Volume 7, Issue 1: KUS: MariNEX1506: xx-xx: 16102025: 2026

KHULNA UNIVERSITY
STUDIES

journal homepage: www.kus.ku.ac.bd

Research article

Effect of Nutmeg (*Myristica fragrans*) on the Growth and Resistant Capacity of Prawn (*Macrobrachium rosenbergii*) against *Vibrio parahaemolyticus* infection

Farhana Nasrin, Shaikh Shaon Ahmmed, Halima Tus Sadia, Uttam Adhikary, Jannatul Ferdaus Jyoti, Md. Mahmudul Hasan, Rajdwip Sarkar, Abul Farah Md. Hasanuzzaman and Alokesh Kumar Ghosh*

Fisheries and Marine Resource Technology Discipline, Khulna University, Khulna-9208, Bangladesh

ABSTRACT

This study assessed the impact of dietary *Myristica fragrans* (MF) extract on the growth, immune function, body composition, and disease resistance in juvenile prawn *Macrobrachium rosenbergii*. Fifteen prawns (2.1 ± 0.25 g) in each tank were given either a control diet or diets with 0.025% (T1) or 0.05% (T2) MF extract. Each treatment was done in three tanks (total n = 135 prawns) and the prawns were fed 5% of their body weight every day for eight weeks. *Vibrio parahaemolyticus* (10^6 CFU/mL) was used to challenge *M. rosenbergii* after the feeding experiment, and they were observed for 14 days post challenge. Prawns fed the T1 diet showed significantly improved growth performance (FW, AWG, SGR, PER, and FCR), higher protein and ash content, and elevated digestive enzyme activities (amylase and protease) compared to the control and T2 groups. Immune parameters (THC, HCT, RB, proPO, SOD, and superoxide anion generation) exhibited a significant elevation in the MF-supplemented groups, both prior to and following bacterial exposure. Post-infection, T1 had the highest survival (86.4%), T2 reached 72.85%, and the control suffered complete mortality (100%) by day 14. These findings indicate a dose-dependent response, with the lower inclusion level (0.025%) being more effective than the higher dose. This study provides preliminary evidence for the optimized use of MF extract in enhancing growth and innate immunity of *M. rosenbergii* under *Vibrio* challenge conditions. However, its application as a replacement for synthetic additives requires further validation.

ARTICLE INFO

Article timeline:
Date of Submission:
16 October, 2025
Date of Acceptance:
13 May, 2026
Article available online:
10 June, 2026

***Keywords*:**
Myristica fragrans
Growth
Survival
Disease
Sustainable

Introduction

Aquaculture plays a crucial role in the global food landscape, bolstering food security, sustaining livelihoods, and aiding economic growth. Bangladesh stands out as a prominent player in aquaculture, exporting fish, shrimp, and various aquatic products, which play a prominent role in enhancing nutritional health and driving economic development (Haque et al., 2025; Salam et al., 2025). FAO (2024) reports showed that worldwide aquaculture production touched a new record of 130.9 million tonnes in 2022, valued at USD 312.8 billion, highlighting the industry's expanding contribution to the world's animal protein needs. Over half of Bangladesh's total fish output for both domestic and export markets come from inland fisheries, which include capture and culture-based systems (Chakraborty, 2021; Shamsuzzaman et al., 2020). Of the many species raised, *M. rosenbergii*, the giant freshwater prawn, has become well-known because of its nutritional value, economic viability, and adaptability to polyculture methods. Due to its tropical climate and abundant water supplies, Bangladesh has one of the best suitable environments for *M. rosenbergii* culture in the world, and this culture creates employment in rural areas. Nowadays, this is the country's second-largest source of foreign currency earnings, behind the ready-made clothing industry (Ahmed et al., 2008, 2010, 2018).

*Corresponding author: < alokesh.ghosh@fmrt.ku.ac.bd>

DOI: https://doi.org/10.53808/KUS.2026.SI.7.01.MariNEX1506-ls

However, crustaceans are more vulnerable to infectious illnesses than vertebrates since they only have innate defenses and lack adaptive immunity (Ghosh et al., 2021). The emergence and spread of opportunistic pathogens, especially Gram-negative bacteria like *Vibrio parahaemolyticus*, a known cause of gastrointestinal disorders in humans and systemic infections in aquatic species, have been aided by intensified farming practices, careless antibiotic use, and inadequate biosecurity measures (Wang et al., 2024; Das et al., 2009**).** In crustacean aquaculture, *Vibrio* spp. are among the most critical pathogens responsible for mass mortality events, leading to severe economic losses in *M. rosenbergii* farming systems through septicemia, hepatopancreatic damage, and immune suppression (Manchanayake et al., 2023; Valente and Wan, 2021; Rao et al., 2015). Such infections' survival poses issues with public health and antibiotic resistance in addition to compromising output and profitability (Ina-Salwany et al., 2019; Saurabh and Sahoo, 2008).

Despite the fact that antibiotics have long been used to control bacterial outbreaks and improve aquaculture species' development performance, their abuse has resulted in the spread of resistant bacterial strains and environmental pollution (Nigam et al., 2014). The world is moving toward safer, more environmentally friendly alternatives as a result of regulatory restrictions, environmental concerns, and rising consumer knowledge. The immunostimulant, growth-promoting, and antibacterial properties of natural bioactive compounds originating from medicinal plants, herbs, and spices are being investigated more and more in aquaculture (AftabUddin et al., 2018; Defoirdt et al., 2011). Among these, the traditionally prized spice and medicinal plant nutmeg (*Myristica fragrans*) has garnered attention because of its phytochemical diversity and proven pharmacological qualities, such as antibacterial, antioxidant, and anti-inflammatory effects (Naeem et al., 2016; Nikolic et al., 2021; Matulyte et al., 2020). Nutmeg contains several bioactive compounds, including myristicin, eugenol, safrole, and various phenolic and flavonoid constituents (Devi, 2024; Trifan et al., 2023; Antasionasti et al., 2021; Ha et al., 2020;)**.** These compounds are known to exert antimicrobial effects through disruption of bacterial cell membranes, inhibition of quorum sensing, and modulation of oxidative stress pathways. In addition, these bioactive components may also enhance innate immunity by activating the prophenoloxidase (proPO) cascade and reactive oxygen species (ROS)-mediated defense mechanisms (Kaur et al., 2025; Cruz et al., 2024; Trifan et al., 2023; Khairan et al., 2023; Ashokkumar et al., 2022; Barman et al., 2021; Antasionasti et al., 2021)**.** In many finfish species, including *Clarias gariepinus* and *Cyprinus carpio*, dietary inclusion of *M. fragrans* has been linked to better growth performance, increased feed consumption, and decreased mortality (Morsy, 2016; Lanari et al., 2018).

However, despite these findings, there remains a lack of systematic studies evaluating the dose-dependent effects of *M. fragrans* extract on immune modulation and disease resistance in freshwater prawns, particularly under *Vibrio* challenge conditions. Moreover, the mechanistic linkage between nutmeg-derived phytochemicals and crustacean immune pathways is still poorly understood. Despite these encouraging results, there are still few thorough assessments of its potential in crab aquaculture, especially when pathogenic stress is present. Therefore, the present study hypothesized that dietary supplementation with *M. fragrans* extract would enhance growth performance, stimulate innate immune responses, and improve resistance against *V. parahaemolyticus* infection in *M. rosenbergii* in a dose-dependent manner. The aim of this research is to assess the effect of *Myristica fragrans* (nutmeg) extracts on *M. rosenbergii's* growth performance, immunological response, and disease resistance in response to *V. parahaemolyticus* challenge. Additionally, by lowering dependency on artificial antimicrobials, the study aims to investigate nutmeg's potential as a natural dietary supplement to support sustainable aquaculture operations.

Materials and Methods

Making of *Myristica fragrans* ethanolic extract

Fresh seeds of *M. fragrans* were obtained from a local market (Khulna). The seeds underwent shade drying at ambient temperatures ranging from 25 to 30°C until they reached a brittle state, signifying that they were fully desiccated. After drying, the seeds were processed into a fine powder with the help of a blender, resulting in a particle size of around 500 µm. A 100 g amount of the powdered nutmeg was mixed with ethanol at a solid-to-solvent ratio of 1:10 (w/v). The blend was kept at room temperature (25°C) and subjected to periodic sonication **(**30 min every 6 h) for a total of 24 hours at 40°C to improve the solubilization of phytochemicals. The resulting slurry underwent vacuum filtration using filter paper to isolate the ethanolic extract from the solid residues. This filtrate underwent into a rotary evaporator (Hahnvapor, Hahnshin, Korea) at 60–70 rpm and 40°C for a duration of 20–30 minutes to eliminate the solvent. The percentage yield of the extract was calculated as (weight of dried extract / initial dry weight of sample) × 100. The extraction yield (%) was calculated based on dry weight and the extract was stored at 4°C in sterile containers until further use to prevent photodegradation and preserve stability.

Evaluation of the extract's toxicity

The toxicity of the ethanolic extract was evaluated through the Brine Shrimp Lethality Assay (BSLA), a well-established preliminary technique for assessing the cytotoxic potential of bioactive compounds (Borja et al., 2016). The experiment was conducted in accordance with established protocols, incorporating certain modifications to accommodate the unique conditions of the laboratory. One gram (1g) *Artemia spp.* Cysts were incubated in the prepared artificial seawater (35 ppt) with continuous aeration with maintaining a controlled temperature of 25–28 °C for a duration of 24 hours. A stock solution prepared from the extract was prepared at a concentration of 100 mg/mL in dimethyl sulfoxide (DMSO), with two-fold serial dilution series (spanning from 1:2 to 1:64) was established. In the process of toxicity screening, 2 µL from each test concentration was introduced into 198 µL of artificial seawater within separate wells of a 96-well

plate with triplicate. The plates were kept at a temperature range of 25–28 °C in constant light for a duration of 24 hours. Clarkson introduced a toxicity index to evaluate and categorize the toxic potential of plant extracts (Hamidi et al., 2014). Following the exposure period, the count of both surviving and deceased nauplii in each well was documented with the aid of a stereomicroscope. The formula of calculating mortality percentage was:

$$\text{“}Mortality\ (\%) = \frac{Total\ number\ of\ death\ naupalli}{Total\ naupalli} \times 100\text{”}$$

Table 1: Toxicity level assessment of Artemia spp. exposed to extract-supplemented diets

Effective toxic concentration range (LC_{50})	Toxicological category
>1000 µg/ml	Non-Toxic
500 - 1000 µg/ml	Low toxic
100 - 500 µg/ml	Medium toxic
0 - 100 µg/ml	Highly toxic

The toxicity index proposed by Clarkson was used to classify the toxicity level of the plant extracts (Hamidi et al., 2014). The LC_{50} value of the extract was found to be 88 ± 9.05 µg/mL, indicating that it falls within the low-toxicity category (Table 1).

Collection and maintenance of experimental *M. rosenbergii*

Juvenile *M. rosenbergii*, with an initial weight of 2.1 ± 0.25 g, were obtained from a commercial hatchery located in Monirampur, Jessore, and were transported to the laboratory under aerated conditions. Upon arrival, the prawns experienced a careful adjustment period to reduce stress and prevent any loss during transport. A 7-day acclimatization period was maintained prior to the initiation of the feeding trial. To ensure proper maintenance, prawns were placed in 80 L tanks, each equipped with a 200W submersible heater (RS Electrical) and a single air stone to provide continuous aeration. Before use, the water underwent UV treatment to reduce microbial contamination. Initially, each tank contained 15 prawns, with extra individuals ready to take the place of any that might perish during the 7-day acclimation period. Prawns received daily feed at 5–10% of their body weight (Sadia et al., 2024), and any uneaten feed and waste were siphoned out prior to each feeding session. The quality of the water was maintained by daily replacing 25% of the tank's water with UV-treated water. Environmental parameters were monitored at daily basis at a continuous range of temperature (28–31°C), pH (7.8–8.5), dissolved oxygen (>6 mg/L), salinity (0–1 ppt), and ammonia (<0.1 mg/L), ensuring optimal conditions for health and experimental consistency (Ghosh et al., 2024b). All experimental procedures were conducted following standard aquaculture animal handling guidelines to minimize stress and ensure welfare.

Experimental design

Control group (C) and two treatment groups were randomly allocated to the prawns, with each treatment conducted in triplicate (three replicate tanks per group). Randomization was performed using a simple random allocation method to ensure unbiased distribution of individuals among treatments. As the LC_{50} value fell within the low-toxicity range, the control group was fed a basic meal devoid of any supplements including plant extracts. The feed supplemented with 0.25g/kg (0.025%) of *M. fragrans* extract was given to Treatment-1 (T1), Treatment-2 (T2) got a greater supplementation level of 0.5g/kg (0.05%) feed. The selected inclusion levels (0.025% and 0.05%) were based on preliminary trials and previously reported effective ranges of phytogenic additives in aquaculture species. The prawns were fed the experimental diets for 8 weeks under the assigned treatment conditions. An ethanolic extract of *M. fragrans* was incorporated into a nutritionally formulated experimental diet containing 33% crude protein, 8% crude fat, and 12% moisture, following the methodology established by Balasubramanian et al.; (2008a). Initially, a predetermined amount of extract was dissolved in ethanol, and the resulting solution was uniformly sprayed onto the feed to ensure thorough coating and homogenous mixing with the feed ingredients. These pellets were initially air-dried and subsequently oven-dried at 40°C to ensure uniform moisture reduction and stability of the bioactive compounds. A control diet, identical in nutritional composition but devoid of the extract, was also prepared. To prevent rapid leaching of the extract upon immersion in water, all diets were coated with a thin layer of edible gel, (gelatin-based coating following standard aquafeed coating procedures)**,** effectively enhancing the retention of active compounds during feeding trials. Pellet stability and leaching loss were assessed following standard aquafeed evaluation protocols. The stocking density of juvenile prawns was 15 per tank and observed for 14 days after the challenge and given an intramuscular injection of 100 µL of *V. parahaemolyticus* (10^6 CFU/mL in PBS; Ghosh et al., 2024b) into the dorso-lateral area of the third abdominal segment after 8 weeks of feeding trial. The bacterial challenge dose (10^6 CFU/mL) was selected based on preliminary trials to induce sub-lethal infection and allow comparative evaluation of survival across treatments (approximate LD_{50} range). Infection was confirmed based on clinical signs and cumulative mortality patterns following bacterial challenge. A sterile intraperitoneal injection of phosphate-buffered saline (PBS) was administered to one group for making negative control. Before the bacterial challenge, growth performance metrics (feed conversion ratio, weight increase, and specific growth rate) were evaluated. Immune responses, such as hemolymph clotting time (HCT), respiratory bursts (RBs), prophenoloxidase (proPO), superoxide dismutase (SOD) activity, total haemocyte count (THC), and superoxide anion generation, were measured both before and after exposure to the *V. parahaemolyticus*.

Growth measurement

At the end of the 8-week feeding trial and prior to the bacterial challenge, growth performance metrics were

recorded, and key parameters were analyzed. To assess how well the diet was used, additional measures such as the feed conversion ratio (FCR) and protein efficiency ratio (PER) were determined.

According to Ahmmed et al., 2025; the formulae are as follows;

"Average weight gain (AWG, g) $= W_t - W_0$

Individual growth rate (GR, g day^{-1}) $= \frac{W_t - W_0}{56}$

Specific growth rate (SGR, % day^{-1}) $= 100 \times \frac{\ln W_t - \ln W_0}{56}$ where W_t and W_0 denote the final and initial mean body weights (g), respectively.

Feed conversion ratio (FCR) $= \frac{\text{Feed intake (g)}}{\text{Weight gain (g)}}$

Protein efficiency ratio (PER) $= \frac{\text{Weight gain (g)}}{\text{Protein intake (g)}}$

Survival rate (SR, %): $= 100 \times \frac{\text{Final prawn count}}{\text{Initial prawn count}}$"

Preparation of bacterial inoculums

Targeted pathogen (*V. parahaemolyticus)* was collected from Shrimp Research Station at the Fisheries Research Institute in Bagerhat, Bangladesh. Pathogen was then cultivated on TCBS agar plates for 18–24 hours at a temperature of 37°C (Morris et al., 1976). A colony was subsequently collected and cultivated in a nutrient broth with 2% NaCl. The concentration of bacteria was assessed using the OD value of 600 nm absorbance via a spectrophotometer, resulting in a measurement of 6×10^8 CFU/mL (Peng et al., 2016). The bacterial suspension was adjusted to a concentration of 1×10^6 CFU/mL in PBS for use in the experimental challenge. The selected bacterial concentration was validated based on preliminary pathogenicity testing to ensure reproducibility of infection. For the experimental infection, each prawn was injected into the third abdominal segment with a 100 µL aliquot of the prepared bacterial suspension using a sterile 1 mL insulin syringe equipped with a 26-gauge needle (Ahmmed et al., 2025; Karnjana et al., 2019).

Immune parameters test

All immune assays were performed in triplicate to ensure reproducibility, and both intra- and inter-assay variations were minimized by maintaining identical experimental conditions.

Procedure of assessing total haemocyte count (THC)

A volume of 100 µL of haemolymph was obtained using a 26-gauge needle attached to a 1 mL pipette that was preloaded with an anticoagulant (Sadia et al., 2024). To conduct an immediate assessment, the hemolymph was diluted in a 1:1 ratio by combining 100 µL of anticoagulant with 100 µL of the collected sample. A single drop of Rose Bengal staining reagent was added to a 20 µL aliquot of the diluted mixture. A small amount of this stained solution was then meticulously placed onto a hemocytometer (Precicolor HBG, Germany), covered with a cover slip, and analyzed under a microscope (Labomed, USA) for the purpose of cell counting. The THC along with the corresponding dilution factor (Dcf) were calculated using the formulas according to Biswas et al. (2023). The THC was determined using a Neubauer hemocytometer and calculated according to the following formula:

"$THC = (\frac{A+B+C+D}{4}) \times 10^4 \times D_{cf}$"

Where A, B, C, and D represent the counts of hemocytes in four separate blocks of the hemocytometer, and Dcf is the dilution correction factor, calculated as:

"$D_{cf} = \frac{\text{Volume of anticoagulant + Volume of hemolymph}}{\text{Volume of hemolymph}}$,,

Determination of hemolymph clotting time (HCT)

According to (Liu et al., 2019; Ahmmed et al., 2025),100 µL of hemolymph was extracted and immediately put into a 1.5 mL microcentrifuge tube that had been frozen to 4°C. A 25 µL aliquot of the cooled hemolymph was carefully transferred into a sterile glass capillary tube using a calibrated micropipette under aseptic conditions. The vertical orientation of the capillary tube allowed the hemolymph to migrate from the upper to the lower end due to gravity. To reverse the direction of flow, the tube was vertically inverted each time when the liquid reached the bottom end. The clotting time is the amount of time that has passed between initial loading and coagulation.

Procedure of assessing prophenoloxidase (ProPO)

Hemolymph samples were collected from experimental prawns in a 1:1 ratio with an anticoagulant solution. Following the protocol adapted from Zhu et al. (2019), the samples were centrifuged at 700 × g for 10 minutes at 5 °C using a pH 7 buffer. The supernatant was carefully removed, and the resulting cell pellet was gently washed and resuspended in cacodylate buffer. An equal volume of zymosan suspension was added to the hemocyte suspension, followed by incubation at room temperature for 1 hour (Ahmmed et al., 2025). After incubation, the mixture was centrifuged for 5 minutes at 700 × *g*, and 60 µL of the supernatant was transferred to individual wells of a microtiter plate. Subsequently, 25 µL of L-DOPA solution (4 mg/mL in cacodylate buffer) was added to each well. After a reaction period of 10 minutes, 300 µL of cacodylate buffer was added to halt the reaction. Optical density was measured at 490 nm using a microplate reader (Multiskan GO, Thermo Scientific, Porto, Portugal).

Assessment of respiratory burst activity

A 100 µL aliquot of prawn hemolymph was combined with 300 µL of anticoagulant solution and incubated at room temperature for 30 minutes, following the procedure described by Munaeni et al. (2020). The cell suspension was centrifuged at 3,000 × *g* for 20 min. The supernatant was discarded, and the resulting cell pellet was resuspended in 100 µL of nitroblue tetrazolium (NBT) solution (0.3% NBT in Hank's Balanced Salt

Solution supplemented with 10 mM $CaCl_2$, 3 mM $MgCl_2$, and 5 mM $MgSO^4$). The final pellet was solubilized in a mixture of 140 µL dimethyl sulfoxide (DMSO) and 120 µL of 2 M potassium hydroxide (KOH). The optical density of the solution was measured at 630 nm using a microplate reader to assess the reduction of NBT.

Measurement of superoxide dismutase enzyme activity

A modified methodology based on Creative BioMart's kit (EC 1.15.1.1) was used to quantify SOD activity, using techniques from Leela et al., (2025). Solutions A (Tris-EDTA buffer) and B (0.2 mM pyrogallol) were used in the experiment to assess the enzyme's ability to prevent pyrogallol autoxidation. Using a UV spectrophotometer, absorbance changes were observed at 325 nm. SOD activity was calculated by 50% suppression of pyrogallol autoxidation at 30 ± 2°C. The activity of superoxide dismutase (SOD) was calculated using the following equation:

$$\text{“SOD activity (U mg}^{-1}) = \frac{(\Delta A_{325,\text{blank}} - \Delta A_{325,\text{sample}})/\Delta A_{325,\text{blank}} \times 100\%}{50\%} \times R \times \frac{D}{V} \times \frac{V_1}{m}$$

Where:

- $\Delta A_{325,\text{blank}}$= autoxidation rate of the blank,
- $\Delta A_{325,\text{sample}}$= autoxidation rate of the sample,
- V= volume of the sample,
- D= dilution factor,
- V_1= total volume of the sample,
- m= weight of the solid sample,
- R= volume of the reaction mixture (4.5 mL).”

Proximate composition assessment

Estimation of protein content

The three essential phases of the classic micro-Kjeldahl method, digestion, distillation, and titration; were used to assess the crude protein concentration. According to Abdissa et al. (2025), crude protein was calculated by multiplying the total nitrogen content obtained from this process by a conversion factor of 6.25. The findings were then represented on a dry weight basis. Following Ahmmed et al. (2025), the amount of gross nitrogen was determined using the following formula:

$$\text{“\% of total N} = \frac{Volume\ of\ Hcl \times 0.014 \times Normality\ of\ Hcl}{Weight\ of\ sample(gm)}$$

% of Crude protein = % N×6.25”

Measurement of Crude Lipid Levels

A modified Folch technique, the crude lipid content was ascertained by solvent extraction using a mixture of methanol and chloroform solution at1:2 (v/v) ratio. The percentage of lipid content was determined using the following formula:

$$\text{“\% of lipid} = \frac{Weight\ of\ lipid}{Weight\ of\ the\ sample} \times 100\text{”}$$

Determination of ash content

To guarantee full combustion of organic materials, the crucibles holding the samples were put in a muffle furnace and burned for eight hours at 550°C. The crucibles were heated, then carefully moved to a desiccator to cool to room temperature. The crucible and any remaining ash were weighed after cooling, and the weight of the empty crucible was deducted from this figure to calculate the amount of ash present. The percentage of ash levels was determined using the following formula.

$$\text{“\% of ash content} = \frac{\text{Weight of crucible PLus sample after ashing- Empty Weight of Crucible}}{Weight\ of sample\ before\ ashing} \times 100\text{”}$$

Assay for digestive enzyme activity

According to Ahmmed et al. (2025), the digestive tract and hepatopancreas of *M. rosenbergii* were homogenized in a beaker with ice-cold distilled water at a ratio of 1:2 (w/v). The homogenate was centrifuged at 9,300 × g for 20 min at 4°C, and the resulting supernatant was collected as the crude enzyme extract. The crude enzyme was stored at −80°C for future use.

Determination of protease activity

Furné et al. (2005) detailed the technique of casein hydrolysis, utilizing casein as the substrate to evaluate protease activity. The reaction mixture consisted of 100 µL of crude enzyme extract, 250 µL of 0.1 M glycine-NaOH buffer (pH 10), and 250 µL of 1% (w/v) casein. The mixture was incubated for one hour at 37°C to promote enzymatic digestion. A standard curve was established using known tyrosine concentrations to express protease activity. A single unit refers to the amount of enzyme that, when tested under specific conditions, produces one µg of tyrosine each minute.

Determination of amylase activity

The assessment of amylase activity was conducted using the method outlined by Sahandi et al. (2025), which relies on the enzymatic hydrolysis of starch and the resulting decrease in absorbance of the starch-buffer solution. Following the incubation period, the absorbance of the reaction mixture was assessed at a wavelength of 600 nm. A standard curve was created utilizing known concentrations of maltose (Muralisankar et al., 2014).

Statistical analysis

All data are presented as mean ± standard deviation (SD). The normality of the data was evaluated using the Shapiro-Wilk test. Statistical analyses were performed using SPSS software (Version 30). Differences among treatment groups were assessed by one-way analysis of variance (ANOVA), followed by Tukey’s post hoc test for multiple comparisons. Paired comparisons between pre- and post-challenge conditions were evaluated using Student’s t-test. Differences were considered statistically significant at $p < 0.05$.

Results

Effect of Nutmeg extract on the growth performance of prawn

Following an 8-week feeding trial, the addition of nutmeg extract to the prawn diet resulted in significant variations in growth performance, feed utilization, and survival rates across the treatment groups. Each group began with comparable starting weights. Nonetheless, prawns in T_1 showed a marked improvement in final weight, weight gain, and specific growth rate when compared to the control and T_2 groups ($p < 0.05$). The feed conversion ratio and protein efficiency ratio were notably more advantageous in T_1, indicating enhanced feed utilization efficiency. While T_2 demonstrated some moderate enhancements compared to the control in certain parameters, the effects were not as significant as those seen in T_1. Furthermore, survival rates were greatest in T_1, with T_2 following closely, as both surpassed the control group. The results indicate that using nutmeg extract in the diet, especially in T_1, can significantly enhance growth, improve feed efficiency, and increase survival rates in prawns.

Immunomodulatory effects of *M. fragrans* extract in freshwater prawns

Total haemocyte count (THC)

A consistent enhancement in THC was observed in the groups supplemented with nutmeg extract when compared to the control (Figure 1; $p < 0.05$). At both the 4th and 8th weeks prior to challenge, the low-dose treatment (T_1) exhibited significantly higher THC values ($p < 0.05$). The high dose group (T_2) also showed increased THC, though to a slightly lesser extent. Post-challenge, THC levels remained elevated in both treatment groups relative to the control, suggesting a sustained immune response. The low-dose group continued to outperform the high-dose and control groups, highlighting its potential to enhance innate immunity in *M. rosenbergii*.

Table 2: Impact of nutmeg diet on the growth, feed efficiency, and survival rates of prawns following an 8-week feeding trial

Observed parameters	**Control (Mean ± SD)**	**T_1 (Mean ± SD)**	**T_2 (Mean ± SD)**
Baseline body weight (g)	2.1 ± 0.25^a	2.1 ± 0.25^a	2.1 ± 0.25^a
Terminal body weight (g)	4.4 ± 0.5^a	5.5 ± 0.7^b	4.6 ± 0.4^a
Net body weight (g)	2.3 ± 0.17^a	3.5 ± 0.05^b	2.5 ± 0.15^a
Specific growth rate (% day^{-1})	1.36 ± 0.07^a	1.78 ± 0.02^b	1.44 ± 0.06^a
Feed conversion ratio	4.1 ± 0.02^a	2.1 ± 0.14^b	3.1 ± 0.01^c
Protein efficiency ratio	0.79 ± 0.00^a	1.52 ± 0.10^b	1.02 ± 0.00^c
Survivability Rate (%)	70±4.7	90.0±9.4	83.3±4.7

(At the 5% level of significance, groups labeled with different superscripts are considered significantly different, with T1 and T2 representing Treatment 1 and Treatment 2, respective

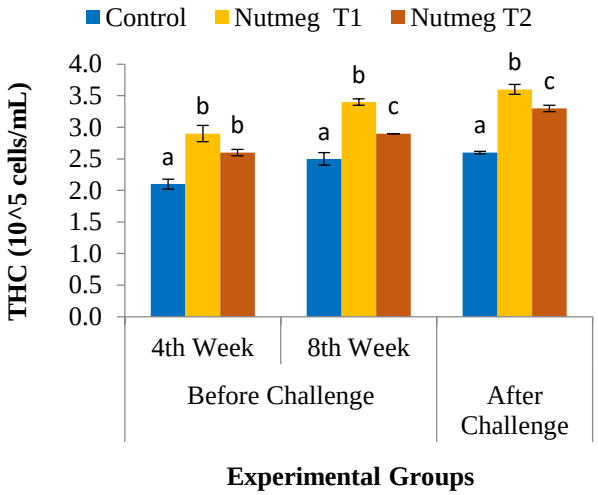

Figure 1: Influence of feeding *M. rosenbergii* with extract-supplemented diets on THC assessed prior to and following exposure to *V. parahaemolyticus*

(At the 5% level of significance, groups labeled with different superscripts are considered significantly different, with T1 and T2 representing Treatment 1 and Treatment 2, respectively)

Haemolymph clotting time (HCT)

The study revealed a significant decrease in clotting time for prawns that were given diets supplemented with nutmeg extract, in contrast to the control group (Figure 2; $p < 0.05$). During the 4th and 8th weeks, the treatment showed significantly reduced clotting times, suggesting an improvement in hemolymph coagulation activity. The decrease was more significant in the low-dose group (T_1), especially at the initial time point. After the challenge, the control group showed a significant reduction in clotting time, while the groups that received the extract exhibited fairly consistent responses.

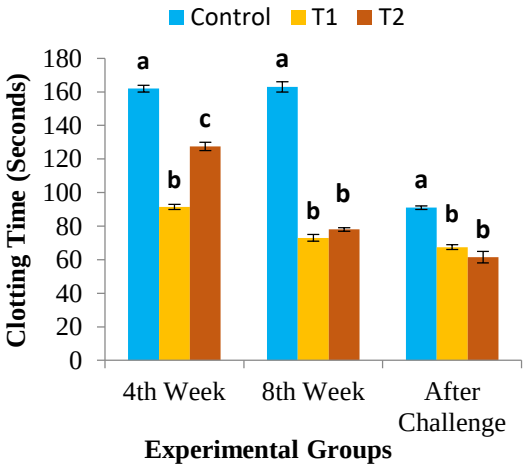

Figure 2: Comparison of HCT values in extract-supplemented and control *M. rosenbergii* under pre- and post-challenge conditions with *Vibrio parahaemolyticus*

(Groups labeled with different superscripts are considered significantly different, with T1 and T2 indicating Treatment 1 and Treatment 2, respectively)

Prophenoloxidase (proPO) activity

Figure 3 illustrates the Prophenoloxidase (ProPO) activity in *M. rosenbergii* that were given extract-supplemented diets compared to control groups, both prior to and following the challenge with *V. parahaemolyticus* ($p < 0.05$). A significant rise in ProPO activity was noted in the extract-fed groups when compared to the control. The greatest activity was observed in prawns that were given the T1 diet, which included a 0.025% extract supplementation. While there was no remarkable difference observed between T1 and T2, both treatments demonstrated significantly elevated ProPO values compared to the control ($p < 0.05$).

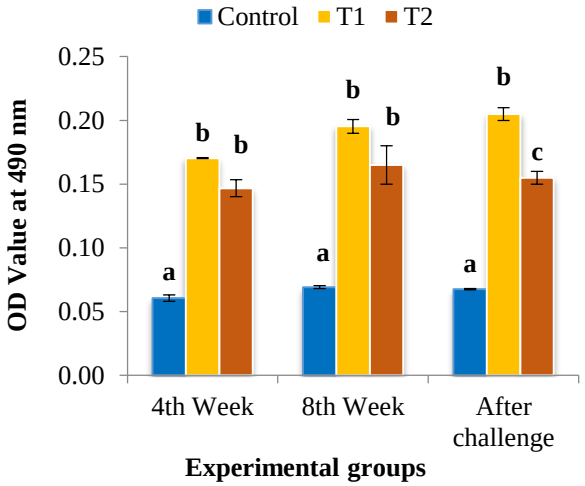

Figure 3: proPO response in *M. rosenbergii* fed extract-supplemented diets prior to and following bacterial challenge

(Groups labeled with different superscripts are considered significantly different (5% significance level), with T1 and T2 representing Treatment 1 and Treatment 2, respectively)

Respiratory burst (RBs) activity

Figure 4 illustrates the respiratory burst (RB) activity of *M. rosenbergii* that were fed diets supplemented with extracts, alongside control groups, both prior to and following the challenge with *V. parahaemolyticus*. The groups that received the extract (T1 and T2) showed increased RB activity when compared to the control, both before and after the challenge conditions. The control group exhibited the lowest RB values, whereas T1 displayed the highest activity, both prior to and following the challenge. A statistically significant difference ($p < 0.05$) was observed among all groups.

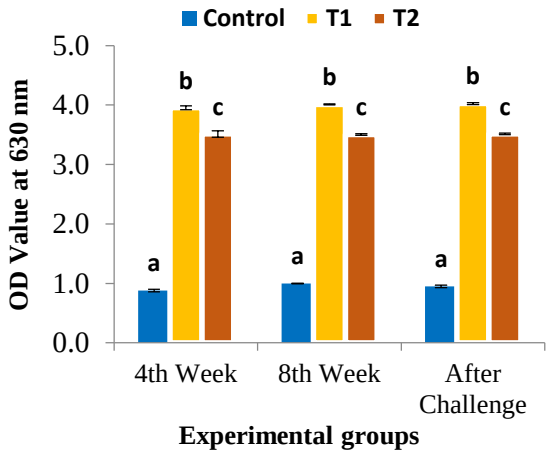

Figure 4: Respiratory burst activity in *M. rosenbergii* fed extract-treated diets: comparison before and after pathogen exposure

(Groups labeled with different superscripts are considered significantly different, with T1 and T2 representing Treatment 1 and Treatment 2, respectively)

Superoxide anion generation

Figure 5 illustrates the levels of superoxide anion production in *M. rosenbergii* that received diets enhanced with extracts at various ratios, compared to control groups, both before and after the challenge with *V. parahaemolyticus*. The control group exhibited lower superoxide levels when compared to the treated groups. Between the two treated groups, T1 (0.025% extract) demonstrated the most notable superoxide anion activity both prior to and following the challenge when compared to T2 (0.05%). A notable difference was found among all groups, with statistical significance ($p < 0.05$).

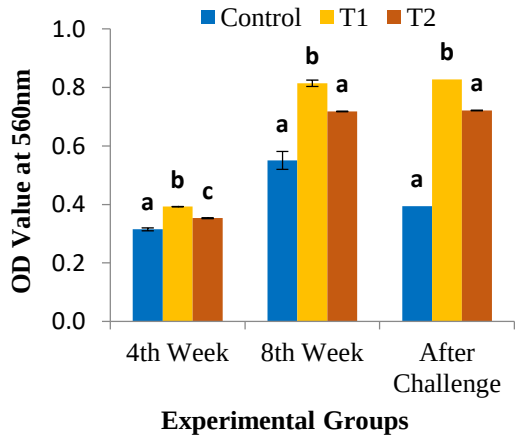

Figure 5: Evaluation of superoxide anion activity in *M. rosenbergii* fed extract-supplemented diets compared to controls before and after *V. parahaemolyticus* exposure

(Groups labeled with different superscripts are considered significantly different, with T1 and T2 representing Treatment 1 and Treatment 2, respectively)

Superoxide dismutase (SOD) activity

Figure 6 illustrates the activity of SOD in *M. rosenbergii* that received diets supplemented with *M. fragrans* extract (T1: 0.025%; T2: 0.05%), in comparison to the control groups, both prior to and after the challenge with *V. parahaemolyticus*. Before the challenge, SOD activity was significantly elevated in the extract-treated groups when compared to the control ($p < 0.05$), suggesting a strengthened antioxidant defense mechanism. After exposure to the pathogen, both treatment groups showed a significant rise in SOD activity in comparison to the control. Interestingly, the activity of SOD decreased in the control group after the challenge, indicating a possible oxidative stress burden. The prawn group that was administered a lower dose of extract (T1 group) exhibited higher values when compared to the higher dose of extract (T2 group) and the control group.

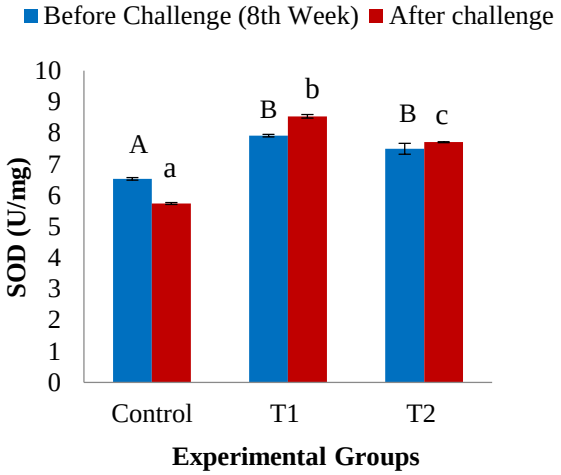

Figure 6: Influence of dietary extract on Superoxide Dismutase (SOD) enzyme activity in *M. rosenbergii* before and after *Vibrio parahaemolyticus* exposure

(Different superscripts are considered significantly different, with T1 and T2 representing Treatment 1 and Treatment 2, respectively)

Impact of dietary extracts on the enzyme activity of prawn

Digestive amylase response

The results indicated that (T_1) exhibited the highest enzymatic activity, with (T_2) and the control group trailing closely behind. T_2 exhibited an increased level of enzymatic activity in comparison to the control, although it was not as pronounced as that of T_1. Despite the numerical discrepancies observed, the statistical analysis revealed no significant difference ($p > 0.05$) in amylase activity among the control, T_1, and T_2 groups.

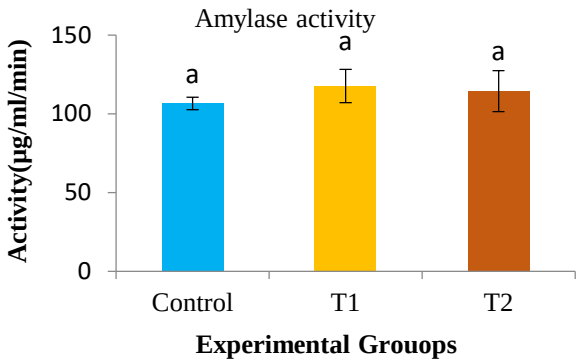

Figure 7: Influence of treatments on the activity of amylase enzyme

(Groups labeled with different superscripts are considered significantly different, with T1 and T2 representing Treatment 1 and Treatment 2, respectively)

Protease enzymatic response

The findings demonstrate that protease activity exhibited a significant treatment effect, with prawns receiving 0.025% extract (T1) showing the highest level of enzymatic activity ($p < 0.05$, Figure 8). Both T1 and T2 groups showed significantly higher protease activity compared to the control group, indicating a positive effect of dietary supplementation. However, no statistically significant difference was observed between T1 and T2 ($p > 0.05$), suggesting a similar response at both inclusion levels.

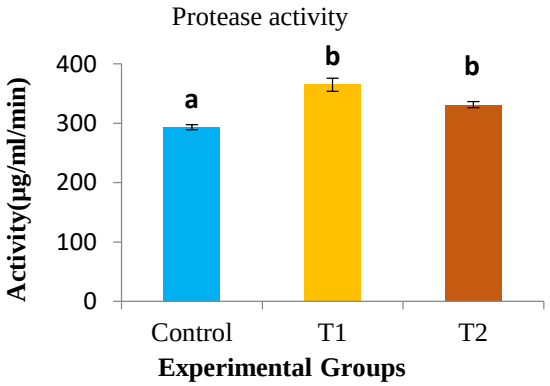

Figure 8: Influence on enzymatic protease function

(Groups labeled with different superscripts are considered significantly different, with T1 and T2 representing Treatment 1 and Treatment 2, respectively)

Impact of M. fragrans extract on proximate composition *of M. rosenbergii*

The analysis of prawn muscle tissue after 8 weeks of dietary supplementation with varying levels of *Myristica fragrans* (nutmeg) extract showed notable differences in protein and ash content, whereas lipid levels did not exhibit any significant changes across the treatments. The dietary treatments had a significant impact on protein content. The T_1 group (0.025%) showed the highest protein concentration; significantly surpassing that of both the control and T_2 (0.05%) groups. Interestingly, the T_2 group exhibited a slight increase compared to the control; however, this value was significantly lower than that of the T_1 group.

Table 3: Modulation of *M. rosenbergii* nutritional profile by *M. fragrans* extract

Treatments	Protein (%)	Lipid (%)	Ash (%)
Control	18.07 ± 0.09^{a}	2.00 ± 0.03^{a}	3.13 ± 0.03^{a}
T1	20.0 ± 0.057^{b}	2.01 ± 0.02^{a}	3.42 ± 0.05^{b}
T2	18.6 ± 0.092^{c}	2.02 ± 0.035^{a}	3.31 ± 0.03^{b}

(At the 5% level of significance, groups labeled with different superscripts are considered significantly different, with T1 and T2 representing Treatment 1 and Treatment 2, respectively)

This indicates a non-linear response to increasing extract concentration and suggests that an optimal dose of 0.025% is effective for promoting protein accretion. The lipid content was stable across all groups, showing no significant differences ($p > 0.05$), which suggests that the addition of nutmeg extract did not affect lipid deposition in muscle tissue. Ash content demonstrated a notable increase in both T_1 and T_2 groups when compared to the control ($p < 0.05$). Nonetheless, there was no notable difference between T_1 and T_2, suggesting that the extract supplementation, irrespective of concentration, had a beneficial effect on mineral accumulation.

Post-challenge survival performance of prawns after infection with *Vibrio parahaemolyticus*

The survivability trends observed post-pathogen challenge reveal distinct differences among the experimental groups. The negative control (NC) group exhibited consistent and complete survivability throughout the entire trial. In contrast, the control group, which did not receive any treatment, showed a progressive and substantial decline in survivability, with mortality increasing steadily from the early days of infection and continuing throughout the observation period.

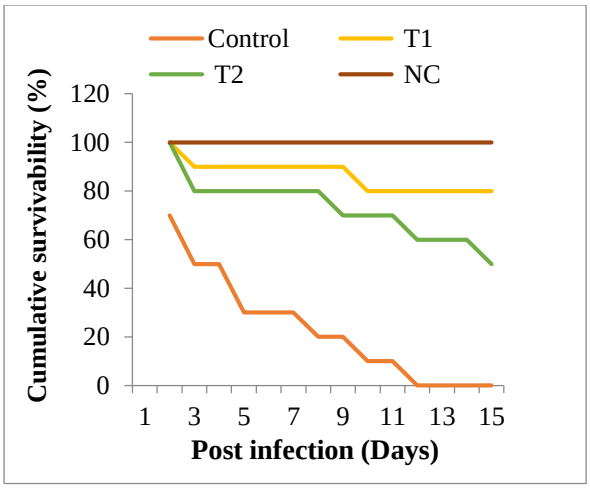

Figure 9: Cumulative Survivability of Extract-Fed and Control Groups *M. rosenbergii* following exposure to *V. parahaemolyticus* (T1 = Treatment 1, T2 = Treatment 2)

Among the treated groups, T1 exhibited superior survivability compared to T2, with only minimal reduction over time. T2, while demonstrating a moderate protective effect, showed a more noticeable decline in survivability than T1 but remained significantly more resilient than the untreated control group. Overall, the results suggest that both T1 and T2 treatments offered protective benefits against pathogen-induced mortality, with T1 being more effective in sustaining survivability throughout the infection period.

Discussion

The aquaculture industry is considered as one of the fastest-growing and leading sectors globally. Currently, the application of natural or feed additives derived from medicinal plants or spices is on the rise each day (Mahboub et al., 2022; Ahmed et al., 2022; Sula et al., 2020). Outbreaks of pathogens remain a significant challenge for prawn aquaculture, frequently leading to considerable economic setbacks. The limited availability of approved preventive measures, most of which rely on antibiotics, highlights the need for alternative strategies, as excessive antibiotic use can have adverse effects on both prawn health and the surrounding environment. As a result, it is crucial to investigate different approaches that could improve disease resistance in cultured species. This study shows that adding *Myristica fragrans* to the diet has a beneficial impact on *Macrobrachium rosenbergii* in various ways, such as enhancing growth performance, improving feed efficiency, boosting immune responses, increasing antioxidant capacity, elevating enzymatic activity, and improving survival rates after exposure to *Vibrio parahaemolyticus*.

The results indicate that *M. fragrans* could be a practical and environmentally friendly substitute for traditional antimicrobials, enhancing health and resilience in prawn culture systems. However, it is essential to recognize that the benefits seen in the study are context-dependent. While a lower dose of the extract (0.025%) enhanced growth performance and feed efficiency significantly, the results could be influenced by the metabolic costs associated with immune responses and oxidative stress. These results align with the observations of several studies such as Sodamola et al. (2017), Zhelyazkov et al. (2018) and Canadianti et al. (2020) who reported that incorporating *Myristica* as a feed additive enhanced growth performance, reduced FCR and reduced mortality in different fish. The metabolic cost of immunity often leads to a trade-off between energy allocation for immune defense and other physiological processes, such as growth. However, due to the presence of toxic or anti-nutritional substances, the addition of a significant number of extracts may limit growth when compared to a smaller amount (Radhakrishnan et al. 2015; Prabu et al. 2018; Kaleo et al. 2019).

The current study demonstrated that supplementation with the extract led to a notable enhancement of total hemocyte count (THC) in *M. rosenbergii* compared to the control group, both prior to and following exposure to *V. parahaemolyticus* (Fig. 1). This increase suggests that the extract may play a role in stimulating the prawn's cellular immune response. The findings of this study indicate that supplementation of *M. rosenbergii* diets with a lower dose (0.025%) of *M. fragrans* extract resulted in the highest THC values. Comparable findings have been also reported in prawns fed diets supplemented with *Withania somnifera* extracts, where inclusion of lower doses led to a significant increase in total hemocyte count (THC). These enhancements in hemocyte levels were associated with improved pathogen resistance relative to the control group (Harikrishnan et al., 2012). The observed decline in hemocyte numbers in infected shrimp may result from the migration and accumulation of hemocytes at the site of injection, where they participate in tissue repair and the phagocytosis of foreign particles (Balasubramanian et al., 2008b; Sarathi et al., 2007).

In the current study, both before to and following exposure to bacterial challenge, prawns supplemented with the extract showed a significant rise ($p < 0.05$) in prophenoloxidase (proPO) activity in comparison to the untreated group (Fig:3). Similarly, the extract of *Z. officinale* and its component zingerone both improved *L. vannamei*'s PO activity when tested against bacteria (Chang et al. 2012; Shahraki et al. 2021). Furthermore, extracts of *Z. officinale* and *A. marmelos* promoted proPO enzyme secretion (Ghosh et al., 2024a). In some cases, lower doses of the extract may lead to enhanced prophenoloxidase (proPO) activity (Isla et al. 2001). Low doses may gradually boost the immune system, increasing proPO activity while reducing stress. In contrast, excessive doses may depress immunity due to toxicity or an oxidative imbalance.

Superoxide dismutase (SOD) activity is frequently employed to evaluate crustacean health (Chang et al., 2012). Nutmeg's beneficial effects on oxidation limitation or inhibition may be attributed to its tribonoid, phenolic, and flavonoid compositions. These chemicals act as antioxidants, eliminating oxygen-free clothing, metals, and radicals (Jukić et al., 2006). The most common bioactive molecule found in nutmeg was sabinene. It has been shown that this chemical has high antioxidant activity (Quiroga et al., 2015). Nutmeg's immunological and antioxidant activity was most effective at 2% and 3% herb-additive levels (Rashidian et al., 2022). In this sudy, both before and after being exposed to the bacterial challenge, where T1 (0.025%) produced a better effect, the prawns that were supplemented with *M. fragrans* extract in their diet showed a significant increase in SOD activity in comparison to the untreated group (Fig:6) ($p < 0.05$).

The reported reduction in immune parameters at the increased doses of *M. fragrans* (T2) may be potentially explained by the toxicity. This brings in the question of the optimal dosage that will maximize the immune response without the point where toxicity will occur. To gain a complete picture of the effects of the extract on the health of prawns it would be of benefit to explore in more detail the dose-dependent effects of *M. fragrans*, and whether it is possible to have a hormetic effect. This type of a response may explain the improvement of immune functions at low doses and may suggest that excessive doses overwhelm the antioxidant defense systems, causing the subsequent decline of immune functions due to toxicity. Such results are consistent with Citarasu et al. (2006) and Balasubramanian et al. (2008a), who discovered that excessive amounts of dietary

immunostimulants may suppress immune function, which could be associated with the oxidative imbalance and metabolic stress.

In the present study, prawns that ate diets with added spice extracts had much higher levels of protein and ash ($p < 0.05$), but their lipid levels stayed the same (Table 3). The increase in protein and ash suggests that the body is better at absorbing nutrients and storing them, which is important for supporting immune functions like hemocyte proliferation and antioxidant enzyme activity. In general, digestive enzymes are considered for playing a crucial role in regulating growth rates in aquatic organisms (Bilen et al., 2020; Reverter et al., 2021). Recently, Pelinson et al. (2025); Ahmmed et al. (2025); and Simtoe et al. (2024); reported that supplementation with turmeric (*Curcuma longa*), Fennel (*Foeniculum vulgare)* and ginger (*Zingiber officinale*) significantly enhanced both weight gain and digestive enzyme activity in *M. rosenbergii*. Similarly, the current study demonstrated that the supplementation of *M. rosenbergii* diets with spice extracts significantly increased the activity of digestive enzymes, especially protease (Fig. 8). In contrast (Fig:7), no significant change was observed in amylase activity in this investigation, which may be attributed to normal physiological regulation or interference from other factors, as suggested by Nothman and Callow (1971). This finding suggests that the incorporation of these bioactive compounds may enhance digestive functions, consequently improving nutrient absorption and overall metabolic efficiency in the prawns.

The current study demonstrated that *M. rosenbergii* fed diets supplemented with plant extracts exhibited enhanced survival following bacterial challenge in comparison to unsupplemented controls (Fig. 9). Similarly, Harlina et al. (2021) demonstrated that the inclusion of *Chromolaena odorata* extract in crustacean diets significantly improved survival during *Vibrio harveyi* infection, thereby reinforcing the efficacy of phytogenic supplements in enhancing disease resistance among aquaculture species. The shrimp that ate diets with the extract had better survival rates. This is probably because the bioactive components of the extract boost the host's immune defenses. Supplementation caused significant increases in the number of hemocytes, the activation of the prophenoloxidase system, the activity of superoxide dismutase, and the strength of respiratory burst responses. Also, there were improvements in the proximate composition and digestive enzyme activities, which made the body stronger overall. All of these improvements to the shrimp's immune system and metabolism seem to make it better able to fight off harmful pathogens.

Conclusions

Prawns fed *M. fragrans*-supplemented diets showed lower mortality, indicating improved resistance under experimental conditions to *Vibrio parahaemolyticus*. Dietary inclusion at 0.025% (T1) and 0.05% (T2) enhanced growth performance, immune responses, digestive enzyme activity (particularly protease), and proximate composition, with the lower dose (T1) showing superior effects. However, these results are based on controlled laboratory conditions and require validation under field conditions. A more extensive study of the hormetic response, the balance of oxidative stress and the long-term metabolic cost of *M. fragrans* supplementation should be carried out in the future in order to gain a complete understanding of the effect that *M. fragrans* supplementation has on aquaculture species. The primary limitation is the potential underestimation of the long-term effects of *M. fragrans* supplementation**,** as the study primarily focused on short-term health markers such as growth and immune response. It is crucial to assess the long-term metabolic and immunological consequences of prolonged exposure to *M. fragrans***,** as chronic supplementation could have varying effects on prawn health, which may differ from short-term outcomes.

Acknowledgement

The authors are grateful to the Head and colleagues in the Fisheries and Marine Resource Technology Discipline. The authors would like to thanks to Md. Sumon Rahman and some undergrad students for their continuous support.

Conflict of Interest

The authors declare no conflict of interest.

Authors' contribution

Farhana Nasrin: Methodology, Data curation, Software, Writing-original draft preparation; **Shaikh Shaon Ahmmed:** Methodology, Data curation, Writing-original draft preparation; **Halima Tus Sadia:** Methodology, Data curation, Software; **Uttam Adhikary:** Methodology, Data curation, Software, Writing-original draft preparation; **Jannatul Ferdaus Jyoti:** Methodology, Data curation; **Md. Mahmudul Hasan:** Methodology, Data curation; **Rajdwip Sarkar:** Methodology, Data curation, Writing-original draft preparation; **Abul Farah Md. Hasanuzzaman**: Supervision, Writing - review & editing; **Alokesh Kumar Ghosh**: Supervision, Monitoring, Funding acquisition, Writing – review & editing.

References

Abdissa, B., Gete, M., & Alemu, E. (2025). Earthworm (*Eisenia Fetida*) Meal as Fishmeal Replacement in the Diet of *Oreochromis Niloticus* (Linnaeus, 1758) Fingerlings. *Aquaculture, Fish and Fisheries*, *5*(4), e70105.

AftabUddin, S., Roman, W. U., Hasan, C. K., Ahmed, M., Rahman, H., & Siddique, M. A. M. (2018). First incidence of loose-shell syndrome disease in the giant tiger shrimp *Penaeus monodon* from the brackish water ponds in Bangladesh. *Journal of Applied Animal Research*, *46*(1), 210-217.

Ahmed, N., Allison, E. H., & Muir, J. F. (2010). Rice fields to prawn farms: A blue revolution in southwest Bangladesh? *Aquaculture International*, *18*, 555-574.

Ahmed, N., Demaine, H., & Muir, J. F. (2008). Freshwater prawn farming in Bangladesh: history, present status and future prospects. *Aquaculture Research*, *39*(8), 806-819.

Ahmed, N., Thompson, S., & Glaser, M. (2018). Transforming organic prawn farming in Bangladesh: Potentials and challenges. *Journal of Cleaner Production*, *172*, 3806-3816.

Ahmed, S. A. A., Nada, H. S., Ibrahim, S. M., Fahmy, E. M., Khedr, M. H. E., Moustafa, S. M., Ismail, T. A., Gesriha, S., Assayed, M. E., & Mahboub, H. H. (2022). Comparative antitoxic potency of honey and natamycin-supplemented diets against aflatoxicosis and their influences on growth, serum biochemistry, immunohistochemistry, and residual deposition in Nile tilapia (*Oreochromis niloticus*). *Aquaculture*, *551*, 737934.

Ahmmed, S. S., Sadia, H. T., Nasrin, F., Adhikary, U., Sarower, M. G., & Ghosh, A. K. (2025). Enhancing Growth and Immune Responses in Giant Freshwater Prawn (*Macrobrachium rosenbergii*) via Oral Administration of Fennel (*Foeniculum vulgare*) Extract against *Vibrio parahaemolyticus*. *Comparative Immunology Reports*, 200247.

Antasionasti, I., Datu, O. S., Lestari, U. S., Abdullah, S. S., & Jayanto, I. (2021). Correlation analysis of antioxidant activities with tannin, total flavonoid, and total phenolic contents of nutmeg (myristica fragrans houtt) fruit precipitated by egg white. *Borneo Journal of Pharmacy*, *4*(4), 301-310.

Ashokkumar, K., Simal-Gandara, J., Murugan, M., Dhanya, M. K., & Pandian, A. (2022). Nutmeg (Myristica fragrans Houtt.) essential oil: A review on its composition, biological, and pharmacological activities. *Phytotherapy Research*, *36*(7), 2839-2851.

Balasubramanian, G., Sarathi, M., Venkatesan, C., Thomas, J., & Hameed, A. S. (2008a). Oral administration of antiviral plant extract of *Cynodon dactylon* on a large-scale production against white spot syndrome virus (WSSV) in *Penaeus monodon*. *Aquaculture, 279*(1–4), 2–5.

Balasubramanian, G., Sarathi, M., Venkatesan, C., Thomas, J., & Hameed, A. S. (2008b). Studies on the immunomodulatory effect of extract of *Cynodon dactylon* in shrimp, *Penaeus monodon*, and its efficacy to protect the shrimp from white spot syndrome virus (WSSV). *Fish & Shellfish Immunology, 25*(6), 820–828.

Barman, R., Bora, P. K., Saikia, J., Kemprai, P., Saikia, S. P., Haldar, S., & Banik, D. (2021). Nutmegs and wild nutmegs: An update on ethnomedicines, phytochemicals, pharmacology, and toxicity of the Myristicaceae species. *Phytotherapy Research*, *35*(9), 4632-4659.

Bilen, S., Altief, T. A. S., Özdemir, K. Y., Salem, M. O. A., Terzi, E., & Güney, K. (2020). Effect of lemon balm (*Melissa officinalis*) extract on growth performance, digestive and antioxidant enzyme activities, and immune responses in rainbow trout (*Oncorhynchus mykiss*). *Fish physiology and biochemistry*, *46*(1), 471-481.

Biswas, J., Ghosh, A. K., Ahmmed, S. S., Sadia, H. T., & Sarower, M. G. (2023). The impact of Vasaka (*Justicia adhatoda*) leaf extract on the growth and immune response of shrimp (*Penaeus monodon*). *Khulna University Studies, 20(1),* 85–93.

Borja, K. B., Buhion, V. L., & Canalita, E. E. (2016). Toxicity test on different brands of food seasonings using brine shrimp (Artemia salina) lethality test. *Biol Chem Res*, *3*, 227-33.

Canadianti, M., Yusiati, L. M., Hanim, C., Widyobroto, B. P., & Astuti, A. (2020). The effect of nutmeg leaves tannin (*Myristica fragrans* Houtt) as protein protecting agents on in vitro nutrient digestibility. *Buletin Peternakan, 44*, 10–14.

Chakraborty, B. K. (2021). Status of fish diversity and production in Bangladesh. *Integrating Biological Resources for Prosperity; Botanical Society of Nepal: Kathmandu, Nepal; Nepal Biological Society: Biratnagar, Nepal*, 85-99

Chang, Y. P., Liu, C. H., Wu, C. C., Chiang, C. M., Lian, J. L., & Hsieh, S. L. (2012). Dietary administration of zingerone to enhance growth, non-specific immune response, and resistance to *Vibrio alginolyticus* in Pacific white shrimp (*Litopenaeus vannamei*) juveniles. *Fish & Shellfish Immunology, 32*(2), 284–290.

Citarasu, T., Sivaram, V., Immanuel, G., Rout, N., & Murugan, V. (2006). Influence of selected Indian immunostimulant herbs against white spot syndrome virus (WSSV) infection in black tiger shrimp, *Penaeus monodon*, with reference to haematological, biochemical and immunological changes. *Fish & Shellfish Immunology, 21*(4), 372–384.

Cruz, A., Sánchez-Hernández, E., Teixeira, A., Oliveira, R., Cunha, A., & Martín-Ramos, P. (2024). Phytoconstituents and ergosterol biosynthesis-targeting antimicrobial activity of nutmeg (Myristica fragans Houtt.) against phytopathogens. *Molecules*, *29*(2), 471.

Das, B., Manna, S. K., Sarkar, P., & Batabyal, K. (2009). Occurrence of *Vibrio parahaemolyticus* in different finfish and shellfish species. *Journal of food safety*, *29*(1), 118-125.

De Souza Valente, C., & Wan, A. H. (2021). Vibrio and major commercially important vibriosis diseases in decapod crustaceans. *Journal of Invertebrate Pathology*, *181*, 107527.

Defoirdt, T., Sorgeloos, P., & Bossier, P. (2011). Alternatives to antibiotics for the control of bacterial disease in aquaculture. *Current Opinion in Microbiology*, *14*(3), 251- 258.

Devi, N. P. (2024). Pharmacological insights into Myristica fragrans (Nutmeg): a systematic review on the therapeutic applications and bioactive compounds. *PLANT SCIENCE*, *11*.

FAO. 2024. *The State of World Fisheries and Aquaculture 2024 – Blue Transformation in action*. Rome. https://doi.org/10.4060/cd0683en

Furné, M., Hidalgo, M. C., López, A., García-Gallego, M., Morales, A. E., Domezain, A., Domezainé, J., & Sanz, A. (2005). Digestive enzyme activities in *Adriatic sturgeon Acipenser naccarii* and rainbow trout *Oncorhynchus mykiss*. A comparative study. *Aquaculture, 250*(1–2), 391–398.

Ghosh, A. K., Ahmmed, S. S., Islam, H. M. R., Hasan, M. A., Banu, G. R., Panda, S. K., L., & Luyten, W. (2024a). Oral administration of *Zingiber officinale* and *Aegle marmelos* extracts enhances growth and immune functions of the shrimp *Penaeus monodon* against the white spot syndrome virus (WSSV). *Aquaculture International*, 32(1), 613–632.

Ghosh, A. K., Islam, H. R., Banu, G. R., Panda, S. K., Schoofs, L., & Luyten, W. (2024b). Effects of *Piper betle* and *Phyllanthus emblica* leaf extracts on the growth and resistance of black tiger shrimp, *Penaeus monodon*, against pathogenic *Vibrio parahaemolyticus*. *Aquaculture International*, 32(3), 3689-3708.

Ghosh, A. K., Panda, S. K., & Luyten, W. (2021). Anti-*Vibrio* and immune-enhancing activity of medicinal plants in shrimp: A comprehensive review. *Fish & Shellfish Immunology, 117*, 192–210.

Ha, M. T., Vu, N. K., Tran, T. H., Kim, J. A., Woo, M. H., & Min, B. S. (2020). Phytochemical and pharmacological properties of Myristica fragrans Houtt.: an updated review. *Archives of Pharmacal Research*, *43*(11), 1067-1092.

Hamidi, M. R., Jovanova, B., & Panovska, T. K. (2014). Toxicological evaluation of the plant products using Brine Shrimp (*Artemia salina L*.) model. *Macedonian Pharmaceutical Bulletin/Makedonsko Farmacevtski Bilten , 60*(1).

Haque, A. M., Khan, M. A., Hossain, M. M., Hossain, M. E., Nahiduzzaman, M., & Islam, M. S. (2025). Improved aquaculture management practices and its impact on small-scale rural aquaculture farmers in Bangladesh. *Aquaculture*, *594*, 741459.

Harikrishnan, R., Balasundaram, C., Jawahar, S., & Heo, M. S. (2012). Immunomodulatory effect of *Withania somnifera* supplementation diet in the giant freshwater prawn *Macrobrachium rosenbergii* (de Man) against *Aeromonas hydrophila*. *Fish & Shellfish Immunology*, *32*(1), 94-100.

Harlina, H., Kasnir, M., Rosmiati, R., Hamdillah, A., & Wamnebo, M. I. (2021). The effectiveness of *Chromolaena odorata* extract and histopathological change in tiger prawn (*Penaeus monodon*) challenged with *Vibrio harveyi*. *Aquaculture, Aquarium, Conservation & Legislation, 14(3),* 1138-1149.

Ina-Salwany, M. Y., Al-saari, N., Mohamad, A., Mursidi, F. A., Mohd-Aris, A., Amal, M. N. A., & Zamri-Saad, M. (2019). Vibriosis in fish: A review on disease development and prevention. *Journal of Aquatic Animal Health*, *31*(1), 3-22.

Isla, M. I., Moreno, M. N., Sampietro, A. R., & Vattuone, M. A. (2001). Antioxidant activity of Argentine propolis extracts. *Journal of Ethnopharmacology*, *76*(2), 165-170.

Jukić, M., Politeo, O., & Miloš, M. (2006). Chemical composition and antioxidant effect of free volatile aglycones from nutmeg (*Myristica fragrans* Houtt.) compared to its essential oil. *Croatica Chemica Acta, 79*, 209–214.

Kaleo, I. V., Gao, Q., Liu, B., Sun, C., Zhou, Q., Zhang, H., & Song, C. (2019). Effects of *Moringa oleifera* leaf extract on growth performance, physiological and immune response, and related immune gene expression of *Macrobrachium rosenbergii* with *Vibrio anguillarum* and ammonia stress. *Fish & Shellfish Immunology, 89*, 603–613.

Karnjana, K., Soowannayan, C., & Wongprasert, K. (2019). Ethanolic extract of red seaweed *Gracilaria fisheri* and furanone eradicate *Vibrio harveyi* and *Vibrio parahaemolyticus* biofilms and ameliorate the bacterial infection in shrimp. *Fish & Shellfish Immunology, 88*, 91–101.

Kaur, V., Kaushal, S., Kalia, A., Jangra, R., & Sharma, P. (2025). Antimicrobial, synergistic and antibiofilm activities of Myristica fragrans Houtt. Bioactive compounds and their derivatives. *Scientific Reports*, *15*(1), 33041.

Khairan, K., Ginting, B., Sufriadi, E., Amalia, A., Sofyan, H., Muhammad, S., ... & Ernawati, E. (2023, May). Studies on the antioxidant activity of safrole, myristicin and terpeniol from myristica fragrans houtt: a review. In *IOP Conference Series: Earth and Environmental Science* (Vol. 1183, No. 1, p. 012062). IOP Publishing.

Lanari, D., Marcotullio, M. C., & Neri, A. (2018). A design of experiment approach for ionic liquid-based extraction of toxic components-minimized essential oil from *Myristica fragrans Houtt*. Fruits. *Molecules*, *23*(11), 2817.

Leela, M., Pushpalatha, V., Swetha, S., Anusuya, S., Madhavan, S., Eswari, S., Thilagam, H., Thiagarajan, R., & Krishnamurthy, R. (2025). Ingestion of weathered high-density polyethylene microplastics induced oxidative stress and modulation of antioxidant responses in post-larval stages of *Litopenaeus vannamei*. *Frontiers in Marine Science, 12*, 1617070.

Liu, P. C., Lin, P. W., Huang, C. L., Hsu, C. H., & Chen, J. C. (2019). Long-term administration of diets containing *Gracilaria tenuistipitata* extract induce the expression of immune-related genes and increase the immune response and resistance against *Vibrio harveyi* in white shrimp *Litopenaeus vannamei. Gene Reports*, *15*, 100378.

Mahboub, H. H., Elsheshtawy, H. M., Sheraiba, N. I., Fahmy, E. M., Masoud, S. R., Mohamed, E. A. A., Abdelnaeim, N. S., Mohamed, D. I., Ismail, T. A., & Ahmed, S. A. A. (2022). Dietary black cumin (*Nigella sativa*) improved hemato-biochemical, oxidative stress, gene expression, and immunological response of Nile tilapia (*Oreochromis niloticus*) infected by *Burkholderia cepacia*. *Aquaculture Reports*, *22*, 100943.

Manchanayake, T., Salleh, A., Amal, M. N. A., Yasin, I. S. M., & Zamri-Saad, M. (2023). Pathology and pathogenesis of Vibrio infection in fish: A review. *Aquaculture Reports*, *28*, 101459.

Matulyte, I., Jekabsone, A., Jankauskaite, L., Zavistanaviciute, P., Sakiene, V., Bartkiene, E., & Bernatoniene, J. (2020). The essential oil and hydrolats from *Myristica fragrans* seeds with magnesium aluminometasilicate as excipient: antioxidant, antibacterial, and anti-inflammatory activity. *Foods*, *9*(1), 37.

Morris, G. K., DeWitt, W. E., Gangarosa, E. J., & McCormack, W. M. (1976). Enhancement by sodium chloride of the selectivity of thiosulfate citrate bile salts sucrose agar for isolating *Vibrio cholera* biotype El Tor. *Journal of Clinical Microbiology*, *4*(2), 133-136.

Morsy, N. F. (2016). A comparative study of nutmeg (*Myristica fragrans Houtt*.) oleoresins obtained by conventional and green extraction techniques. *Journal of food science and technology*, *53*, 3770-3777.

Munaeni, W., Yuhana, M., Setiawati, M., & Wahyudi, A. T. (2020). Effect in white shrimp *Litopenaeus vannamei* of *Eleutherine bulbosa* (Mill.) Urb. powder on immune genes expression and resistance against *Vibrio parahaemolyticus* infection. *Fish & shellfish immunology*, *102*, 218-227.

Muralisankar, T., Bhavan, P. S., Radhakrishnan, S., Seenivasan, C., Manickam, N., & Shanthi, R. (2014). Effects of dietary supplementation of fish and vegetable oils on the growth performance and muscle compositions of the freshwater prawn *Macrobrachium rosenbergii*. *The Journal of Basic & Applied Zoology, 67(2),* 34–39.

Naeem, N., Rehman, R., Mushtaq, A., & Ghania, J. B. (2016). Nutmeg: A review on uses and biological properties. *International Journal of Chemical and Biochemical Sciences*, 9, 107-110.

Nigam, A., Gupta, D., & Sharma, A. (2014). Treatment of infectious disease: beyond antibiotics. *Microbiological Research*, *169*(9-10), 643-651.

Nikolic, V., Nikolic, L., Dinic, A., Gajic, I., Urosevic, M., Stanojevic, L., & Danilovic, B. (2021). Chemical composition, antioxidant and antimicrobial activity of nutmeg (*Myristica fragrans Houtt*.) seed essential oil. *Journal of Essential Oil Bearing Plants*, *24*(2), 218-227.

Nothman, M. M., & Callow, A. D. (1971). Investigations on the origin of amylase in serum and urine. *Gastroenterology, 60(1),* 82-89.

Pelinson da Fonseca, A. P., Retcheski, M. C., Tormen, L., Romão, S., & Cazarolli, L. H. (2025). Metabolic and stress resistance effects in *Macrobrachium rosenbergii* juveniles supplemented with *Curcuma longa* extract. Veterinary Research Communications, 49(1), 20.

Peng, W., Shi, Y., Li, G. F., He, L. G., Liang, Y. S., Zhang, Y., & Lu, D. Q. (2016). *Tetraodon nigroviridis*: A model of *Vibrio parahaemolyticus* infection. Fish & Shellfish Immunology, 56, 388-396.

Prabu, D. L., Chandrasekar, S., Ambashankar, K., Dayal, J. S., Ebeneezar, S., Ramachandran, K., et al. (2018). Effect of dietary *Syzygium cumini* leaf powder on growth and non-specific immunity of *Litopenaeus vannamei* (Boone 1931) and defense against virulent strain of *Vibrio parahaemolyticus*. *Aquaculture, 489*, 9–20.

Quiroga, P., Asensio, C., & Nepote, V. (2015). Antioxidant effects of the monoterpenes carvacrol, thymol and sabinene hydrate on chemical and sensory stability of roasted sunflower seeds. *Journal of the Science of Food and Agriculture, 95*, 471–479.

Radhakrishnan, S., Saravana Bhavan, P., Seenivasan, C., Muralisankar, T., & Shanthi, R. (2015). Effects of native medicinal herbs (*Alternanthera sessilis*, *Eclipta alba* and *Cissus quadrangularis*) on growth performance, digestive enzymes and biochemical constituents of the monsoon river prawn *Macrobrachium malcolmsonii*. *Aquaculture Nutrition, 21*(4), 496–506.

Rao, R., Bing Zhu, Y., Alinejad, T., Tiruvayipati, S., Lin Thong, K., Wang, J., & Bhassu, S. (2015). RNA-seq analysis of Macrobrachium rosenbergii hepatopancreas in response to Vibrio parahaemolyticus infection. *Gut Pathogens*, *7*(1), 6.

Rashidian, G., Shahin, K., Elshopakey, G. E., Mahboub, H. H., Fahim, A., Elabd, H., & Faggio, C. (2022). The dietary effects of nutmeg (*Myristica fragrans*) extract on growth, hematological parameters, immunity, antioxidant status, and disease resistance of common carp (*Cyprinus carpio*) against *Aeromonas hydrophila*. *Journal of Marine Science and Engineering, 10*(3), 325.

Reverter, M., Tapissier-Bontemps, N., Sarter, S., Sasal, P., & Caruso, D. (2021). Moving towards more sustainable aquaculture practices: a meta-analysis on the potential of plant-enriched diets to improve fish growth, immunity and disease resistance. *Reviews in Aquaculture, 13(1),* 537-555.

Sadia, H. T., Ghosh, A. K., Ahmmed, S. S., Biswas, J., Hasanuzzaman, A. F. M., & Banu, G. R. (2024). Sesbania grandiflora leaf extract enhances growth and non-specific immune responses of shrimp (*Penaeus monodon*). *Khulna University Studies*, 30-38.

Sahandi, J., Sorgeloos, P., Tang, K. W., Jafaryan, H., Yang, W., Mai, K., & Zhang, W. (2025). Highlighting antibiotic-free aquaculture by using marine microbes as a sustainable method to suppress Vibrio and enhance the performance of brine shrimp (Artemia franciscana). *Archives of Microbiology*, *207*(2), 26.

Salam, M. A., Anik, A. R., Khan, M. A., & Sharna, S. C. (2025). Production risk and efficiency of fish farms in Bangladesh: A panel data analysis. *Aquaculture Economics & Management*, 1-25.

Sarathi, M., Ahmed, V. I., Venkatesan, C., Balasubramanian, G., Prabavathy, J., & Hameed, A. S. (2007). Comparative study on immune response of *Fenneropenaeus indicus* to *Vibrio alginolyticus* and white spot syndrome virus. *Aquaculture, 271*(1–4), 8–20.

Saurabh, S., & Sahoo, P. K. (2008). Major diseases and the defence mechanism in giant freshwater prawn, *Macrobrachium rosenbergii* (de Man). *Proceedings of the National Academy of Sciences, India, Section B*, *78*, 103-121.

Shahraki, N., Imanpour, M. R., Akbary, P., Safari, R., & Jafari, V. (2021). Dietary administration of aqueous *Zingiber officinale* extract on growth performance, antioxidant activity, and resistance of shrimp *Litopenaeus vannamei* against *Photobacterium damselae*. *Iranian Journal of Fisheries Sciences, 20*(1), 32–44.

Shamsuzzaman, M. M., Mozumder, M. M. H., Mitu, S. J., Ahamad, A. F., & Bhyuian, M. S. (2020). The economic contribution of fish and fish trade in Bangladesh. *Aquaculture and Fisheries*, *5*(4), 174-181

Simtoe, A. P., Lugendo, B. R., & Mgaya, Y. D. (2024). Efficacy of aromatic plants *Zingiber officinale* and *Syzygium aromaticum* extracts as natural immunostimulants on survival, growth performance, and immune response of farmed prawn *Penaeus monodon* juveniles. *Aquaculture International*, *32*(4), 4499-4513.

Sodamola, M. O., Adejola, Y. A., Akanni, K. T., Ibrahim, I. K., Fajobi, A. K., & Aniche, D. C. (2017). Effect of nutmeg (*Myristica fragrans*) as an additive on the growth performance of juvenile catfish (*Clarias gariepinus*). *Journal of Fisheries*, *11*, 60.

Sula, E., Aliko, V., Barceló, D., & Faggio, C. (2020). Combined effects of moderate hypoxia, pesticides and PCBs upon crucian carp fish (*Carassius carassius*) from a freshwater lake—in situ ecophysiological approach. *Aquatic Toxicology*, *228*, 105644.

Trifan, A., Zengin, G., Korona-Glowniak, I., Skalicka-Woźniak, K., & Luca, S. V. (2023). Essential oils and sustainability: In vitro bioactivity screening of Myristica fragrans Houtt. post-distillation by-products. *Plants*, *12*(9), 1741.

Wang, G., Guo, X., Zhou, C., Lou, H., Li, X., Yao, J., & Huang, J. (2024). Coinfection with infectious precocity virus and Decapod iridescent virus 1 in farmed giant freshwater prawn (*Macrobrachium rosenbergii*) . *Aquaculture*, *586*, 740830.

Zhelyazkov, G., Stoyanova, S., Sirakov, I., Velichkova, K., & Staykov, Y. (2018). Effect of nutmeg extract supplementation on some productive traits and economic efficiency of common carp (*Cyprinus carpio* L.) cultivated in recirculation system. *Agricultural Science and Technology*, *10*, 54–56.

Zhu, F., Twan, W. H., Tseng, L. C., Peng, S. H., & Hwang, J. S. (2019). First detection of white spot syndrome virus (WSSV) in the mud shrimp *Austinogebia edulis* in Taiwan. *Scientific Reports*, *9*(1), 18572.