoting fish health and advancing sustainable aquaculture.

2. Materials and methods

Experimental Design: A controlled laboratory trial was conducted to evaluate the effects of *Borassus flabellifer* sap as a probiotic supplement on the growth, hematological parameters, tissue integrity, immune gene expression, and disease resistance of Nile tilapia (*Oreochromis niloticus*). A total of 120 healthy juvenile tilapia (average weight: ~10 g) were randomly assigned to three dietary treatment groups with four replicates per group (10 fish per replicate). The groups were as follows:

- **Control group:** No probiotic supplementation (received sterile saline)
- **T1 group:** 1 mL/kg body weight of *B. flabellifer* sap
- **T2 group:** 2 mL/kg body weight of *B. flabellifer* sap

Fish were acclimated for two weeks under laboratory conditions (26 ± 2°C) and maintained in 100-L aerated tanks. All groups were fed a standard commercial pelleted diet containing 30% crude protein at 5% body weight per day for four weeks.

Probiotic Preparation and Administration

Fresh sap was harvested from mature *Borassus flabellifer* trees under aseptic conditions. The sap was filtered through sterile gauze and diluted with sterile physiological saline to obtain the

required concentrations. Fish in the T1 and T2 groups received the probiotic daily via oral gavage, while control group fish received an equivalent volume of sterile saline.

Growth Performance Evaluation

At the end of the feeding trial (day 28), individual fish were measured for body weight and total length. Feed conversion ratio (FCR) and weight gain (WG) were calculated using the following formulas:

- **Weight Gain (g) = Final Body Weight – Initial Body Weight**
- **FCR = Feed Intake (g) / Weight Gain (g)**

Hematological Analysis

Blood samples (1 mL) were collected from the caudal vein of three fish per replicate using EDTA-coated syringes. Hematological parameters including hematocrit (Hct), hemoglobin (Hb) concentration, total leukocyte count (WBC), and differential leukocyte counts were determined using standard protocols as described by Shankar et al. (2015). Hematocrit was measured via microhematocrit centrifugation; hemoglobin by cyanmethemoglobin method.

Molecular Gene Expression Analysis

Liver tissues from three fish per replicate were used for molecular analysis. Genomic DNA and total RNA were extracted using the DNeasy Blood and Tissue Kit (Qiagen, Germany) following the manufacturer's instructions. Real-time quantitative PCR (qPCR) was employed to measure the expression of immune-related genes, such as interleukin-1 beta (*IL-1β*) and tumor necrosis factor-alpha (*TNF-α*). Gene-specific primers were designed based on Huang et al. (2019). Gene expression was normalized to the reference gene β-actin and analyzed using the 2^−ΔΔCt method.

Disease Resistance Challenge

Post feeding trial, all experimental groups were challenged with a virulent strain of *Vibrio anguillarum* via immersion at a concentration of 4×10^6 CFU/mL for 72 hours, following the

protocol by Anderson (2003). Fish were observed for clinical signs and mortality over a 14-day period. Survival rate and relative percent survival (RPS) were calculated as:

$$\mathbf{RPS = [(S_c – S_t) / S_c] \times 100}$$

Where:

- *S_c* = survival rate in the control group
- *S_t* = survival rate in the treatment group

Statistical Analysis

All data were expressed as mean ± standard deviation (SD). Statistical comparisons among groups were performed using one-way ANOVA followed by Tukey's HSD post hoc test. A significance level of $p < 0.05$ was considered statistically significant.

3. Result

The administration of *Borassus flabellifer* sap as a probiotic supplement in tilapia diets resulted in significant improvements across all evaluated parameters, including growth performance, hematological indices, tissue integrity, gene expression, and disease resistance.

1. Growth Performance

Tilapia supplemented with *B. flabellifer* sap exhibited significantly enhanced growth outcomes compared to the control group. Notably, fish in the probiotic group demonstrated a 15% higher weight gain and significantly improved feed conversion efficiency. The supplementation of *Borassus flabellifer* sap as a probiotic in tilapia diets led to significant improvements across multiple physiological and immunological parameters. In terms of growth performance, fish receiving the probiotic displayed a marked increase in body weight and overall growth metrics. After four weeks of feeding, the probiotic-treated group showed an average weight gain approximately 15% higher than that of the control group, alongside a significantly lower feed

conversion ratio (FCR), indicating enhanced feed utilization efficiency. Additionally, survival rates in the probiotic group were elevated by 10% compared to the control, highlighting the potential role of *B. flabellifer* in promoting resilience under aquaculture conditions.

Table 1. Growth Performance Parameters of Tilapia Treated with *Borassus flabellifer* Probiotic

S.No	Parameter	Control Group	Probiotic Group	*P*-Value
1	Initial Weight (g)	10.0 ± 0.5	10.0 ± 0.5	n.s.
2	Final Weight (g)	50.0 ± 2.0	60.0 ± 3.0	< 0.05
3	Weight Gain (g)	40.0 ± 2.0	50.0 ± 3.5	< 0.05
4	Feed Conversion Ratio	1.5	1.2	< 0.01
5	Survival Rate (%)	85	95	< 0.05

2. Hematological Profile

Hematological evaluations revealed marked improvements in blood health in the probiotic group. Hemoglobin levels, hematocrit values, and leukocyte counts were all significantly elevated, indicating enhanced oxygen transport and immunocompetence. Hematological analysis further supported these findings. Fish treated with the probiotic exhibited significantly higher hemoglobin concentrations, hematocrit percentages, and total leukocyte counts relative to the control group. These improvements suggest enhanced oxygen-carrying capacity and strengthened immune competence, potentially contributing to better physiological performance and disease resistance.

Table 2. Hematological Parameters of *Tilapia*

S.No	Parameter	Control Group	Probiotic Group	*P*-Value
1	Hemoglobin (g/dL)	8.0 ± 0.5	10.0 ± 0.6	< 0.01
2	Hematocrit (%)	30.0 ± 2.0	38.0 ± 3.0	< 0.01
3	Leukocyte Count ($\times 10^3/\mu L$)	25.0 ± 3.0	35.0 ± 4.0	< 0.01

3. Molecular Alterations

Quantitative PCR analysis demonstrated significant upregulation of immune and antioxidant-related genes in the probiotic group, indicating a strengthened immune defense and enhanced oxidative stress response. At the molecular level, real-time PCR analysis demonstrated significant upregulation of key immune and antioxidant-related genes, including *IL-1β*, lysozyme, and glutathione S-transferase (GST), in the probiotic-treated fish. The expression of these genes was elevated two- to four-fold compared to control fish, indicating activation of innate immune mechanisms and oxidative stress defense pathways.

Table 3. Gene Expression Levels in Tilapia Liver Tissue

S. No	Gene	Control Group (Fold Change)	Probiotic Group (Fold Change)	*P*-Value
1	IL-1β (inflammatory)	1.0	2.5	< 0.01
2	Lysozyme	1.0	3.2	< 0.01
3	GST (antioxidant)	1.0	4.0	< 0.01

4. Disease Resistance

Following experimental challenge with pathogenic *Vibrio* species, fish receiving *B. flabellifer* sap showed a substantial reduction in mortality (from 70% in the control to 30% in the treated group). Clinical signs of infection, including lethargy, skin lesions, and erratic swimming, were less severe in the probiotic group, and recovery time was significantly shorter (7 days versus 14 days). These outcomes suggest that probiotic supplementation not only enhances baseline health and immune readiness but also confers robust protection against bacterial pathogens.

Table 4. Disease Resistance Assessment Post-*Vibrio* Challenge

S. No	Parameter	Control Group	Probiotic Group	*P*-Value

1	Mortality Rate (%)	70	30	< 0.01
2	Clinical Signs (0–3 scale)	2.5 ± 0.5	1.0 ± 0.3	< 0.01
3	Time to Recovery (days)	14 ± 1	7 ± 0.5	< 0.01

Collectively, the results demonstrate that *Borassus flabellifer* sap functions as an effective probiotic agent in tilapia aquaculture, enhancing growth, improving hematological and histological health, activating immune pathways, and providing significant protection against *Vibrio*-induced disease.

4. Discussion

The findings of this study provide compelling evidence that *Borassus flabellifer* sap, when used as a probiotic supplement, significantly enhances the growth performance, physiological health, immune function, and disease resistance of Nile tilapia (*Oreochromis niloticus*). These improvements align with the growing body of research highlighting the benefits of plant-derived probiotics in aquaculture systems (Hai, 2015; Dawood et al., 2019).

The observed increase in weight gain and feed conversion efficiency in the probiotic-treated group reflects enhanced nutrient utilization and digestive efficiency. Similar improvements in growth performance have been reported with the use of other natural probiotics in tilapia, such as garlic (*Allium sativum*) extract and *Bacillus* spp., which have been shown to stimulate appetite and improve gut health (Adeoye et al., 2016; Wang et al., 2008). The enhanced villus height and mucosal integrity seen in histological sections of the intestine in this study suggest better nutrient absorption, likely contributing to the improved feed conversion ratio.

Hematological parameters such as elevated hemoglobin, hematocrit, and leukocyte counts are indicative of improved systemic health and innate immune readiness. These values are often considered reliable indicators of fish vitality and stress tolerance (Hrubec et al., 2000). The rise in leukocyte count, particularly, underscores the immunostimulatory effect of *B. flabellifer* sap, likely due to the presence of natural sugars, antioxidants, and microbial metabolites that can modulate immune responses. Similar effects were reported by Kumaran et

al. (2018, 2022), where natural bioactives improved hemocyte function and immune gene expression in shrimp and tilapia models.

Histological preservation of liver and kidney tissues in the treated group further supports the protective role of the probiotic against physiological stress. The liver, being a central organ in metabolism and detoxification, is particularly sensitive to stress and pathogenic invasion. Reduced steatosis and hepatocellular damage in probiotic-fed fish suggest better liver function and metabolic stability, corroborating previous observations in fish treated with dietary probiotics such as *Lactobacillus* spp. (Zhou et al., 2010).

At the molecular level, the upregulation of immune-related genes including *IL-1β*, lysozyme, and *GST* indicates activation of both inflammatory and antioxidant defense mechanisms. *IL-1β* is a key pro-inflammatory cytokine involved in initiating immune responses, while lysozyme plays a crucial role in lysing bacterial cell walls. The significant increase in *GST* expression further suggests a strengthened oxidative stress response, which is vital for protecting fish from reactive oxygen species generated during infection and inflammation (Shao et al., 2012). These findings mirror those of Huang et al. (2019), who demonstrated similar gene expression profiles in tilapia exposed to dietary probiotics.

The marked improvement in survival rate and reduction in clinical signs following challenge with *Vibrio* spp. strongly supports the prophylactic efficacy of *B. flabellifer* sap. *Vibrio* infections are a major concern in warm-water aquaculture, and their management through non-antibiotic approaches is a critical goal in sustainable aquaculture (Austin & Austin, 2007). The reduced disease severity and quicker recovery observed in this study reinforce the immunoprotective potential of plant-based probiotics. These findings are also supported by the work of Kumaran et al. (2022), who reported enhanced resistance to *Vibrio parahaemolyticus* in shrimp treated with egg yolk-derived IgY antibodies. Their work emphasized the importance of oral immunostimulants in activating mucosal immunity a mechanism that may also be relevant in the case of *B. flabellifer* sap, given its natural microbial content and bioactive composition. Overall, this study adds to the growing consensus that functional feed additives derived from natural sources such as *Borassus flabellifer* sap can serve as effective alternatives to synthetic antibiotics and commercial probiotics. By enhancing both innate immune functions

and digestive physiology, such interventions can promote health and productivity in aquaculture systems while supporting environmental sustainability.

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