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Potential of Bioactive Compounds of Noni Leaf Extract on the Performance of Tiger Prawn Larvae Through *Artemia salina* Bioenrichment

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ABSTRACT

The tiger shrimp (*Penaeus monodon*) is a high-value species widely cultivated in aquaculture ponds. However, recent years have seen a decline in its production, primarily due to low survival rates. To address this issue, strategies to increase survival rates must focus on improving feed nutrition, enhancing immune resistance, and minimizing both space requirements and environmental impact. One promising approach is the use of noni leaf extract. The study aimed at analyzing the improvement of tiger shrimp larvae performance through bioenrichment of noni leaf extract in *Artemia salina*. The research was conducted experimentally and involved several stages, including sampling, extraction, phytochemical analysis, proximate analysis, toxicity testing, and larval rearing. The larval rearing phase was designed using a completely randomized design (CRD) with 4 experimental treatments and 3 replicates for each treatment. The measured parameters included growth, toxicity, survival rate and water quality. The results indicated that the addition of noni leaf extract does not contain toxicity and is able to increase nutrition in *Artemia salina* as natural food to increase the growth and survival rate of the tiger prawn larvae. An increased performance of the tiger prawn larvae was recorded with growth of 64.48%, while the survival rate was 53.33%. The optimal result were achieved with a dosage of 150ppm of noni leaf extract.

INTRODUCTION

The tiger shrimp (*Penaeus monodon*) is a highly valuable species that is extensively cultivated in aquaculture ponds due to its premium quality and strong consumer demand **(Aftabuddin *et al*., 2018)**. In 2016, the global production of Penaeidae shrimp exceeded 4.200 million tons, generating a substantial revenue of USD 4,8 billion **(GAA, 2017)**. However, despite the increasing global demand for shrimp, Indonesia has experienced a significant decline in shrimp production. Data from the Ministry of Marine Affairs and Fisheries **(KKP, 2021)** revealed a sharp decrease in shrimp production from 1.371.22,7 tons in 2017 to 434.872,7 tons in 2020. This decline in tiger shrimp

production is largerly due to the limited availability of larvae, primarily caused by low survival rates.

Several factors contribute to the low survival rate of the tiger shrimp larvae, including weak immune system resistance and vulnerability to bacterial disease **(Leobert *et al.*, 2015; Dabu *et al.*, 2017)** as well as water quality management and poor feed nutrition **(Jaffer *et al.*, 2020)**. To address these challenges, efforts to improve survival rates should focus on enhancing feed nutrition **(Pisuttharachai *et al.*, 2022)**, boosting immune resistance, optimizing space utilization, and reducing the environmental impact **(Villarreal & Juares 2022)**. One promising approach to improving tiger shrimp performance involves the use of herbs or phytopharmaceuticals. The application of these natural supplements has been widely recognized, with examples including *Melastoma malabathricum* **(Awaluddin & Ridwan, 2016; Farizah *et al.*, 2017)**, seaweed **(Aftabuddin *et al.*, 2021)**, fern plants **(Maulianawati & Suhami 2022)**, as well as the noni plant. According to **Phan *et al.* (2023)**, noni extract is able to increase growth, survival, and resistance to environmental stress in *Litopenaeus vannamei*. **Abarike and Addah (2024)** stated that noni leaf extract was able to improve the performance in *Oreochromis niloticus*.

The noni plants (*Morinda citrifolia*) has attracted considerable interest for its potential as food supplements, functional food ingredients, or as immunostimulants, prompting researchers to further explore its bioactive compounds. According to **Lavanya and Veerappan (2011)** and **Esquer-Miranda *et al.* (2016)**, noni contains bioactive compounds such as sterols, hormones, vitamins, and structural components of biomembranes, which can enhance immunostimulatory effects. Phytochemical screenings of noni extracts from various parts of the plant, including fruit, leaves and stems, have identified the presence of alkaloid, flavonoids, saponins, tannins, quinones, steroids and terpenoids **(Pahlani *et al.*, 2022)**. Research has also been conducted on noni leaves (**Zhang *et al.*, 2016; Ene *et al.*, 2021)**, fruit **(Kang & Song, 2019)**, seeds **(Jahurul *et al.*, 2021)**, and roots **(Sam-ang *et al.*, 2023)**. However, the utilization of noni extract in the field of fisheries remains underexplored. Consequently, there is a compelling need to explore the potential of noni leaf extract in enhancing the performance of the tiger shrimp larvae. This study aimed at analyzing the improvement of the tiger shrimp larvae performance through bioenrichment of the noni leaf extract in *Artemia salina*.

MATERIALS AND METHODS

1. Material

The materials used in this study include: tiger prawn larvae, *Artemia salina*, noni leaf, 80% ethanol, sterile sea water, alcohol, aquadest, magnesium, hydrochloric acid, methanol, and ferric chloride reagent. The equipments used in this study include: analytical balance (Mettler Toledo AL204), waterbath, evaporator, DO, pH, (LUTRON YK-2001PHA Conductivity/TDS/Salt - PH-ORP-DO Meter), thermometer, and aeration.

2. Method

2.1. Noni leaf extract preparation

Mature noni leaves, characterized by their dark green color, were selected for this study. According to **Yang *et al.* (2011)**, mature noni leaves contain higher total phenol content (±16.1mg/ g) compared to younger leaves (±14.6mg/ g). The extraction process was carried out using maceration method. The leaves were macerated in 80% ethanol at leaf-to-solvent ratio of 1:3. The mixture was then incubated for 3 days at room temperature, ranging 30 to 37ºC. After incubation, the extract was filtered and concentrated using a rotary evaporator set at 40°C, resulting in a thick paste **(Farizah *et al.*, 2017)**.

2.2. Phytochemical analysis

Phytochemical tests were conducted to qualitatively identify the presence of bioactive components in the noni (*M. citrifolia*) leaf extracts. The testing was performed according to the methods outlined by **Harborne (1998)**.

2.3. Proximate analysis of A. salina

The proximate analysis was performed on *A. salina* samples both with and without the addition of noni leaf extract. This was done to asses any changes in proximate composition resulting from the extract's inclusion. The analysis followed the procedure described by **AOAC (2005)**.

2.4. Toxicity test (LC_{50} test)

The lethal concentration (LC) test was conducted based on Brine Shrimp Letal Toxicity Test (BSLT) to determine the toxic dose of noni leaf extract. Solution of noni leaf extract were prepared at a concentration of 0.5, 1, 3, 5, 10mg/ ml. For the LC_{50} test, 1ml of each extract solution was mixed with 4ml of seawater containing 20 *A. salina.* The mixtures were incubated for 24 hours, after which the number of dead larvae was counted. LC_{50} values were calculated using probit analysis

2.5. Research design

The experimental design to evaluate the performance enhancement of tiger shrimp larvae was based on a completely randomized design (CRD). Four treatments were tested, each with three replicates, and larvae were fed 3 times daily: Treatment A: Artemia without extract immersion, Treatment B: Artemia soaked with 50ppm extract, Treatment C: Artemia soaked with 100ppm extract, and Treatment D: Artemia soaked with 150ppm extract.

2.6. Enrichment of Artemia salina and rearing of tiger shrimp larvae

To administer noni leaf extract to tiger shrimp larvae, *Artemia salina* were enriched by culturing them in extract-infused water. The cultured were divided into three

containers, each soaked in noni leaf extract at difference concentrations according to the treatment dose. The enriched *A. salina* were then fed to tiger shrimp larvae three times daily, with 20 larvae per feeding. To maintain optimal feed quality, the *A. salina* culture was refreshed every three days. Post-larvae (PL-12) tiger shrimp were sourced from the CV. Nurwindu Jaya hatchery. Each aquarium used for rearing contained a shrimp density of 10 larvae per liter. Before rearing, initial sampling was conducted to measure the length and weight of the larvae, followed by a 1-2 days acclimatization. The rearing period lasted 21 days, during which the larvae fed with *A. salina* enriched with noni leaf extract.

2.7. Growth, survival rate and water quality parameters

2.7.1. Absolute growth

Absolute growth in terms of weight and length was calculated using the formula provided by **Effendi (2002)**:

$$W = Wt - Wo \qquad L = Lt - Lo$$

2.7.2. Specific growth

The growth rate was determined using the specific growth rate formula as described by **Steffens (1989)**:

$$SGR = \frac{InWt - InWo}{Ti - To} X100\%$$

2.7.3. Survival rate (SR) of shrimp fry

The survival rate of shrimp fry was calculated using the following formula from **Effendi (2002)**:

$$SR = \frac{Number\ of\ shrimp\ at\ the\ end\ of\ the\ study}{Number\ of\ shrimp\ at\ the\ beginning\ of\ the\ study} x100\%$$

2.8. Water quality measurement

Water quality parameters including temperature, pH, ammonia, salinity and DO (dissolved oxygen) were measured every 5 days, with the exception of temperature which was monitored daily. These measurements were conducted *in situ* using instrument such as DO meter, thermometer, pH meter, hand refractometer, and spectrophotometer.

RESULTS

1. Phytochemical content of noni extract

The results of the phytochemical test (Table 1) show that the of noni leaf and fruit extracts have differences in terms appearance of the test results. In the noi leaf extract, the color that appears is more concentrated than of the noni fruit.

Table 1. Secondary metabolite test results of noni extracts

Extract	Phytochemical test				
	Alkoloids	Flavonoids	Saponins	Steroids	Tannins
Leaves	+++	+++	-	+++	+++
Fruit	++	+++	-	+	++

Description: - (Not contained), + (Less strong); ++ (Strong); +++ = (Very strong)

2. Proximate content of noni extract

The proximate analysis results of noni fruit and leaf extracts indicated that noni leaf extract had higher nutrional content (Table 2), especially in protein fat and carbohydrates. While, the proximate test using arthemi salina enriched with the extract can be seen in Table (3).

Table 2. Proximate analysis results of noni extract

Extract	Proximate test results (%)				
	Ash	Water	Protein	Fat	Carbohydrates
Leaves	0,78 %	0,12%	27,4%	6,6%	34,9%
Fruit	1,12%	0,19%	25,2%	3,2%	29,7%

Table 3. Proximate test results of *Arthemia salina* through enrichment of noni leaf extract

No.	Proximate Test	No extract	Extract 50ppm	Extract 100ppm	Extract 150ppm
1	Protein	52,15%	53,9%	54,6%	55,3%
2	Fat	15,2%	15,2%	15,6%	16,4%
3	Water content	6%	6%	4%	3%
4	Ash content	10,9%	10,5%	11,6%	11,7%

3. Toxicity of noni leaf extract

The toxicity of noni leaf extract was evaluated using BSLT and probit analysis methods, as shown in Table (4) and the probit value of LC50 in Fig. (1).

Table 4. Results of toxicity test with BSLT method

Treatment	Log 10	Number of test animals	Number who live	Mortality (%)	Probit value
0.5 mg	-0,30	20 tails	20 tails	0%	0
1 mg	0	20 tails	20 tails	0%	0
3 mg	0,47	20 tails	20 tails	0%	0
5 mg	0,69	20 tails	19 tails	5%	3,36
10 mg	1	20 tails	19 tails	5%	3,36

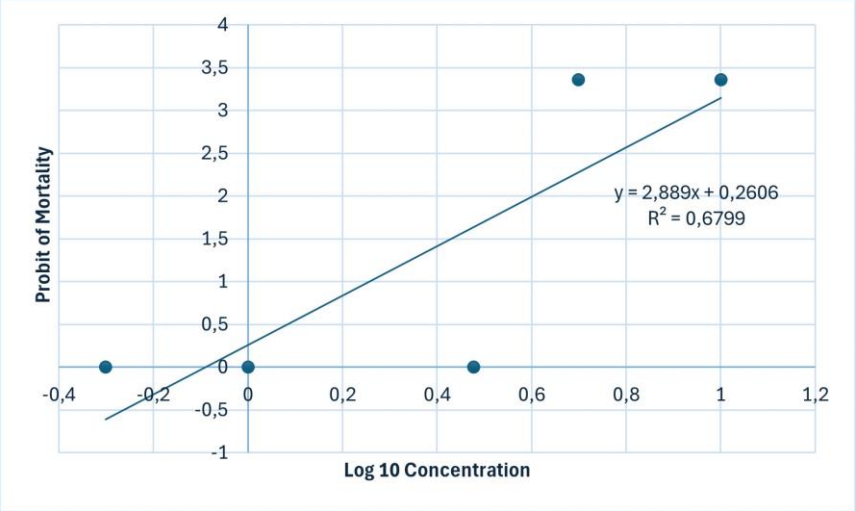

Fig 1. Graph of probit value

4. Growth performance of tiger shrimp larvae

The results of the analysis of the growth of shrimp larvae can be seen in Fig. (2). Tiger shrimp larvae fed with *A. salina* enriched with noni leaf extract showed significant improvement compared to those not supplemented with the extract. Based on statistical analysis with ANOVA, Treatment B (50ppm) was not significantly different from Treatment C (100ppm), but Control Treatment A (without extract immersion) was significantly different from Treatment D (150ppm) compared to Treatment B (50ppm) and Treatment C (100ppm). This proves that noni leaf extract can affect the growth of shrimp weight.

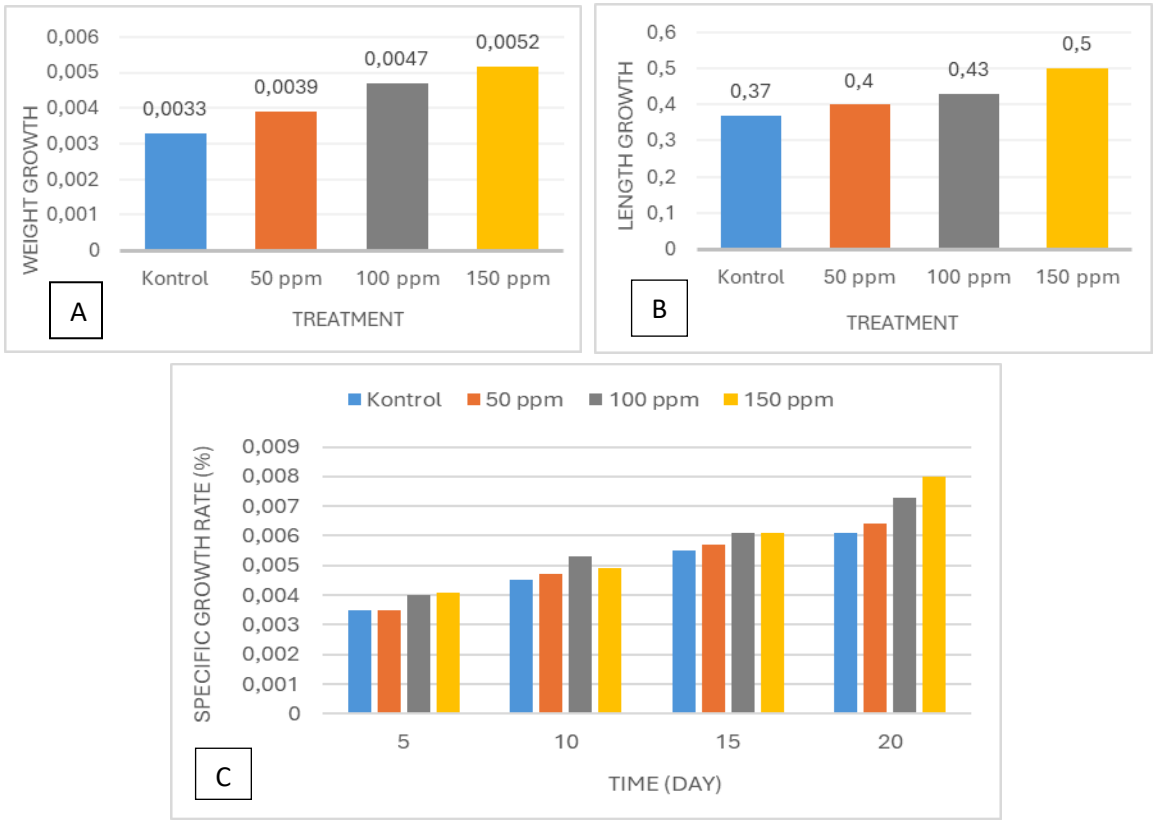

Fig. 2. Growth of tiger shrimp larvae showing: (A) Length growth, (B) Weight growth, (C) Specific growth

5. Survival rate

The results of measurements of survival rates of tiger prawn larvae are shown in Fig. (3). The highest survival rate was observed in the 150ppm treatment group, with a survival rate of 53.33%.

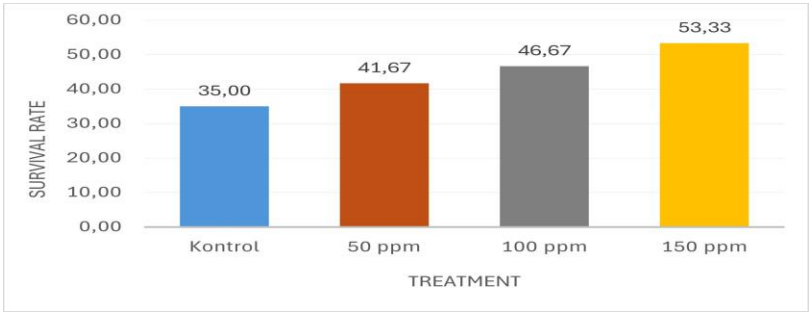

Fig. 3. Survival rate of tiger shrimp larvae

6. Water quality

The results of water quality measurements met the standards for cultivating tiger prawn larvae (Table 5).

Table 5. Water quality measurement results

Parameters	Unit	Observation Range	SNI-8556-4-2021
Temperature	^{o}C	27 - 30	26 - 32
Salinity	ppt	25 - 30	10 - 35
pH	-	7,9 - 8,2	7,8 - 8,4
DO	mg/L	4,6 - 4,8	>4
Ammonia	mg/L	0,02 - 0,16	<0,1

DISCUSSION

1. Phytochemical content of noni extract

The extract of noni leaves exhibited a more intense coloration compared to the noni fruit extract, suggesting a higher concentration of bioactive compounds in the leaves. Phytochemical screening of noni leaf extract, as reported by **(Sogandi *et al.*, 2020)**, identified the presence of tannins, triterpenoids, anthraquinones, flavonoids, steroids, alkaloids and saponins. **Halimah *et al.* (2019)** noted that noni leaves processed into powder contain phytochemicals such as flavonoids, tannins, saponins, phenols, steroids and triterpenoids, although alkaloid compounds were not detected. These phytochemicals are recognized for their antibacterial properties, which function by disrupting bacterial cell membranes, inhibiting metabolic processes, and interfering with protein transport within cells. Moreover, these compounds can inhibit the enzymatic activities of reverse transcriptase and DNA topoisomerase, further hindering bacterial survival **(Mulyani *et al.*, 2021)**.

2. Proximate content of noni extract

Proximate analysis was performed to determine the nutritional composition of the feed, specifically focus on protein, fat, carbohydrates, water content, ash, fiber and nitrogen-free extract. These three compounds are essential nutrients for enhancing the growth and overall performance of tiger shrimp larvae. A study by **Berek *et al.* (2022)** demonstrated that the inclusion of noni leaves in artificial feed can significantly boost growth. Additionally, research by **Julia *et al.* (2020)** found that tilapia fed a diet supplemented with noni leaf powder showed improvement in growth and survival rates of tilapia larvae.

Proximate tests were also conducted on *Artemia salina* that had been enriched with noni leaf extract. The results showed an increase in nutrient content across all

parameters tested, indicating that noni leaf extract effectively enhances the nutritional quality of natural feed for *A. salina*. This increase in nutrients content can contribute to improving growth, as shown by studies involving fermented noni juice occurred in all tests. This allows noni leaf extract to improve nutrition in natural food that has been enriched with extracts. The addition of noni extract is able to provide increased growth **(Wang *et al.*, 2021; Pisuttharachai *et al.*, 2022)**. The growth of shrimp is closely linked to the availability of protein in their diet. The observed increase in growth is largely attributed to protein synthesis, which is essential for both the formation of new tissues and the maintenance of existing ones **(Yunita *et al.*, 2022)**. Furthermore, the biosynthesis of key macromolecules such as proteins, lipids, and nucleotides is vital for building biomass, a fundamental driver of cellular, tissue, and organismal growth **(Ben-Sahra & Manning, 2017)**.

3. Toxicity of noni leaf extract

Mortality was observed in *A. salina* at extract concentration of 5 and 10mg, while no mortality occured at lower concentrations. According to **Mayer *et al.* (1982)**, an extract is considered toxic if it causes 50% mortality in larvae at concentration <1000mg/ L. The addition of noni leaf extract to *A. salina* did not induce toxicity at lower doses, but higher doses showed toxicity effects. The LC_{50} value, determined through probit analysis, was calculated by converting the percentage of mortality into probit value. The analysis resulted in LC_{50} value of 43.7017 (Fig. 1) indicates that noni leaf extract exhibits toxicity. According to **Mayer *et al.* (1982)**, LC_{50} value within the range of 30-1000ppm is considered toxic. Although the noni leaf extract shows some levels of toxicity, it does not result in significant mortality.

4. Growth performance of tiger shrimp larvae

The growth measurements of tiger shrimp larvae fed with *A. salina* enriched with noni leaf extract showed a significant improvement compared to those not supplemented with the extract. This indicates that noni leaf extract effectively enhances the growth performance of tiger shrimp larvae. According to **Moh *et al.* (2021)**, incorporating noni extract into feed mixed with pellets can increase the growth of the caname shrimp. **Hambali *et al.* (2019)** and **Phan *et al.* (2023)** stated that noni extract is able to increase growth, survival, and resistance to environmental stress. In addition, according to **Rosidah *et al.* (2022)**, noni extract is able to increase specific growth rate, growth rate, decrease feed conversion ratio, and increase protein retention in red tilapia.

The most effective dose of noni leaf extract was found to be 150ppm, which resulted in the most significant growth among the treatments. This indicates a dose-dependent improvement in the growth performance of the tiger shrimp larvae, likely due to the presence of active compounds in the noni leaf extract. Research by **Nya and Austin (2009)**, **El-Desouky *et al.* (2012)**, **Harikrishnan *et al.* (2012)** and **Shabana *et al.* (2019)** suggests that these active compounds stimulate growth by enhancing enzyme

secretion during digestion, which in turn increases appetite and improves nutrient absorption. Additionally, the therapeutic properties of noni leaf extracts as anti-pathogens and stress reducer further contribute to enhanced growth performance **(Tan *et al.*, 2017)**. The ability of noni leaf extract to increase protein retention **(Azizah *et al.*, 2020)** also plays a critical role in supporting better growth outcomes in the tiger shrimp larvae.

5. Survival rate

The survival rate of tiger shrimp larvae increased with the addition of noni leaf extract. The highest survival rate was observed in the 150ppm treatment group, with a survival rate of 53.33%. This increase is attributed to the bioactive component in noni leaf extract. **Moh *et al.* (2024)** stated that the survival rate of *Penaeus vannamei* shrimp enriched with noni fruit extract was better than without the extract, reaching 26.7%. **Wardani *et al.* (2021)** found that soaking tilapia in noni leaf extract improved their survival rate by 26.66%. Moreover, adding noni leaf extract to feed has been shown to enhance survival by stimulating and regulating immune response and immune gene expression in gala shrimp **(Halim *et al.*, 2018)**. The increase in survival rate is likely influenced by higher total hemocyte count (THC) and differential hemocyte count (DHC), as reported by **Halim *et al.* (2017)**.

6. Water quality

The aquatic environment plays a crucial role in the growth and survival of the tiger shrimp larvae. The water quality measurements conducted during the study showed an optimal condition according to SNI-8556-4-2021 standard. With stable and optimal water quality parameters, the positive effects of noni leaf extract on the growth and survival of tiger shrimp larvae are clearly evident.

CONCLUSION

The study concluded that noni leaf extract significantly enhances the performance of tiger shrimp larvae by improving their nutrition, growth and survival rate. The optimal dose was found to be 150ppm.

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