4

by A F Pratiwi A F Pratiwi

Submission date: 09-Jun-2021 05:54PM (UTC+0800)
Submission ID: 1603373408
File name: -Pratiwi_2021_IOP_Conf._Ser.__Earth_Environ._Sci._679_012068.pdf (1.1M)
Word count: 3534
Character count: 17872

PAPER · **OPEN ACCESS**

The administration of *Caulerpa racemosa* extract on total bacteria and survival rates of white shrimp (*Litopenaeus vannamei*) after infected by *Vibrio parahaemolyticus*

The administration of *Caulerpa racemosa* extract on total bacteria and survival rates of white shrimp (*Litopenaeus vannamei*) after infected by *Vibrio parahaemolyticus*

A F Pratiwi[1], W H Satyantini[2]*, G Mahasri[2], L Sulmartiwi[3], A T Mukti[2], Sudarno[2]

[1]Aquaculture Program, Faculty of Fisheries and Marine, Universitas Airlangga Campus C UNAIR, Jl.Mulyorejo, Surabaya, Indonesia 60115
[2]Department of Fish Health Management, Faculty of Fisheries and Marine, Universitas Airlangga, Campus C UNAIR, Jl.Mulyorejo, Surabaya, Indonesia 60115
[3]Departement of Marine, Faculty of Fisheries and Marine,UniversitasAirlangga, Campus C UNAIR, Jl.Mulyorejo, Surabaya, Indonesia 60115

*Corresponding author: woro_hs@fpk.unair.ac.id

Abstract. The purpose of this study was to determine the effect of administration *Caulerpa racemosa* extract on total bacteria and survival rate of white shrimp (*Litopenaeus vannamei*) after infected by *Vibrio parahaemolyticus*. This study used a Completely Randomized Design with five treatments and three replications. Shrimp were divided into four groups, one control group without *C. racemosa* extract, another three groups were administrated with *C. racemosa* extract in different doses; 3 μg/g (P1), 6 μg/g (P2) and 9 μg/g (P3) by injection. After 24 hours, the control group was divided into two groups, negative control (K-) was injected by PBS and positive control (K+) also another three groups (P1, P2 and P3) were infected by *V. parahaemolyticus*. Observed parameters were total bacteria and survival rates. On the 1st day, the total bacteria was lower in the treatment with *C. racemosa* extract than K+ and significantly different ($P < 0.05$). On the 7th day, total bacteria increased again, but in P3 (20.46 $\times 10^4$ CFU/ml) was lower than K+ and significantly different ($P < 0.05$). The highest survival rates was 93.33% (P2) and the lowest was 56.67% (K+). It can be concluded that *C. racemosa* extract can be given to *L.vannamei* at dose 6 μg/g.

1. Introduction

White shrimp (*Litopenaeus vannamei*) is a fishery commodity that is profitable, including fast growth, high stocking density and has a high selling price [1]. World white shrimp production in 2015 was above 3.5 million tons with the largest contributor to China 1.62 million tons, India 416,000 tons and Indonesia 410,000 tons [2]. The increasing market demand causes white shrimp to be cultured intensively.

Disease is a limiting factor in shrimp farming. Diseases can be caused by bacteria, viruses, fungi or parasites. Vibriosis attacks can cause mortality up to 100% in the larval or juvenile stage [3]. Several studies have reported that acute hepatopancreatic necrosis disease or AHPND caused by *V. parahaemolyticus* caused 80% mortality in China in 2011 [4], while AHPND cases in Mexico resulted in 81% mortality [5].

Antibiotics as disease control have been prohibited from being used, this is because they can cause the emergence of antibiotic-resistant pathogens and leave a residue in the environment and on the body of shrimp [6]. One of the efforts to control and prevent disease in shrimp is through increasing the shrimp defense system by using immunostimulants [7]. Immunostimulants are biological in nature and are synthesized from compounds that can induce a non-specific immune system in shrimp.

Caulerpa racemosa has potential as a source of phytochemicals that have immunological activity, namely sulfate polysaccharides. Sulfate polysaccharides have biological activities such as immunology, antiviral, and anti-oxidation [8]. Sulfate polysaccharides can boost the immune system by activating macrophage activity [9]. The purpose of this study was to determine the effect of administrating *Caulerpa racemosa* extract to total bacteria and survival rate of white shrimp (*Litopenaeus vannamei*) after infected with *Vibrio parahaemolyticus*.

2. Material and methods

2.1. Materials

There are 20 aquariums used with a size of 40 x 30 x 30 cm^3 for this research. White shrimp (*Litopenaeus vannamei*) DOC 30 with a weight of 2- grams and a length of 5-7 cm. Previously, the shrimp were acclimatized for 15 days, then before using the shrimp, they were isolated first whether they were infected with *Vibrio* or not. *Caulerpa racemosa* is obtained from Balai Besar Perikanan Budidaya Air Payau Jepara. While the *Vibrio parahaemolyticus* isolate was obtained from Balai Perikanan Budidaya Air PayauSitubondo.

2.2. Methods

2.2.1 Research procedures

a. Extraction of C. racemosa by hot water method

Caulerpa racemosa powder is put into Erlenmeyer mixed with distilled water with a ratio (g: v / 1:20) then put into a beaker glass filled with hot water 85ºC. Extraction was carried out for 2 hours at a temperature of 85ºC above the hotplate stirrer. Then the extract was separated between the residue and the supernatant using a refrigerator centrifuge at 4 ºC at 3500 rpm for 15 minutes. The supernatant is then separated with the residue and then filtered. The filtrate obtained was then carried out by solvent evaporation using a rotary evaporator and then put in an oven at 35ºC to get the extract in the form of a paste.

b. Aquarium preparation and water maintenance media

Before use the aquarium is cleaned by brushing and rinsing water until clean. The aquarium is filled with water that has been added with 10 ppm of chlorine as a disinfectant. The aquarium was immersed for 24 hours, then the chlorine water was removed and rinsed with clean water. Furthermore, drying for 24 hours with an inverted aquarium position.

c. Preparation of anticoagulant

The preparation of the anticoagulant is by mixing the following ingredients 30 mM trisodium citrate, 0.34 M sodium chloride, 10 mM EDTA while maintaining an atmosphere of pH 7.5.

d. Vibrio parahaemolyticus Culture and Density Measurement

V. parahaemolyticus culture was carried out by taking one ose of isolate and culturing it on TSB media aseptically, then incubating for 24 hours at room temperature (33 ° C). After 24 hours, the concentration of bacteria was measured using a spectrophotometer.

e. White shrimp rearing

The use of feed in the maintenance of white shrimp uses artificial feed in the form of pellets with a minimum protein content of 36%. White shrimp feed is given as much as 3% of the total body weight. The frequency of feeding was three times a day, namely at 09.00 AM, 01.00 PM and 05.00 PM.

f. Injection with C. racemosa

White shrimp was injection with *C. racemosa* intramuscularly in the third segment of the abdomen as much as 0.1 ml / head. Meanwhile, for the control treatment, injected with PBS as much as 0.1 ml / shrimp. *C. racemosa* extract was administered at the beginning of maintenance (T0) on shrimp.

g. Challenge test with V.parahaemolyticus
The challenge test with *V. parahaemolyticus* was carried out 24 hours after given *C. racemosa* extract by injection (T1), which was injected intramuscularly into the third segment of the abdomen as much as 0.1 ml / shrimp, for control treatment injected with PBS as much as 0.1 ml / shrimp.

h.Collecting the hepatopankreas
Shrimp hepatopancreas was taken for total bacterial observation. The hepatopancreas was taken by sterilizing the body of the shrimp with alcohol and then the shrimp was dissected to extract the hepatopancreas. The hepatopancreas was placed in a sterile microtube containing 0.9 ml of PBS and then homogenized.

2.2.2 Parameter of Research
The main parameters observed were total bacteria and survival rate. The supporting parameters observed were clinical sign and water quality which included temperature, pH, Dissolved Oxygen and salinity.

2.2.3 Data Analysis
Data processing from the research used Analysis of Variance (Anova) to determine the effect of the treatment given. If the results are significantly different ($p < 0.05$), the calculation is continued with the Duncan Test (DMRT) using the SPSS 20 tool [10].

3. Result and discussion

3.1 Result

3.1.1 Total Bacteria

The average of total bacteria in hepatopancreas of *L. vannamei* could be seen in Table 1.

Table 1. The average of total bacteria in hepatopancreas of *L. vannamei*

Treatment	Total bacteria ($\times 10^4$ CFU/ml)		
	T0 ± SD	T1 ± SD	T7 ± SD
K-	$24.56^{bc} \pm 0.49$	$3.33^{d} \pm 0.21$	$14.20^{e} \pm 0.26$
K+	$24.86^{ab} \pm 0.15$	$26.20^{a} \pm 0.43$	$23.46^{c} \pm 0.45$
P1	$24.13^{c} \pm 0.15$	$17.03^{c} \pm 0.15$	$24.90^{a} \pm 0.10$
P2	$24.56^{bc} \pm 0.35$	$21.36^{b} \pm 0.32$	$24.26^{b} \pm 0.25$
P3	$25.26^{a} \pm 0.31$	$21.26^{b} \pm 0.25$	$20.46^{d} \pm 0.50$

Description: (K-) sterile PBS injection; (K +) infection with VP 10^6 CFU / ml per shrimp; (P1) injection *of C. racemosa* 3 µg / g + VP infection 10^6 CFU / ml per shrimp; (P2) injection of *C. racemosa* 6 µg / g + VP infection 10^6 CFU / ml per shrimp; (P3) injection of *C. racemosa* 9 µg / g + VP infection 10^6 CFU / ml per shrimp; (T0) 24 hours after *C. racemosa* extract administration and prior to infection with *V. parahaemolyticus*; (T1) one day after being infected with *V. parahaemolyticus*; (T7) seven days after being infected with *V. parahaemolyticus*. Different letter notations in the same column showed significantly different results ($p < 0.05$).

Total bacteria on T1 or one day after *V.parahaemolyticus* infection, the K + treatment was significantly different ($p < 0.05$) against K-, P1, P2 and P3. The highest total bacteria shown in the positive control (K +) treatment**,** shrimp without *C. racemosa* extract and being infected with *V. parahaemolyticus* (26.20 x 10^4 CFU / ml). Whereas at T7 or seven days after being infected with *V.parahaemolyticus*, the total bacteria in treatment P1 was significantly different ($p < 0.05$) to treatment K-, K +, P2 and P3. Total bacteria decreased at T7, namely in the P3 treatment, namely 20.46×10^4 CFU / ml.

3.1.2 Survival Rates

The survival rates of white shrimp can be seen in Table 2. The survival rates of white shrimp during maintenance after administration of *C. racemosa* extract onT1 or one day after being infected with *V. parahaemolyticus* ranged from 83.33 (P1) to 100% (P2) and the results of the analysis of variability (ANOVA) showed significantly different results ($p<0.05$). Shrimp survival in P2 treatment (100%) was significantly different from treatment P1 (83.33%), but not significantly different (p>

0.05) with treatment K-, K + and P3. The survival rates of white shrimp ranged from 56.67(K +) to 93.33% (P2) and showed significantly different results ($p<0.05$) at T7.

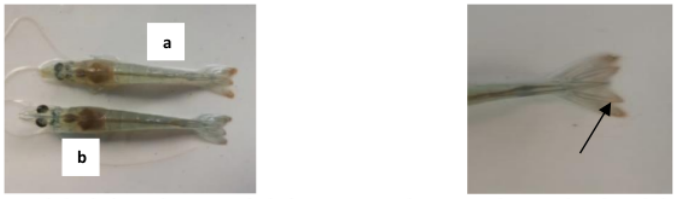
Table 2. Survival rates of white shrimp (*L. vannamei*)

Treatment	Survival rates (%)		
	T0 ± SD	T1 ± SD	T7 ± SD
K-	$100^{a} \pm 0$	$90.00^{ab} \pm 0.00$	$90.00^{a} \pm 0.00$
K+	$100^{a} \pm 0$	$86.67^{ab} \pm 11.5$	$56.67^{c} \pm 11.5$
P1	$100^{a} \pm 0$	$83.33^{b} \pm 5.77$	$73.33^{b} \pm 11.5$
P2	$100^{a} \pm 0$	$100.00^{a} \pm 0.00$	$93.33^{a} \pm 5.77$
P3	$100^{a} \pm 0$	$90.00^{ab} \pm 10.0$	$86.67^{ab} \pm 5.77$

Description: (K-) sterile PBS injection; (K +) infection with VP 10^{6} CFU / ml per shrimp; (P1) injection *of C. racemosa* 3 µg / g + VP infection 10^{6} CFU / ml per shrimp; (P2) injection of *C. racemosa* 6 µg / g + VP infection 10^{6} CFU / ml per shrimp; (P3) injection of *C. racemosa* 9 µg / g + VP infection 10^{6} CFU / ml per shrimp; (T0) 24 hours after *C. racemosa* extract administration and prior to infection with *V. parahaemolyticus*; (T1) one day after being infected with *V. parahaemolyticus*; (T7) seven days after being infected with *V. parahaemolyticus*. Different letter notations in the same column showed significantly different results ($p < 0.05$).

3.1.3 Clinical Sign

The clinical sign emerged at the third day after infected by*V. parahaemolyticus*. Based on the results of the study, white shrimp infected with *V. parahaemolyticus* showed clinical sign, namely the hepatopancreas was pale, the intestines were empty, the uropods turned red and the gnats.



a. sick shrimp; b. normal shrimp the uropod turned red and the gnats

Figure 1. Clinical sign ofshrimp infected by*V. parahaemolyticus*

3.1.4 Water Quality

The data of water quality range value can be seen in Table 3.

Table 3. Water quality during the experiment

Parameter	Treatment					References
	K-	K+	P1	P2	P3	
Temperature(°C)	28-29.2	28.2-29.8	28.2-30.1	28.2-30.1	28.1-29	28-33(SNI. 2014)
Salinity (ppt)	15	15	15	15	15	15-32 (Hukom*et al*. 2020)
pH	7-8	7-8	7-8	7-8	7-8	7.5-8.5(SNI. 2014)
DO (ppm)	3.95-5.22	4.14-5.42	4.27-6.26	4.05-5.69	3.87-5.33	>3.0(Mohanty *et al*.2018)

Water quality as a supporting parameter in this study was measured in all treatments. Water quality supports the life of aquatic organisms.The water quality all treatments are in accordance with the references which is in the normal range. The temperature of 28-30.1 °C, salinity is 15 ppt, pH is 7-8 and Dissolved Oxygen is in the range of 3.87-6.26 ppm.

3.2 Discussion

Total bacteria were isolated from hepatopancreas of shrimp because of changes in clinical symptoms that appear is pale hepatopancreas. Acute hepatopancreatic necrosis disease (AHPND) or known as Early mortality syndrome (EMS) is known to attack this organ. Total bacteria on T1 (one day after

V.parahaemolyticus infection) decreased in all treatments with the administration of *C.racemosa* extract in P1, P2 and P3 treatments, namely 17.03 × 10^4 CFU / ml, 21.36 × 10^4 CFU / ml and 21.26 × 10^4 CFU / ml, respectively. Meanwhile. in the positive control (K +) treatment, shrimp without *C. racemosa* extract and being infected with *V. parahaemolyticus* showed the highest total bacteria (26.20 x 10^4 CFU / ml). This indicates that the sulfate polysaccharides contained in *C.racemosa* extract are starting to work as an immunostimulant which can reduce the total bacteria in the hepatopancreas. According to [11]. the addition of sulfated polysaccharides from *Ulva lactuta* extract can increase the activity of the immune system in shrimp. This is due to an increase in total hemocytes and phagocytosis activity in shrimp after giving the extract. Hemocytes are a form of cellular defense from shrimp. Haemocyte can extinguish the infection caused pathogen by the synthesis and exocytosis of microbicidal protein bioactive molecule. According to [12].hemocytes consist of granular.semigranular and hyalin cells. Hyalin cells play a role in phagocytosis.semigranular cells play a role in melanization. encapsulation and coagulation. while granular plays a role in activating the ProPO system. The results showed that at T7 or 7 days after being infected with *V.parahaemolyticus* there was an increase in the total bacteria back in treatment P1 (24.90 × 10^4 CFU / ml) and P2 (24.26 × 10^4 CFU / ml) but the extract was given at a dose of 9 μg / g showed a lower total bacterial yield than K + (Table 1). This condition indicates that the immune system is back to normal conditions so that in this condition it is necessary to re-administer the *C.racemosa* extract (booster). According to [13]. that giving immunostimulants through feed once every 3 days is more effective than giving once every 7 and 10 days. This is because the shrimp do not have memory cells and the short-term nature of the shrimp immune response. So it is necessary to provide periodic immunostimulants so that it can increased non-specific immune systems in shrimp.

The highest survival rates at T1 (one day after being infected with *V. parahaemolyticus*) was found in the treatment with the addition of 6 μg / g (P2) of *C. racemosa* extract which reached 100% and the lowest value was in P1 treatment (83.33%). On the 7th day. the survival rates value in P2 treatment (93.33%) was higher than that of K + treatment (56.67%). According to [14]. 6-20 µg / g of *Spirulina platensis* extract injected into shrimp resulted in a higher survival rates compared to the control. This is because the polysaccharide sulfate content in seaweed can stimulate the shrimp immune system. Polysaccharides sulfate in *Gracilaria verrucosa* can stimulate the immune response of shrimp by increasing resistance to disease so that the survival rate of shrimp is high [15].

The results of the observation of clinical sign of shrimp infected with *V. parahaemolyticus* were among other things: the hepatopancreas was pale, the intestine was empty, the uropod turned red and the gnats. *Vibrio parahaemolyticus* is a halophilic bacterium that attacks white shrimp and causes lethargy, irregular swimming movements, empty intestine and hepatopancreas blanching [16] as shown in Figure 1. The results of measurement of water quality parameters are still in the optimum value and tolerance for the life of white shrimp. The temperature during maintenance is 28-30.1 ºC. this value is suitable for white shrimp culture, which is between 28 - 33 ºC [17]. Salinity during the maintenance of white shrimp is 15 ppt. This value is still within the tolerance limit for the maintenance of white shrimp. namely salinity 15 - 32 ppt [18]. The results of the average pH parameter measurement are 7 - 8 where these results are in the optimum value for white shrimp culture, namely 7.5 - 8.5 [17]. The average result of Dissolved Oxygen parameters is 3.87-5.33 ppm. The optimum Dissolved Oxygen value in white shrimp culture is > 3 ppm [19].

4 Conclusion

The administration of *C. racemosa* extract by injected can affect the total bacteria in white shrimp after being infected with *Vibrio parahaemolyticus*. On the first day after infection. the total bacteria in the treatment with the administration of *C. racemosa* extract was lower than the positive control. The administration of *C. racemosa* extract can affect the survival rate of white shrimp and the highest was achieved in treatment with a dose of 6 μg / g.

5 References

[1] Putri FM. Sarjito. Suminto 2013 J of *Aquacult Manag and Tech*.. 2 102-112.

[2] Food and Agriculture Organization 2017 Fishstat Plus Version 2.30. FAO Fisheries Department. Fishery Information. Data and Statistics Unit. http://www.fao.org./fi/ statist/FISOFT/FISHPLUS asp. 24 February 2017.
[3] Kementerian Kelautan dan Perikanan Indonesia 2018 Diakses dari http://www.kkp.go.id
[4] Zorriehzahra MJ and Banaederakhshan 2015 *Advanc Anim and Vet Sci*. 3 64-72.
[5] Nunan L, Lightner D, Pantoja C, Gomez-Jimenez S 2014 *Aquatic Organ*. 111 81- 86.
[6] Satyantini W H, Ananta K, dan Rahayu K 2016 J *Akuakul Indo*. 1(2) 120-129.
[7] Pujiati. Sarjito dan Suminto 2013 *Jurnal Manaj Akuakul dan Tek*. 2(1) 66-74.
[8] Xie JH. Wang ZJ, Shen MY, Nie SP, Gong B. Li H 2016 *Food Hydrocolloids*.53 7–15.
[9] Huang L. Shen M. Morris GA. & Xie J 2019 *Tren in Food Scie & Tech*. 92 1-11.
[10] Kusriningrum 2008 *Dasar Perancangan Percobaan dan Rancangan Acak Lengkap*. *Fakultas Kedokteran Hewan* (Universitas Airlangga: Surabaya) p 92.
[11]Suleman S. Sri A. and Ating Y 2018 *Res J of Life Sci*. 5(3) 156-162.
[12] Cheng W, Ka YW and Chang CC 2017 *Fish & Shellfish Immun*. 68 92-101.
[13] Ojerio VT. Corre VL, Toledo NA, Andrino-Felarca KGS, Nievales LM & Traifalgar RFM 2018 Aqua inter. 26(1) 267-278.
[14] Tayag C, Yong C, Chang C, Chyng H, Jiann C 2010 *J of Fish & Shellfish Immun*. 28 764-773.
[15] Jasmanindar Y, Sukenda S, Alimuddin A, Junior MZ, and Utomo NBP 2018 *Omni-Akua*. 14(3).
[16] Tawut R, Nantavadee B, Somluk A, Boonsirm W, Kanokpan W 2017 *J of Fish and Shellfish Immun*. 65 186-197.
[17] SNI 8037.1 2014 *Udang Vaname (Litopenaeus vannamei. Boone 1931) Bagian 1: Produksi Induk Model Indoor* Badan Standardisasi Nasional (Jakarta) p 7.
[18] Hukom V, Nielsen R, Asmild M, and Nielsen M 2020 *Ecologic Eco*. 176 106717.
[19] Mohanty RK, Ambast SK, Panigrahi P, and Mandal KG 2018 *Aquaculture*. 485 210-219.

6 Acknowledgement

The author would like to thank the supervisors and lecturers, who helped this research. Futhermore thanks to Faculty of Fisheries and Marine, Universitas Airlangga, for conducting the seminar.

4

ORIGINALITY REPORT

12%	10%	9%	0%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

MATCH ALL SOURCES (ONLY SELECTED SOURCE PRINTED)

2%

★ repository.wima.ac.id

Internet Source

Exclude quotes	On	Exclude matches	Off
Exclude bibliography	On		