

THE EFFECTS OF *Sonneratia caseolaris* EXTRACT ON GROWTH PERFORMANCE AND INNATE IMMUNE RESPONSE OF WHITE SHRIMP *Penaeus vannamei*

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Abstract – This research evaluates the effects of supplementary *Sonneratia caseolaris* extract on the growth performance, survival rate, and innate immune responses in white shrimp, *Penaeus vannamei*, in an experiment conducted from May to September 2024 at Tra Vinh University, Vietnam. White shrimp with an initial weight of 2.60 ± 0.24 g were separated into five groups and fed diets containing *Sonneratia caseolaris* leaf extract at 0 (control), 2.5, 5, 10, and 20 g/kg for a 56-day experimental period. Following the feeding trial, the growth performance and innate immune parameters, including total haemocyte count, phenoloxidase activity, and phagocytic activity, were examined to determine the extract's efficacy. The results showed that white shrimp fed a diet of 10 g/kg improved growth performance. In addition, total haemocyte count increased in white shrimp fed a diet containing 5 g/kg, while those fed diets containing 2.5 and 5 g/kg showed increased phenoloxidase activity. Furthermore, white shrimp fed diets supplemented with 2.5, 5, and 10 g/kg showed an increase in phagocytic rate. The results demonstrate that *Sonneratia caseolaris* leaf extract supplementation at a concentration of 5 g/kg diet could be applied to enhance growth and immune response of white shrimp.

Keywords: growth performance, innate immune response, *Penaeus vannamei*, *Sonneratia caseolaris* leaf extract.

I. INTRODUCTION

White shrimp, *Penaeus vannamei*, is one of the most economically important crustacean species farmed in various countries and led global aquaculture production in 2022 at 6.8 million tonnes [1]. However, intensified shrimp farming has contributed to increased disease outbreaks, causing substantial economic losses in major shrimp-producing regions worldwide [2, 3].

The use of immunostimulants to enhance and activate both nonspecific and specific defense mechanisms within an organism's immune system has received considerable attention [3, 4]. Several studies indicated that immunostimulants derived from medicinal plants could be an alternative approach for disease control because these extracts are rich in phytochemicals and bioactive compounds that not only stimulate the growth, immune system, and maturation of aquatic animals but also inhibit pathogenic bacteria, viruses, fungi, and parasites [5–7].

Sonneratia caseolaris (*S. caseolaris*) leaf extract is rich in bioactive compounds, such as phenolic acids, flavonoids, and tannins, as well as terpenoids, steroids, fatty acids, fatty alcohols, aldehydes, hydrocarbons, and polysaccharides [8]. This study also focused on the antioxidant and antimicrobial activities of *S. caseolaris* leaf extract, showing strong free-radical scavenging capacity and broad-spectrum antibacterial and antifungal effects [8]. The evaluation of antimicrobial activities of *S. caseolaris* extracts has been conducted *in vitro* against Gram-positive (*Staphylococcus aureus*, *Bacillus subtilis*, *Micrococcus luteus*) and Gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*, *Vibrio* spp.) [8]. Existing evidence indicates that the

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effects of *S. caseolaris* leaf extract on aquatic animals remain largely unexplored; therefore, this research aims to determine the effect of *S. caseolaris* leaf extract on growth and immune response in white shrimp.

II. LITERATURE REVIEW

The defense mechanisms of crustaceans are less studied compared to those of finfish and other vertebrates. More specifically, crustaceans have no adaptive memory. In other words, they do not have the ability to produce immunoglobulin, so they apparently depend on an innate defense system [9]. Three types of circulating hemocytes have been identified, including hyaline cells, semi-granular cells, and granular cells [10, 11]. These hemocytes are involved in the pattern-recognition system, phagocytosis, prophenoloxidase (proPO) activating system, encapsulation, nodule formation, and the release of antimicrobial peptides and lysozyme [12].

Medicinal plants, called immunostimulants, are used as an alternative approach to prevent disease in aquatic animals [3]. Studies indicated that medicinal plants contain specific active substances that stimulate growth performance, innate immune response, and resistance against pathogens [7, 13–15]. *S. caseolaris* leaf extract, which contains biologically active compounds, phenolic acids, flavonoids, and tannins, alongside terpenoids, steroids, fatty acids, fatty alcohols, aldehydes, hydrocarbons, and polysaccharides, can be used for the evaluation of antioxidant and antimicrobial activities [8].

III. RESEARCH METHODOLOGY

A. Time and location of the study

This research was conducted from May to September 2024 at the Department of Aquatic Pathology, School of Agriculture and Aquaculture, Tra Vinh University, Vietnam. The study focused on investigating the effects and applications of *S. caseolaris* leaf extract on whiteleg shrimp (*Penaeus vannamei*).

B. Preparation of *Sonneratia caseolaris* leaf extract

The *S. caseolaris* leaf extract was obtained following the method described by Lim et al. [16], with a slight modification. One hundred grams of dry powder was suspended in 1 L of double-distilled water and boiled at $100\pm 2^{\circ}\text{C}$ for an hour. The resulting suspension was then filtered through Whatman No. 1 filter paper to obtain the crude extract. The filtrate was subsequently evaporated and concentrated at room temperature until a solid extract was obtained. The extract was kept at 4°C for subsequent experiments.

C. Preparation of experimental diets

Commercial diet (Grobest No. 2; crude protein: 40%, crude fat: 5%, crude fiber: 4%, moisture: 11%, total lysine: 2%, and total phosphorus: 0.9%) was supplemented with *S. caseolaris* leaf extract at five levels: 0 (control), 2.5, 5, 10, and 20 g/kg diet. Each diet was then coated with squid oil at 5 ml/kg of feed before being fed to the shrimp.

D. Experimental design

Shrimp were fed diets containing *S. caseolaris* leaf extract at 0 (control), 2.5, 5, 10, and 20 g/kg for 56 days. Then, growth performance and immune parameters were examined. During the experiments, the water temperature, pH, and salinity were maintained at $26\pm 1^{\circ}\text{C}$, 8.06–8.34, and 15‰, respectively.

E. Effect of dietary *Sonneratia caseolaris* on the growth performance of white shrimp

Shrimp were cultured at the brackish water hatchery of Tra Vinh University and acclimated in 6 m^3 tanks for three days prior to the experiment. There were five experimental diets carried out in 0.5 m^3 /tank with triplicates for each diet. Thus, fifteen 0.5 m^3 tanks equipped with an air filter were used in this study. A total of 750 shrimp (mean weight: $2.60\pm 0.24\text{ g}$) were randomly selected and stocked into tanks containing 350 L of aerated brackish water with 50 shrimp

per tank. During the experiment, shrimp were fed at 8 a.m. and 5 p.m. at a rate of 2% of body weight. Uneaten feed and fecal matter were siphoned out before each feeding, and one-third of the water volume was exchanged daily. At the end of the feeding trial, growth performance such as final weight (FW), daily weight gain (DWG), specific growth rate (SGR), and survival rate (SR) were calculated as described by Cárdenas et al. [17].

F. Effect of *Sonneratia caseolaris* on immune response

The hemolymph of each shrimp was collected using a 1-mL sterile syringe fitted with a 25-gauge needle. A total of seven shrimp per treatment (2–3 shrimp/tank) were sampled for each immune parameter. They were then diluted in anticoagulant solution to determine immune parameters of white shrimp, including total haemocyte count (THC) [18], phenoloxidase activity (PO) [19], and phagocytic activity [20].

G. Statistical analysis

All data were analyzed using one-way ANOVA. When significant differences were detected, Duncan's multiple comparison test was applied to compare treatment means. All statistical tests were conducted using IBM SPSS version 20.0, with a significance level at $p < 0.05$.

IV. RESULTS AND DISCUSSIONS

A. Effect of dietary *Sonneratia caseolaris* on growth performance and survival rate of white shrimp

No significant differences in initial weight were found among treatments at the beginning of the experiment. At the end of the experiment, shrimp fed a diet containing 10 g/kg showed the highest FW, DWG, and SGR, followed by 5 g/kg, while the lowest in FW, DWG, and SGR were found in shrimp fed a diet containing 20 g/kg. No significant difference in FW, DWG, and SGR was found between shrimp fed diets containing 2.5 and 5 g/kg. The survival rate of shrimp

ranged from 78.7% to 85.7%, and no significant difference in survival rate was observed among diets (Table 1).

The positive effects of medicinal herbs have been demonstrated in growth promotion, survival rate, and nutrient absorption ability of aquatic animals [3, 4, 7, 21]. The nutrients from medicinal herbs could promote growth performance and physiological functions of aquatic animals [20]. The ethanol extract of diets containing 250 mg/kg of each medicinal herb, *Artemisia herba-alba*, *Lonicera japonica*, and *Lilium candidum*, was significantly higher than that of the control group for four months [7]. Similarly, shrimp fed diets coated with *Wedelia chinensis* ethanol extract at levels of 31.25, 312.5, or 625 mg/kg showed significant differences in growth performance compared to the control diet after a 21-day period [22]. In this study, shrimp fed a diet containing of *S. caseolaris* leaf at 10 g/kg showed significantly improved FW, DWG, and SGR. Similarly, shrimp fed diets containing 1, 5, and 10 g/kg of guava leaf extract showed significantly higher growth performance than those fed the control diet [23]. However, the growth performance of shrimp fed a diet containing *S. caseolaris* leaf at 20 g/kg in this study was lower than that of other diets (Table 1). This may be attributed to the presence of both nutrients and anti-nutritional compounds in *S. caseolaris* leaves, the latter of which have a negative effect on the growth of shrimp [24].

B. Immune responses of shrimp fed diets containing *Sonneratia caseolaris*

The highest THC was found in shrimp fed a diet containing *S. caseolaris* leaf extract at 5 g/kg, followed by a diet of 10 g/kg. However, THC did not differ significantly between shrimp fed diets containing 5 and 10 g/kg *S. caseolaris* leaf extract. Similarly, there was no significant difference in THC among shrimp fed diets containing *S. caseolaris* leaf extract at 0, 2.5, and 20 g/kg after 56 days of feeding (Figure 1).

Shrimp fed diets supplemented with *S. caseolaris* leaf extract at 2.5 and 5 g/kg showed significantly higher PO activity compared with

Table 1: The growth performance and survival rate of white shrimp fed diets containing *S. caseolaris* leaf extract at 0 (control), 2.5, 5, 10, and 20 g/kg after a 56-day period

<i>S. caseolaris</i> leaf extract supplementation levels (g/kg)	Initial Weight (g)	Final Weight (g)	DWG (g/day)	SGR (%/day)	Survival rate (%)
0	2.60±0.24	20.23±1.56 ^b	0.28±0.04 ^b	2.64±0.46 ^b	82.7±3.1 ^a
2.5	2.60±0.24	20.02±1.42 ^b	0.28±0.03 ^{bc}	2.74±0.42 ^{ab}	82.7±8.1 ^a
5	2.60±0.24	20.69±1.76 ^{ab}	0.29±0.03 ^{ab}	2.81±0.37 ^{ab}	82.7±9.2 ^a
10	2.60±0.24	21.44±1.57 ^a	0.31±0.03 ^a	2.89±0.40 ^a	85.7±5.0 ^a
20	2.60±0.24	20.02±1.20 ^b	0.27±0.03 ^c	2.63±0.33 ^b	78.7±5.0 ^a

Note: Data are expressed as mean ± SD. Values within the same column with different superscripts indicate statistically significant differences ($p < 0.05$).

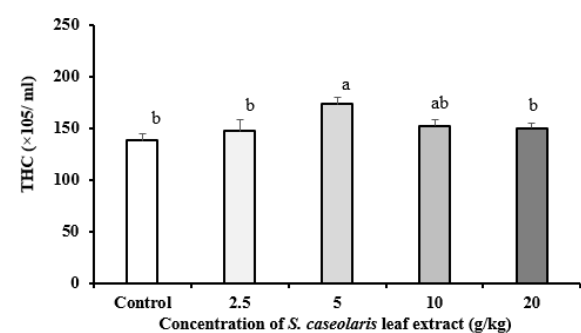


Fig. 1: The THC of white shrimp fed diets containing *S. caseolaris* leaf extracts at 0 (Control), 2.5, 5, 10, and 20 g/kg after a 56-day experimental period

Note: Each bar is expressed as mean ± SE ($n = 7$); bars with different letters differ significantly ($p < 0.05$) among concentrations.

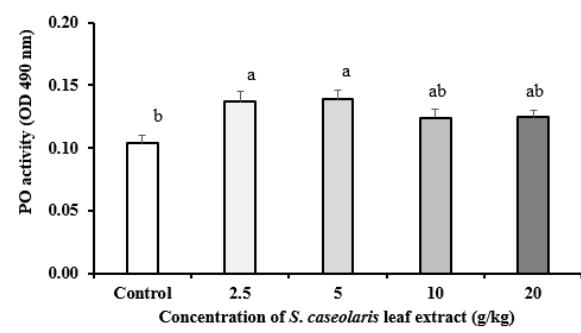


Fig. 2: PO activity of white shrimp fed diets containing *S. caseolaris* leaf extracts at 0 (Control), 2.5, 5, 10, and 20 g/kg for a 56-day experimental period

Note: Each bar is expressed as mean ± SE ($n = 7$); bars with different letters differ significantly ($p < 0.05$) among concentrations.

those fed the control diet. However, no significant differences in PO activity were observed between the 2.5 and 5 g/kg groups or between the 10 and 20 g/kg groups after the 56-day experimental period (Figure 2).

In the phagocytic rate (PR) observation, shrimp fed diets containing different concentrations of *S. caseolaris* leaf extracts (2.5, 5, 10, and 20 g/kg) had considerably higher PR than that of the control diet. PR of shrimp fed the control diet was 51.32%, while the PR of shrimp fed diets containing *S. caseolaris* leaf extracts at 2.5, 5, 10, and 20 g/kg was 57.39%, 68.66%, 68.13%, and 65.10%, respectively (Figure 3A). In the

phagocytic index (PI) observation, PI of shrimp fed diets containing *S. caseolaris* leaf extracts (2.5, 5, 10 and 20 g/kg) was significantly higher than that of the control diet. The highest PI was found in shrimp fed a diet of 5 g/kg, followed by 2.5 g/kg, while the lowest one was recorded in the control diet. No significant difference in PI was observed between shrimp fed diets containing 10 and 20 g/kg supplementation (Figure 3B).

Haemocytes are the fundamental components of the immune system in shrimp. These cells are involved in key defense processes, including phagocytosis, encapsulation, nodule formation, wound healing, coagulation, cell-to-cell commu-

nication, and activation of the proPO system [25–27]. PO, a key enzyme in the proPO activating system, plays a vital role in humoral defense by facilitating the elimination of invading pathogens [28], whereas phagocytosis serves as an important cellular defense mechanism in crustaceans [29]. This study indicated that the THC of shrimp fed a diet of 5 g/kg was significantly greater than that of shrimp fed diets of 0, 2.5, and 20 g/kg (Figure 1). Additionally, the PO activity of shrimp fed diets containing *S. caseolaris* leaf extracts at 2.5 and 5 g/kg was significantly higher than that of shrimp fed the control diet (Figure 2). Similar results have been reported in previous studies, where THC and PO activity increased in shrimp fed diets containing various extracts such as *Artemisia herba-alba*, *Lonicera japonica*, and *Lilium candidum* [7], *Excoecaria agallocha* [15], *Wedelia chinensis* [22], *Pandanus tectorius* [30], *Chromolaena odorata* [31], *Syzygium nervosum* [32], *Ocimum tenuiflorum* [33].

S. caseolaris leaf extract is rich in diverse chemical constituents, including flavonoids, tannins, phenolic compounds, steroids, terpenoids, fatty acids, alcohols and aldehydes, polysaccharides, hydrocarbons, and other bioactive compounds [8], which have been shown to inhibit five microbial strains *in vitro*. Recently, shrimp receiving diets supplemented with *Excoecaria agallocha* extract at a concentration of 20 g/kg showed significantly higher PR than those fed the other diets from day 1 to 14 [15]. Similarly, shrimp receiving diets supplemented with *Wedelia chinensis* extract (12.5, 25.0, and 37.5 g/kg) showed significantly higher PR compared to those fed the control diet [22]. In this study, PR and PI of shrimp fed diets containing *S. caseolaris* leaf extracts at 2.5, 5, 10, and 20 g/kg were noticeably higher than those of the control diet (Figure 3).

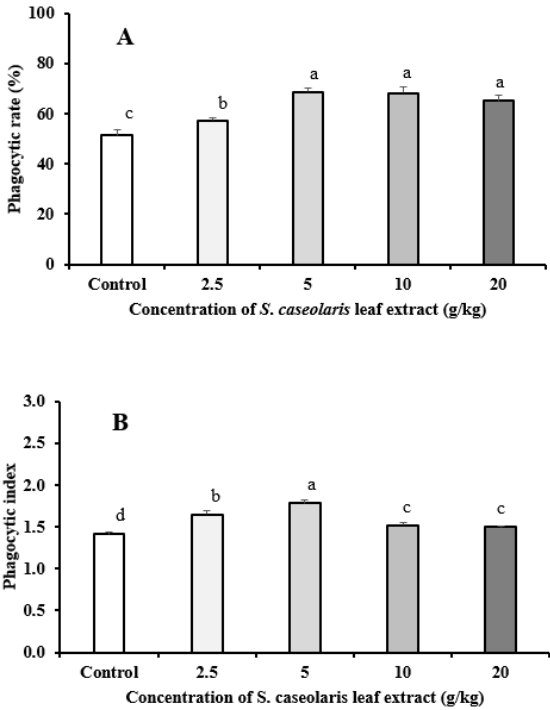


Fig. 3: PR (A) and PI (B) of white shrimp fed diets containing *S. caseolaris* leaf extracts at 0 (Control), 2.5, 5, 10, and 20 g/kg for a 56-day experimental period

Note: Each bar is expressed as mean ± SE (n = 7); bars with different letters differ significantly (p < 0.05) among concentrations.

V. CONCLUSIONS AND RECOMMENDATION

The present study indicates that *S. caseolaris* leaf extract supplementation at 10 g/kg diet enhanced the growth performance of shrimp. However, *S. caseolaris* leaf extract-supplemented diets at a concentration of 5g/kg showed an increase in the immune response by increasing THC, PO activity, and PR after a 56-day experimental period. This result demonstrates that *S. caseolaris* leaf extracts supplemented diets at a concentration of 5 g/kg can be used to promote the growth and innate immune response of shrimp.

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