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The Use of (*O. Basilicum*) Extract to Treat *Litopenaeus vannamei* Disease the Infected Bacteria *Vibrio parahaemolyticus*

Harlina Harlina [a*], Muhammad Saenong [a], Syahrul Syahrul [a], Andi Hamdillah [a] and Inda Safitri Tangdilintin [a++]

[a] Aquaculture Study Program, Faculty of Fisheries and Marine Sciences, Universitas Muslim Indonesia, Makassar 90231, Indonesia.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

This research aims to treat vaname shrimp disease infected with bacteria*Vibrio parahaemolyticus*. In this research, we used Completely Randomized Design (CRD) 4 treatments and 3 replications. The treatment being tested was the treatment of vaname shrimp disease by soaking in basil (*O. basilicum*) leaf extract with different concentrations, namely: Treatment A (0 mg/ml), treatment B (1.56 mg/ml), treatment C (3.12 mg/ml) and treatment D (12.5 mg/ml). The parameter observed was the number of bacterial colonies*V. parahaemolyticus* maintenance media and in the hepatopancreas of vaname shrimp. Apart from that, observations were made on the survival rate of vaname shrimp and water quality parameters. The research results found that the best

[++] Student;
**Corresponding author: Email: harlina.harlina@umi.ac.id;*

concentration in the treatment of disease*V. parahaemolyticus* in vaname shrimp at a concentration of 12.5 mg/ml with the lowest number of bacterial colonies in the rearing media, namely 1.20 x 10^2 CFU/ml. In the hepatopancreas of vaname shrimp, the lowest number of colonies was obtained at a treatment concentration of 12.5 mg/ml, namely 1.12 x 10^2 CFU/ml. The highest survival rate was obtained at a treatment concentration of 12.5 mg/ml of 86,67±5,77%. The range of water quality in research observations was obtained as follows salinity 28.72-29.05 ppt, temperature 26.71-27.25^{O}C, oxygen ranges from 5.72-7.81, pH 7.18-8.13 and ammonia 0.033-0.041ppm. Water quality during the research was in the optimal range for the life of vaname shrimp.

Keywords: Basil (*O. basilicum*) *leaf extract; vaname shrimp disease (Litopenaeus vannamei); Vibrio parahaemolyticus.*

1. INTRODUCTION

Vaname shrimp (*Litopenaeus vannamei*) is one of Indonesia's export commodities and is in demand by various countries, including the United States, the European Union and Japan [1]. Indonesia was the 4th largest exporter of frozen shrimp in the world in 2019. The Ministry of Maritime Affairs and Fisheries has a target for the export value of frozen shrimp products to increase to 250 percent by 2024. To achieve this, steps are being taken to increase vaname shrimp production through cultivation in ponds. However, in developing this business, we were faced with the problem of decreasing vaname shrimp production. In 2021, vaname shrimp production will reach 102 thousand tons. However, in 2022 it will experience a decline, where production will only be 58 thousand tons a year.

The decline in vaname shrimp production was mainly caused by disease attacks which caused mass deaths of vaname shrimp. One type of disease that attacks white shrimp is the Vibrio bacteria. Bacteria*Vibrio parahaemolyticus* is the most common disease agent, and is one of the causes of Acute Hepatopancreatic Necrosis Disease (AHPND). AHPND is a disease that is very feared by all farmers in the world because it can cause 40-100% shrimp death. Vibriosis outbreaks can cause up to 100% mortality in the larval or juvenile stages.Taukhid, [2] explained that researchers Try to find the source of the bacteria*V. parahaemolyticus* which is the source of infection. until the discovery of plasmids*V. parahaemolyticus* strain (Vp-AHPND), EMS changed its name to AHPND (*Acute Hepatopancreatic Necrosis Disease*).

So far, efforts to control the disease have been carried out using antibiotics. The success of disease control is very dependent on accurate diagnosis, but currently antibiotics have been proven to cause resistance to bacteria if they continue to be used, and the presence of antibiotic residues in shrimp and humans means that it is necessary to look for other alternatives that are more effective and safer [1]. One approach that can be applied is the use of broad-spectrum natural bioactive compounds without adverse side effects.

The use of natural bioactive compounds with a broad spectrum is one method that can be applied to control disease without any adverse side effects.Basil leaves have (*O. basilicum*) various benefits, one of which is antibacterial, the main element of basil leaves is essential oil and ethanol extract that can inhibit the growth of bacteria [3]. This is reinforced by research done by Harlina et al. [4,5] that leaves *P. betle* and *O. basilicum* demonstrated bacterial inhibitory properties against various pathogens affecting economically important shrimp farming species. Therefore, trials are being conducted to treat infected vaname shrimp*V. parahaemolyticus* to obtain an effective concentration used in treating diseases in vaname shrimp.

2. RESEARCH METHODS

2.1 Research Time

This research was carried out in February-April 2023 at the Laboratory of the Faculty of Fisheries and Marine Sciences, Indonesian Muslim University, Makassar, South Sulawesi.In this research, we used Completely Randomized Design (CRD) 4 treatments and 3 replications. The treatment being tested was the treatment of vaname shrimp disease by soaking in basil leaf extract (*Leisure center*) with different concentrations namely: Treatment A (0 mg/ml), treatment B (1.56 mg/ml), treatment C (3.12 mg/ml) and treatment D (12.5 mg/ml).

2.2 Culture Tank

The container used for culture in this research is a large aquarium (40 cm × 25 cm × 28 cm) with a volume of 10 L of water added. Before use, the aquarium is washed thoroughly then dried, after that each is given an aeration hose that has been connected to the aerator hose as an oxygen supply.

2.3 Preparation of Test Animals

In this study vaname shrimp (*L. vannamei*) is a test animal that will be used with a weight of ±15 grams and free of vibriosis with a length of 9 cm. Before rearing, vaname shrimp will be acclimatized for 3 days. The aim is to give white vaname shrimp time to adapt to their new environment and to determine the body condition of the shrimp that will be used in research [6].

2.4 Extraction of Basil Leaves

The basil is first washed clean, then drained and air-dried without being exposed to sunlight with the aim of maintaining the bioactive compounds contained in basil leaves. Then separate the leaves from the stem. Extraction was carried out by blending the basil leaves, then weighing the basil leaf powder and then extracting it using the maceration method with a solvent, namely methanol. For the soaking (maceration) process, soak for 1×24 hours at room temperature and stir occasionally. Next, the extract is filtered to separate the filtrate and debris using Whatman filter paper, then the filter results are concentrated using a tool*rotary evaporator* [7].

2.5 Preparation of Test Bacteria

In this study the bacteria used were *V. parahaemolyticus* who came from the Maros Brackish Water Fisheries Research Institute. The isolate was then cultured in nutrient broth media, then incubated for 24 hours before use.

2.6 Vaname Shrimp Challenge Test

Vaname shrimp which have passed the acclimatization stage later injected intramuscularly with*V. parahaemolyticus* with a concentration of 0.1 ml/head. After 5 minutes of injection and the shrimp showing clinical morphological symptoms, the shrimp were immersed in basil leaf extract for 15 minutes according to the treatment dose of basil leaf extract (*O. basilicum*).

2.7 Test of Parameter

2.7.1 Observation of clinical symptoms

In observing clinical symptoms in shrimp will be observed abnormalities or changes in the macroanatomy of the test shrimp after the challenge test*V. parahaemolyticus* in each treatment. Shrimp clinical symptoms were observed on day 0, day 4, hour 8, hour 24, day 2, day 4, and day 8.

2.7.2 Total bacterial colonies

Calculation total bacterial colonies*V. parahaemolyticus* contained in the rearing media and hepatopancreas of vaname shrimp were calculated using the Total Plate Number Determination (ALT) technique or*Total Plate Count* (TPC) in water and shrimp media samples for each aquarium.

$$N = \frac{\sum C}{[(1 \times n1) + (0{,}1 \times n2)] \times d}$$

N = Number of product colonies, expressed in colonies per ml
ΣC = Number of colonies in all petri dishes counted
n1 = Number of plates in the first dilution calculated
n2 = Number of plates in the second dilution calculated
d = first dilution calculated

2.8 Survival Rate (SR)

Survival rate udang vaname (*L.* vannamei) was obtained by manually counting the total number of shrimp that were still alive in each experimental unit at the beginning and end of the study. Survival rate is calculated using the following formula [8]:

$$SR = \frac{Nt}{N0}.100\%$$

SR = survival (%)
N_t = number of test shrimp alive at the end of observation (individuals)
N_0 = number of test shrimp stocked at the start of observation (individuals)

2.9 Water Quality

The water quality that will be observed in the research is: dissolved oxygen (DO), temperature, pH, salinity and ammonia. Measured from the beginning of the observation and the end of the observation.

3. RESULTS AND DISCUSSION

3.1 Clinical Symptoms

Observation of clinical symptoms of vaname shrimp infected with bacteria*V. parahaemolyticus* showed changes in the behavior of vaname shrimp after two hours of injection, where the shrimp began to be seen swimming to the surface of the water. Shows clinical symptoms of vaname shrimp such as reddish pleopods, reddish preopods, reddish scale antennae and necrosis, the hepatopancreas has a yellowish or brownish pallor and melanosis on the abdomen. Based on Apriliani's research [9], that white vaname shrimp are attacked by bacteria*Vibrio* sp. will show behavior such as decreased appetite, swimming towards air stones and swimming to the side, becoming weak and dying. Apriliani further et al. [10] explained that the clinical symptoms of vaname shrimp such as reddish pleopods, reddish preopods, reddish scale antennae and necrosis, pale yellowish or brownish hepatopancreas and melanosis on the abdomen indicated a bacterial infection of the genus*Vibrio* sp (Austin, 2007). Similar clinical symptoms have also been reported in shrimp that have been attacked by vibriosis. According to Apriliani et al. [10] from the results of morphological and biochemical characterization, there are five bacterial agents that cause vibriosis in vaname shrimp, including*V. vulnificus, V. mimicus, V.damsella, V. parahaemolytics* and *V. Fluvial*

In treatment A (control), vaname shrimp that were not soaked in basil extract did not experience healing. Shrimp can only survive 1 x 48 hours, then the shrimp goes limp and dies. Meanwhile, in treatments B, C and D, the vaname shrimp recovered until the final day of observation. This is related to the soaking of basil leaf extract (*O. basilicum*) in aquariums B, C and D with different doses for each aquarium. The presence of bioactive compounds contained in basil leaves is thought to be antibacterial for healing vaname shrimp that are attacked by bacteria.*V. parahaemolyticus.* Solomon (2020), stated that basil leaves contain chemical compounds such as essential oils and ethanol extract which can inhibit bacterial growth.

Based on research results from Agung [11]**,** that the higher the dose of extract, the higher the antibacterial inhibition zone formed. This proves that the active compounds contained in basil leaves (*O. basilicum*) functions as an antibacterial in vaname shrimp that have been injected with bacteria*V. parahaemolyticus*.

3.2 Total Bacteria Colonies

Total Plate Number (ALT) is a method used to facilitate testing of microorganisms on certain products. With ALT, it can be seen whether there are pathogenic or non-pathogenic microorganisms through visual observation or using a magnifying glass on the planting medium being studied. Next, the ALT calculation results are based on the base plate for the standard test for bacteria. In a sample, the calculation is expressed in CFU/ml units. Results from bacterial counts*V. Parahaemolyticus* The Total Plate Number (ALT) in white shrimp hepatopancreas samples and shrimp rearing water samples can be seen in Table 1.

From the results obtained, the number of colonies*Vibrio* sp. in the highest water sample in treatment A: 2.18 x 10^3 CFU/ml, treatment B: 1.88 x 10^3 CFU/ml, treatment C: 1.67 x 10^2 CFU/ml and the lowest in treatment D: 1.20 x 10^2 CFU/ml. The highest number of hepatopancreatic colonies was in treatment A with a number of 2.13 x 10^3 CFU/ml, then followed by treatment B: 1.54 x 10^3 CFU/ml, C: 1,17 x 10^2 CFU/ml and treatment D: 1.12 x 10^2 CFU/ml.

Vibrio sp. It is known that it is a gram-negative bacteria that is cumulatively anaerobic and is the main cause of disease*Vibriosis* even if the abundance exceeds the threshold and continues to increase over time, it can cause mass death of affected shrimp [12]. Based on government regulation No.75/PERMEN-KP/2016, the number of bacterial densities*Vibrio* sp. at a maximum threshold of 10^3 CFU/ml. This is also reinforced by Ambat, et al. [13], that the bacterial density is 10^3 can cause farmed animals to suffer from vibriosis and even mass death. According to, Sukenda et al. [14] that bacteria*Vibrio* which causes vibriosis are bacteria *Vibrio injurious* and*Vibrio parahaemolyticus.* Calculation of the number of bacterial colonies*Vibrio* sp. Each

treatment was carried out to determine the density of bacteria in the water and hepatopancreas of vaname shrimp (*L. vannamei*).

The research results show that the number of densities *Vibrio* sp. on treatment A achieves 2,13 x 10^3 CFU/ml, this amount is above normal, causing clinical symptoms in shrimp. Meanwhile for treatments B, C and D it is in the range of 10^3 CFU/ml, meaning bacterial density*Vibrio* sp. This is still in the safe range and is not harmful either to the environment or to the cultivated shrimp.

3.3 Survival Rate

After the vaname shrimp is infected by bacteria*V. parahaemoliyticus* Shrimp survival rates varied greatly from each treatment. In treatment A, the control (without basil leaf extract) showed the lowest percentage of survival 23,33±11,55%, then followed by treatment B with a dose of basil leaf extract (1.56 mg/ml) showing a survival rate of 40,00±17,32%, while treatment C with a dose of basil leaf extract (3.12 mg/ml) showed a survival rate of 86,67±0,82% and the highest percentage of survival towards the end of the review was in treatment D with a dose of basil leaf extract (12.5 mg/ml) of 86,67±5,77%.

Based on the results of the analysis of variance, it showed that the treatment with basil leaf extract showed a significant difference in the survival of vaname shrimp. The results of further tests or BNJ (Honest Significant Difference) showed a real difference between treatments A, B, C and D. This was caused by the low survival in treatment A because in this treatment no basil leaf extract was given and the higher survival in treatment D than B and C because of the high concentration of the extract given in the aquarium/immersion, so that the bioactive compounds contained in basil leaf extract are able to be absorbed well in the shrimp's body so that they can inhibit bacterial growth.*V. parahaemolyticus.* The survival rate of vaname shrimp can be seen in Fig. 1.

The survival of vaname shrimp in this study was influenced by the dose of extract given. The higher the concentration given, the greater the antibacterial activity that is formed, the lower the shrimp mortality. One of the compounds contained in basil leaves is flavonoids which play an important role in the healing process as antibacterials, by denaturing protein cells and damaging bacterial cell walls, so that bacteria cannot reproduce in the shrimp's body and prevent the release of toxic substances so that they can kill bacteria [15].

3.4 Water Quality

The results of measuring water quality parameters during the research are presented in Table 2.

Table 2 shows that during the research the salinity was in the range of 28.72-29.05 ppt, the temperature was in the range of 26.71-27.25^{O}C, oxygen ranges from 5.72 to 7.81 ppm, pH ranges from 7.18 to 8.13 and ammonia ranges from 0.033 to 0.041. Temperature and salinity are one of the main limiting factors for shrimp life and growth. Zulfikar [16], stated that shrimp grow well in the salinity range of 36-40 ppt, temperature 26-32^{O}C, dissolved oxygen > 4 ppm, [17,18]. Meanwhile, the optimum dissolved oxygen concentration for shrimp is above 4 mg/l, pH is in the range of 7.0-8.5 and ammonia is in the range of <0.01 ppm [19-21,3,4]. Based on the results of measuring water quality parameters, it can be explained that the range of water quality parameters during the research was in the optimal range for the survival of vaname shrimp [22-25].

Table 1. Results total number of *Vibrio sp.* colonies

No	Treatment	Number of colonies *V. parahaemolyticus* (CFU/ml)	
		Water Media	**Hepatopankreas**
1	A	2,18 x 10^3	2,13 x 10^3
2	B	1,88 x 10^3	1,54 x 10^3
3	C	1,67 x 10^2	1,17 x 10^2
4	D	1,20 x 10^2	1,12 x 10^2

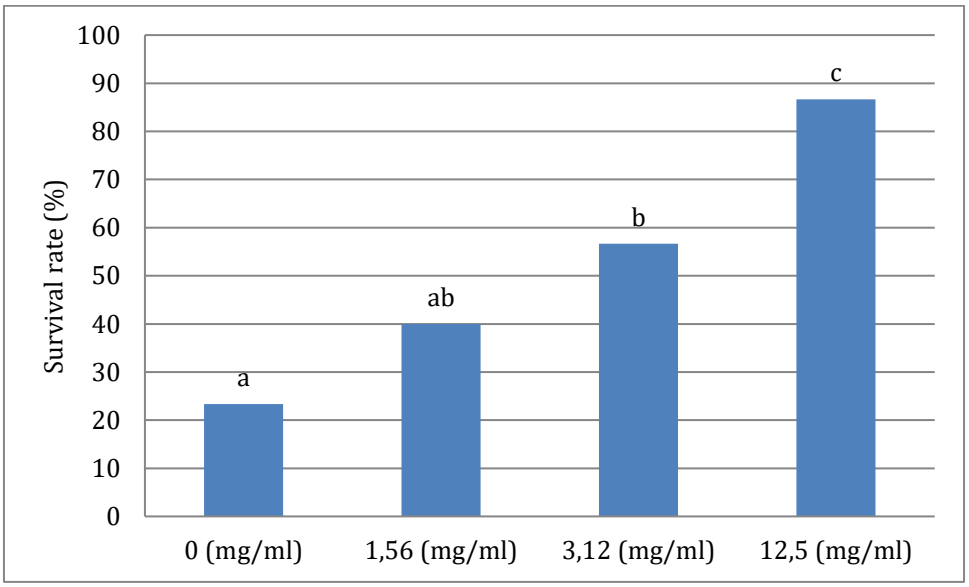

Fig. 1. Survival Rate vaname shrimp (*Litopanaeus vannamei*)

Table 2. Water quality measurement results

Treatment	Salinity (ppt)	Temperature (^{O}C)	Dissolved Oxygen (ppm)	pH	Ammonia (ppm)
A (0 ppm)	28,72±0,19	26,94±0,38	5,76±0,12	7,26±0,13	0,039±0,013
B(1,56 ppm)	28,67±0,25	26,77±0,32	5,81±0,28	7,21±0,05	0,035±0,021
C(3,12 ppm)	29,05±0,19	27,25±0,34	5,72±0,23	7,18±0,05	0,041±0,022
D(12,5 ppm)	28,71±0,30	26,71±0,17	5,78±0,30	8,13±0,06	0,033±0,019

4. CONCLUSION

Clinical symptoms appear post-injection*V. parahaemolyticus* soaked in basil leaf extract (*O. basilicum*) namely pale yellowish hepatopancreas, black/reddish antennal scales, reddish uropods, reddish pleopods and preopods and the abdomen has melanosis.Effect of basil leaf extract (*O. basilicum*) with different doses on bacterial survival*V. parahaemolyticus* in hepatopancreas samples was highest in treatment A (control) equal to 2.13 x 10^3 CFU/ml dan D (12,5 mg/ml) 1,12 x 10^2 CFU/ml. For the highest water sample in treatment A (control) 2.18 x 10^3 CFU/ml and the lowest in treatment D 1.20 x 10^2 CFU/ml (12,5 mg/ml). The highest survival was in treatment D with a dose of basil leaf extract (12.5 mg/ml) with a survival rate of 86,67±5,77% and the lowest was in treatment A (control) at 11.11 ± 47.14%.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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