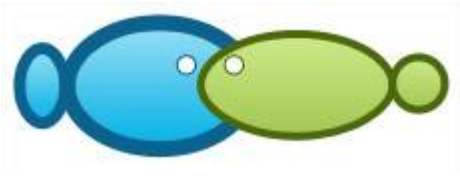

Antibacterial and immunostimulatory activities of sodium alginate isolated from brown seaweed, *Sargassum* sp.

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Abstract. Sodium alginate was isolated from brown seaweed *Sargassum* sp. to identify its antibacterial and immunostimulating activities. The antibacterial activity of sodium alginate was evaluated *in vitro* against *Vibrio harveyi*. *In vitro* test was carried out by first preparing a sodium alginate solution with a dose of 0; 1; 2.5; 5; 7.5; 10; and 15 mg mL^{-1} PBS. *In vitro* antibacterial activity test resulted in three selected doses with the strongest ability to inhibit the growth of *V. harveyi* based on the area of inhibition zone formed on TSA agar with 1.5% NaCl; 10 mg mL^{-1}, 5 mg mL^{-1} and 7.5 mg mL^{-1} of sodium alginate, in which the number of growing colonies was approximately 3.3 x 10^5, 4.2 x 10^5, and 2.9 x 10^5 CFU mL^{-1}, respectively. A significant increase in the total hemocyte count (THC), phagocytic index (PI), and phenoloxidase (PO) was observed during the *in vivo* evaluation via intramuscular injection of the *Litopenaeus vannamei* shrimp at the dose of 10 µg g^{-1}, 15 µg g^{-1}, and 20 µg g^{-1}, respectively. The highest phagocytic index was 37.0±1.41% exhibited by 10 µg g^{-1} of injection dosage. The result showed the presence of antibacterial activity of sodium alginate from *Sargassum* sp. and its potential as an immunostimulator to enhance shrimp's nonspecific immune response.
Key Words: immunostimulator, *Litopenaeus vannamei*, non-specific immune response, sodium alginate, *Vibrio harveyi*.

Introduction. Vannamei shrimp (*Litopenaeus vannamei*) is one of the main commodities that has been extensively farmed in Indonesia. One of the advantages of vannamei shrimp is that they are genetically healthier and tend to be more resistant to pathogen attacks than tiger shrimp (*Penaeus monodon*), thus having faster growth, and rearing periods until harvest (Balasubramanian et al 2018). The growth of vannamei shrimp cannot be dissociated from bacterial diseases including vibriosis. Shrimp luminescent disease or luminescent vibriosis caused by *Vibrio harveyi* is a major issue in shrimp farming in Indonesia. Vibriosis in vannamei shrimp is characterized by loss of appetite, red coloration of the body and pleopods, gills are often seen as red to brown, reduced appetite, and empty intestines (Mastan & Begum 2016). The disease is typically triggered by the influence of stress from the cultural environment (de Souza Valente & Wan 2021), thus *Vibrio* infects the shrimp bodies that are in a weakened condition (Zhang et al 2020).

Various ways can be used to prevent and control bacterial attacks on shrimp and fish, including the use of vaccines and antibiotics. Vaccination is very effective, but not against several bacteria or viruses simultaneously. While treatment with antibiotics and other chemotherapeutic agents may induce resistance to pathogenic microorganisms, as well as build-up in fish and their environment (Pepi & Focardi 2021). A lot of attention has been given to the use of immunostimulants in aquaculture to combat infectious diseases (Kela 2022). Immunostimulants can increase shrimp resistance to disease. These immunostimulants may be obtained from seawater macroalgae and have been used in fish and shrimp (Kumar et al 2023). Brown seaweed sodium alginate contains fucoidan, which is an anti-tumor, antibacterial, and antiviral drug that can increase immunity and stimulate immune cells (Reyes et al 2020). The use of seaweed as an

immunostimulant is relatively safe because it is neither toxic nor pathogenic to shrimp and is environmentally friendly (Abdel-Latif et al 2022).

Indonesia has vast waters and one of the most important products of fishing is the abundance of seaweed. Therefore, the isolated sodium alginate of *Sargassum* sp. will be investigated for its antibacterial properties as well as an immunostimulator, hence it can increase the non-specific immune response and the resistance of vannamei shrimp to *Vibrio harveyi* infection.

Material and Method

Extraction of Sargassum sp. The production of sodium alginate using the alginic acid extraction method refers to the method of Husni et al (2012). Dissolved sodium alginate was separated from the rest of the seaweed which was not soluble in alkali, especially cellulose. The separation was done using a filter press consisting of fine filter cloth, filter aid, or metal filters (120-200 mesh). Precipitation of calcium alginate was carried out by the addition of sodium alginate filtrate into the calcium alginate solution in the form of fibers. Then the fiber was separated from the solution by a filter. Furthermore, bleaching was carried out by adding sodium hypochlorite (NaOCl) to a suspension of calcium alginate in water. While the conversion of calcium alginate to alginic acid was performed by putting calcium alginate into a vessel containing acid (HCl) with a pH of less than 2 resulting in a solid consisting of alginic acid, then filtered, the solid was washed with water, the result was alginic acid. Conversion of alginic acid to sodium alginate was done by mixing alginic acid with a solid alkali and sodium carbonate (Na_2CO_3), the mixture was stirred to form a sodium alginate paste, dried, and made into flour. Sodium alginate flour was then ready to use.

Vanname shrimp acclimatization. The test animals used in this study were vannamei shrimp from the cultivation location in Pangkep Regency. Before being used in the study, the shrimp were acclimatized in rearing tanks equipped with aerators for two weeks. During rearing, the shrimp were given commercial feed twice a day at a dose of 3% of the shrimp biomass. The seawater used in the culture containers was filtered and processed. The treatment container for research was an aquarium equipped with an aeration device. Before use, the aquarium was cleaned and sterilized using a chlorine solution, then rinsed with clean water and dried. Then, the aquarium was filled with clean seawater and aerated.

Preparation for the V. harveyi suspension. The pathogenic strain *V. harveyi* MR 3559 Rf^R was obtained from the Pathology Laboratory of BBRBAP Maros. Before being used for the test, the virulence of the bacteria was increased by reinfection of healthy live shrimp and then isolated again in thiosulfate citrate bile salts sucrose (TCBS) media which had been mixed with 50 μg mL^{-1} of the antibiotic rifampicin, and incubated at room temperature. Then the pathogen was cultured on sea water complete (SWC) agar for 24 h at room temperature, then transferred to 20 mL SWC liquid (broth) for 12-16 hours at room temperature as the stock culture for the test. The culture stock was centrifuged at 7155 x *g* for 15 min. The supernatant was transferred and the bacterial pellet was suspended in a solution of phosphate-buffered saline (PBS) as a bacterial suspension stock for antibacterial activity and challenge test.

Antibacterial activity of sodium alginate. The test was conducted to determine the antibacterial activity of sodium alginate against *V. harveyi.* The test was carried out by first preparing a sodium alginate solution with a dose of 0; 1; 2.5; 5; 7.5; 10; and 15 mg mL^{-1} PBS. *V. harveyi* MR3559 Rf^R was spread evenly on 0.1 mL SWC-agar medium. Paper discs (Whatman antibiotic assay paper 42) with a diameter of 6 mm were immersed in each concentration of sodium alginate solution for 5 min. After that, the disc paper was taken using tweezers and placed on the surface of the media containing bacteria and incubated at room temperature for 24 hours. PBS solution was used as a control. After 24 hours of incubation at room temperature, the diameter of the clear zone formed around

the paper disc was measured (in mm). The dose of sodium alginate that produces a clear zone was 5, 7.5, and 10 mg mL^{-1}. These results indicate the antibacterial activity of sodium alginate can inhibit *V. harveyi*. To strengthen the results of the antibacterial activity test, the cup count method was used to see the antibacterial activity of the three selected doses of sodium alginate. A volume of 9 mL of SWC agar medium was mixed with 1 mL of sodium alginate solution. After that, the mixture of media and extract was poured into a Petri dish. After cooling, 0.1 mL of *V. harveyi* bacteria was poured into each medium and spread evenly. After that, it was incubated for 24 h to see the number of bacterial colonies and counted. Later, the three doses of sodium alginate that have been tested were applied to further tests (*in vivo*).

Evaluation of the effect of sodium alginate as an immunostimulator on the non-specific immune response of vannamei shrimp*. In vivo* tests were performed to evaluate the immunostimulatory effects of three doses of sodium alginate on non-specific immune response parameters of shrimp vanname. The *in vivo* test included five treatments and three replicates, three doses of sodium alginate 10, 15, and 20 μg g^{-1} $shrimp^{-1}$, and one positive control (with the addition of sterile PBS) and one negative control (without the addition of any substances). The dose of sodium alginate injected into the shrimp's body was 10, 15, and 20 μg g^{-1} $shrimp^{-1}$. The doses were obtained by making a stock solution by dissolving 5, 7.5, and 10 mg of sodium alginate into each 1 mL of PBS. A volume of 20 μL of stock solution was injected into shrimp that had been acclimatized for 2 weeks, with an average weight of 10.40-11.90 g $shrimp^{-1}$. Furthermore, the shrimp were randomly distributed into the aquaria (5 shrimps/aquaria), thus a total of 75 shrimp were used. Feeding the shrimp was 2 times daily with a feeding rate (FR) of 3% of the weight of the biomass. Analysis of shrimp immune parameters was carried out by taking hemolymph using a syringe from the base of the first swimming leg of each test shrimp on days 0, 1, 3, 5, and 7 post-injection of sodium alginate. The shrimp immune parameters measured were total hemocyte count (THC), differential hemocyte count (DHC), phagocytic index (PI), and phenoloxidase (PO) activity.

Measurement of immunity parameters

Total hemocyte count. The number of hemocytes was counted by taking 0.1 mL of hemolymph from the base of the swimming legs, using a 1 mL syringe containing 0.3 mL of Na-citrate anticoagulant, then homogenizing, by moving the hands to form a figure of eight for 5 min. One drop of the solution was placed on the hemocytometer and the number of cells per mL was counted (McCarthy et al 1973).

Total hemocyte count = average number of cells counted x (1/volume of grid; 0.1 mm^3) x dilution factor x 1000

Differential hemocyte count. It was calculated by dripping the hemolymph into a glass object, then drying it and fixing it with methanol for 5 minutes. Then air-dried and stained with Giemsa solution for 10 min, washed with running water, and allowed to dry then examined under a microscope with a magnification of 100 times (Martin & Graves 1985):

Percentage of hemocyte cell types = (number of each type of hemocytes / total hemocytes) x 100

Phagocytic index. A volume of 0.1 mL of shrimp hemolymph was put into the microplate and mixed evenly with 25 μL of *Staphylococcus aureus* and incubated for 20 min. Then 5 μL was dripped onto a glass object and a smear preparation was made. Then fixed with 100% methanol for 5 min and stained with Giemsa (10%) for 15 min. Phagocytosis activity was measured based on the percentage of phagocytic cells that showed phagocytosis (Anderson & Siwicki 1995).

Phagocytic index = number of phagocytic cells that carry out phagocytosis / number of phagocytic cells) x 100

Phenoloxidase activity. Measurement of PO activity was carried out using a spectrophotometer to record the formation of dopachrome produced from L-dihydroxyphenylalanine (L-DOPA) (Hernandez-Lopez et al 1996), which was carried out by centrifuging diluted hemolymph at 700 x *g* at 4°C for 20 min. The supernatant liquid was discarded and the pellet was rinsed, then slowly resuspended in cacodylate-citrate buffer (0.01 M sodium cacodylate, 0.45 M sodium chloride, 0.10 M trisodium citrate, pH 7.0) and centrifuged. Next, the pellet was resuspended with 200 µL cacodylate buffer (0.01 M sodium cacodylate, 0.45 M sodium chloride, 0.01 M calcium chloride, 0.26 M magnesium chloride, pH 7.0) and 100 µL aliquots. Incubated with 50 µL trypsin (1 mg mL^{-1}), as an activator for 10 minutes at 25-26°C; then added 50 µL L-DOPA, and 5 min later added 800 µL cacodylate buffer. Optical density at 490 nm was measured using a Hitachi U-2000 spectrophotometer. The optical density of shrimp PO activity for all test conditions was expressed as the formation of dopachrome in 50 µL of hemolymph.

Results

Antibacterial activity of sodium alginate against V. harveyi*. In vitro* antibacterial activity test of sodium alginate was conducted at several doses; 0; 1; 2.5; 5; 7.5; 10; and PBS 15 mg mL^{-1}. The results showed 3 (three) selected doses; 5; 7.5 and 10 mg mL^{-1} which indicated the ability to inhibit the growth of *V. harveyi*. To strengthen the results of the test, the cup count method was used to determine the antibacterial activity of those three (3) selected doses. The diameter of the inhibition zone formed around the disc paper at doses 5; 7.5 and 10 mg mL^{-1}, as well as the number of bacterial colonies growing on agar media containing sodium alginate, are presented in Table 1.

Table 1

Inhibition zone of sodium alginate against *V. harveyi* and the number of bacterial colonies growing on agar media

No.	*Doses of sodium alginate (mg mL^{-1})*	*Diameter of inhibition zones (mm)*	*V. harveyi colonies (CFU mL^{-1})*
1	5	7.5	3.3×10^5
2	7.5	7.3	4.2×10^5
3	10	7.6	2.9×10^5

Evaluation of the effect of sodium alginate*.* The immune-stimulating effect of sodium alginate was evaluated based on the non-specific immune responses of vannamei shrimp, including THC, DHC, PI, and PO activities. The results of the evaluation are described below:

Total hemocyte count. The total hemocyte count of vannamei shrimp on day 0 and day 1 post-injection of sodium alginate, experienced a slight increase but did not show a significant difference between the dose treatment and the control (Figure 1).

The highest increase in total hemocytes was found on day 3. All sodium alginate treatment doses were significantly different ($p < 0.05$) from both controls. The highest total hemocytes value was obtained at the dose of 10 µg g^{-1}. On day 5, there was a slight decrease but the doses of 10 and 15 µg g^{-1} were still significantly different from the both controls and the dose of 20 µg g^{-1}. The results of the analysis of variance showed that the highest increase in total hemocytes was on day 3, where the dose of 10 µg g^{-1} was 110.3±3.01 (x 10^5 cells mL^{-1}), the dose of 15 µg g^{-1} was 99.2±5.11 (x 10^5 cells mL^{-1}) and the dose of 20 µg g^{-1} was 92.7±3.82 (x 10^5 cells mL^{-1}) significantly different from the both controls. Furthermore, 10 µg g^{-1} was significantly different from 15 and 20 µg g^{-1}. Total hemocytes decreased on days 5 and 6, but the doses of 10 µg g^{-1} and 20 µg g^{-1} were still significantly different from the controls.

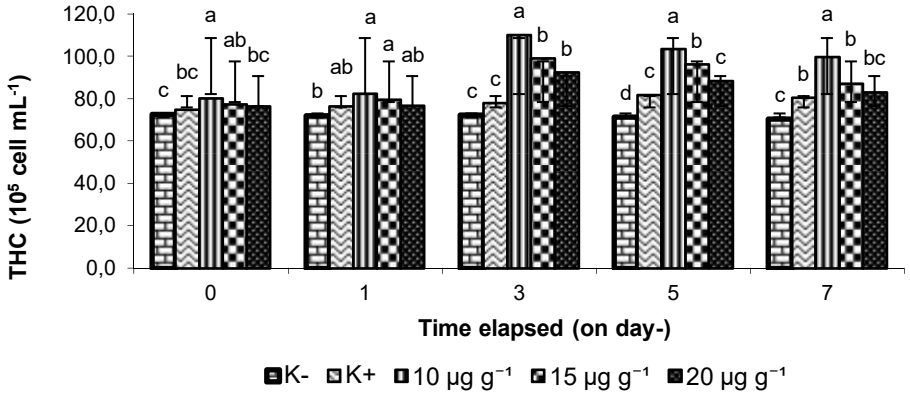

Figure 1. Vanname shrimp total hemocytes in each treatment (data (mean±SD) on the same day of observation with different letters shows a significant difference ($p < 0.05$)).

Differential hemocyte count

Hyaline. The percentage of hyaline cells on day 0 and day 1 did not show a significant difference between the alginate and control doses (Figure 2). The percentage of hyaline cells increased on day 3 and the 10 µg g^{-1} treatment was significantly different from all treatments and controls.

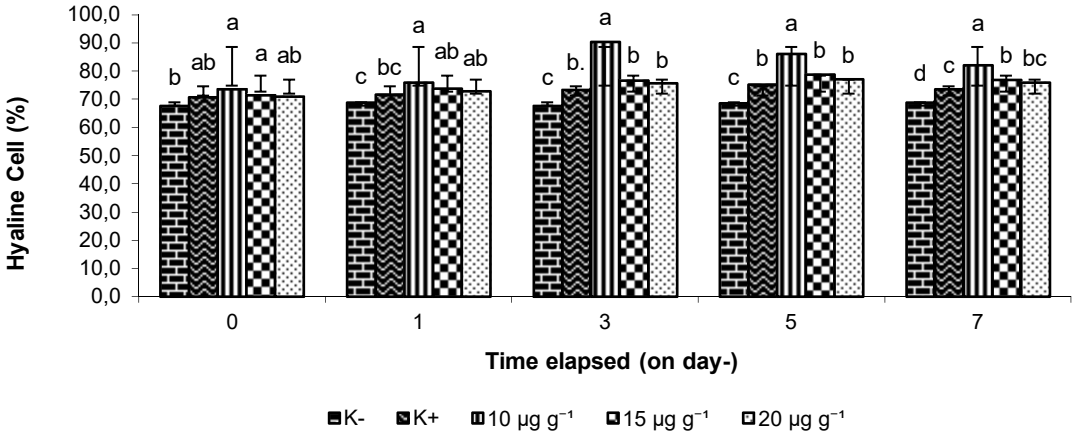

Figure 2. Hyaline cells of vanname shrimp in each treatment (data (mean±SD) on the same day of observation with different letters shows a significant difference ($p < 0.05$)).

The increase in the percentage of hyaline cells indicates that sodium alginate can increase the vannamei shrimp's immune response because hyaline cells carry out more of the function of phagocytosis. The percentage of hyaline cells began to decrease on days 5 and 7. The treatment dose of 10 µg g^{-1} was found to be significantly different from all treatments and controls. The 10 µg g^{-1} sodium alginate dose treatment showed the highest percentage of hyaline cells on day 3 of 90.4±3.37%, significantly different from the negative control which was only 67.7±2.52%, the 15 µg g^{-1} treatment 76.6±2.57% and 20 µg g^{-1} treatment of 75.6±2.80%.

Semi-granular. The percentage of semi-granular cells on day 0 showed no significant difference between the treatments and the controls (Figure 3). The percentage of semi-granular cells began to increase on day 1 where the 10 µg g^{-1} treatment was significantly different ($p < 0.05$) from the 20 µg g^{-1} treatment.

The highest increase in the percentage of semi-granular cells was seen on day 3 where the 10 µg g^{-1} treatment was 30.3±2.56% significantly different from the 15 µg g^{-1} treatment at 26.0±1.50% and the 20 µg g^{-1} treatment was 25.2±0.80% and the positive

control. The decrease in the percentage of semi-granular cells occurred starting on day 5 and continued to decrease on day 7 but the 10 µg g^{-1} treatment was generally significantly different from all treatments and controls.

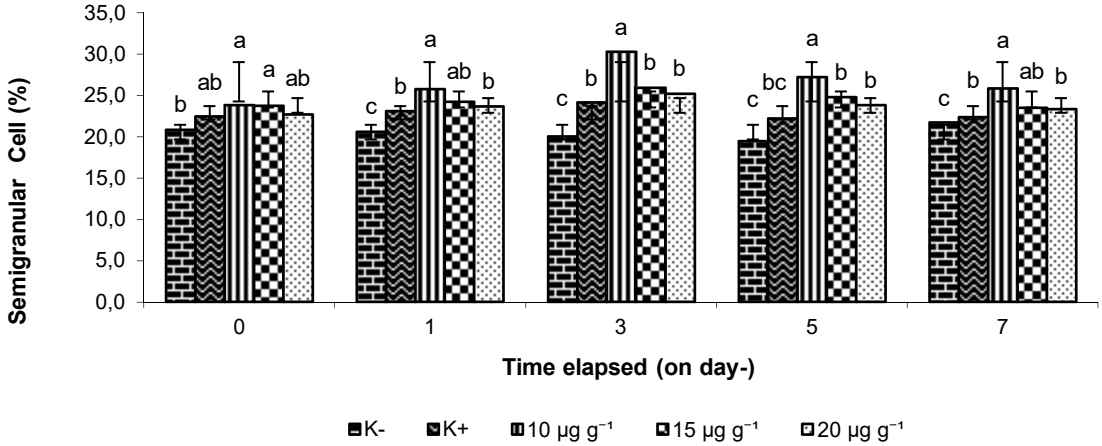

Figure 3. Vannamei shrimp semi-granular cells in each treatment (data (mean±SD) on the same day of observation with different letters shows a significant difference ($p < 0.05$)).

Granular. The percentage of granular cells on day 0 and day 1 showed no significant difference between all treatment and control doses (Figure 4). The percentage of granular cells experienced the highest increase on day 3, where the treatment with a dose of 10 µg g^{-1} was 31.8±2.11% significantly different ($p < 0.05$) with a dose of 15 µg g^{-1} which was 27.3±0.75% and 20 µg g^{-1} dose of 25.6±0.79% and both controls. Furthermore, the percentage of granular cells decreased on day 5 and day 7. On day 5 only the 10 µg g^{-1} treatment was significantly different from the 20 µg g^{-1} treatment and both controls, whereas on day 7 all dose treatments did not show a significant difference between controls and treatments.

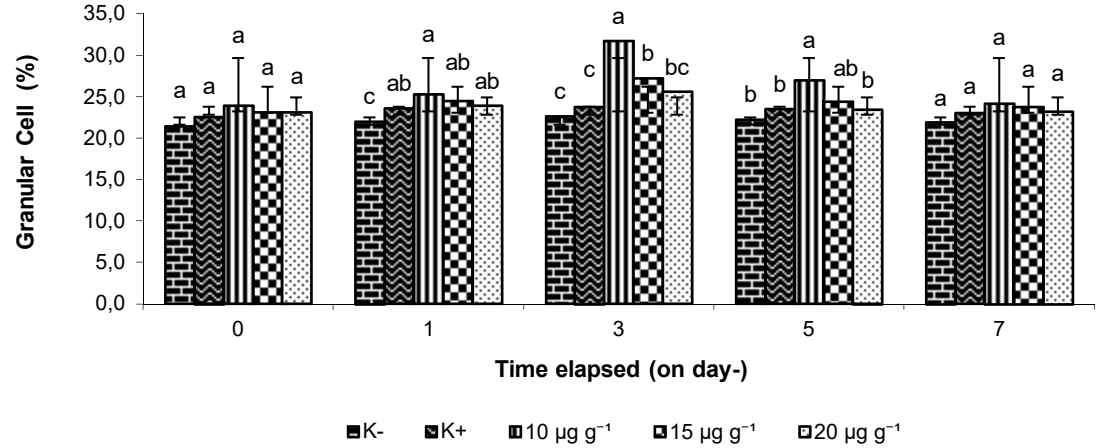

Figure 4. Vanname shrimp granular cells in each treatment (data (mean±SD) on the same day of observation with different letters show a significant difference ($p < 0.05$).

Phagocytic index. The percentage of the PI on day 0 and day 1 showed no significant difference between all treatments and controls (Figure 5). An increase in the percentage of the PI was seen on day 3, where the treatment at a dose of 10 µg g^{-1} (37±1.41%) was significantly different ($p < 0.05$) from the treatment at a dose of 15 µg g^{-1} (32.5±1.50%) and a dose of 20 µg g^{-1} (31.5±1.36%) and both controls. The percentage of PI began to decrease on the 5th and 7th day. The percentage of the PI on day 5 showed that the 10 µg g^{-1} treatment was still significantly different from the 15 and 20 µg g^{-1} treatments and

the controls. Whereas on day 7 there was generally no significant difference between the treatment and the control.

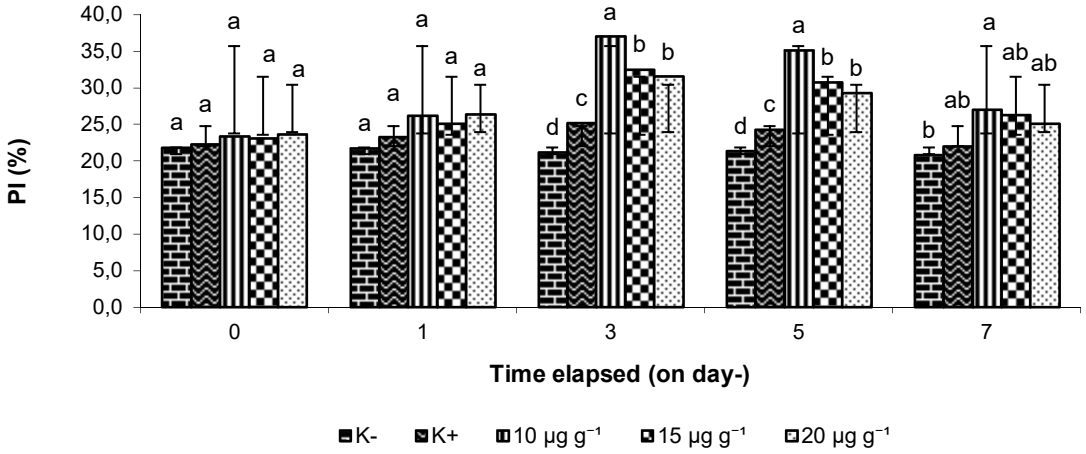

Figure 5. Phagocytic index of vanname shrimp hemocytes in each treatment (data (mean±SD) on the same day of observation with different letters shows a significant difference ($p < 0.05$).

Phenoloxidase (PO) activity. PO *activity* on day 0 showed no significant difference between all treatments. The increase in PO activity began to be seen on day 1 and continued to increase on day 3, where the 10 µg g^{-1} (0.31±0.06) treatment was significantly different ($p < 0.05$) with a dose of 15 µg g^{-1} (0.22±0.03) and a dose of 20 µg g^{-1} (0.21±0.04) as well as the control. Furthermore, all treatments decreased from day 5 to 7.

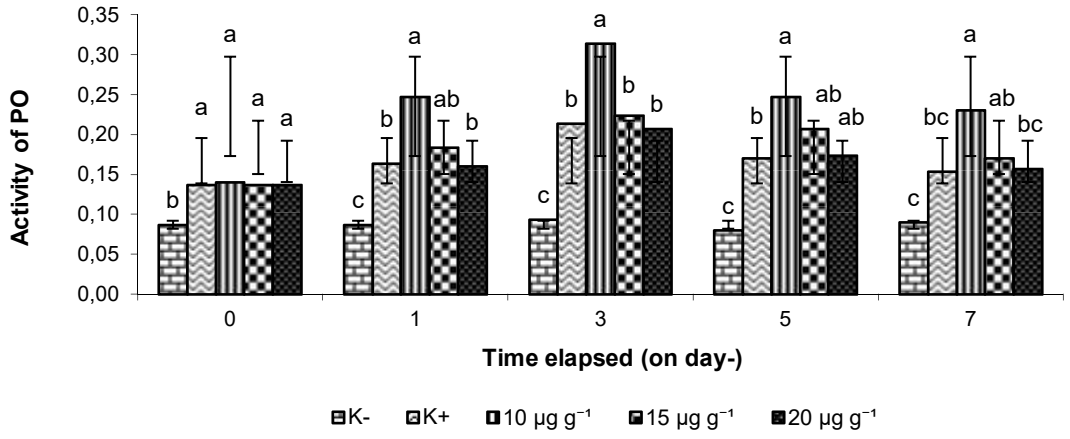

Figure 6. Vanname shrimp PO activity in each treatment (data (mean±SD) on the same day of observation with different letters shows a significant difference ($p < 0.05$).

Discussion. The ability of sodium alginate to inhibit the growth of *V. harveyi* was shown by the clear zone formed around the disc paper containing sodium alginate. The formation of an inhibition zone and the presence of antibacterial activity proves that sodium alginate has antibacterial properties. According to Bubonja-Šonje et al (2020), the formation of a bacteria-free inhibition zone by observing the clear area around the disc paper is evidence of antimicrobial activity. The sensitivity of *V. harveyi* to sodium alginate is indicated by the extent of the clear area around the growth of *V. harveyi*. Based on the diameter of the inhibition zone in Table 1, the treatment dose of sodium alginate is 5, 7.5, and 10 mg mL^{-1} indicating the inhibition zone with a value of 7.5, 7.3,

and 7.6 mm respectively. Inhibition of sodium alginate on the growth of *V. harveyi* after 24 hours of incubation with the dose of 5, 7.5, and 10 mg mL^{-1} indicated by the number of bacterial colonies growing on agar media with a density of 3.3 x 10^5; 4.2 x 10^5 and 2.9 x 10^5 CFU mL^{-1} respectively.

Three doses of sodium alginate showed the presence of an inhibition zone. This indicates that sodium alginate produces antibacterial compounds, although the diameter of the inhibition zone produced from the three doses is included in the medium category, where the highest inhibition zone value is at a dose of 10 mg mL^{-1} with a diameter of 7.6 mm. This also indicates that even though it is extracted in a dry state, sodium alginate can inhibit bacterial growth. The results obtained support the findings of Rohmah (2011), that the average diameter of the clear zone of *Sargassum* extract for *Vibrio parahaemolyticus* was 7.73 mm. However, it is different from the statement of Thanigaivel et al (2015) that the dried *Sargassum* and *Padina* extracts did not show any antibacterial activity. It was further explained that fresh *Sargassum* extract was very effective in suppressing the growth of *Vibrio* sp. These results were also reinforced by Siregar et al (2012) that the fresh extract of *Sargassum* sp. is the most active extract against *Pseudomonas aeruginosa* and *Micrococcus luteus*.

The effect of sodium alginate as an immunostimulator on the parameters of the vaname shrimp's non-specific immune response can be seen from its immune parameters. Parameters of non-specific immunity are one of the important criteria for diagnosis in determining the health of fish and shrimp (Bagheri et al 2023). Observation of vannamei shrimp total hemocytes on day 1 post-injection of sodium alginate increased until day 3, then decreased from day 5 to day 7. The increase in the THC of vannamei shrimp indicates that sodium alginate can increase the shrimp's immune response. Unlike the research of Fu et al (2007) who used *Gelidium amansii* extract, where THC decreased on days 2 and 4 of exposure.

Hemocytes in shrimp have an important role as red and white blood cells in fish. The THC in crustaceans plays a role in pathogen resistance, if there is a decrease in THC it will open up opportunities for acute infection which can cause death (Cui et al 2020). The increase in the number of shrimp hemocytes indicated that the injected sodium alginate was able to increase the immune response. Sodium alginate can stimulate the formation of hemocyte cells which will be released into the shrimp hemolymph. The increase in THC has implications for increasing the ability of the phagocytosis process because the hemocyte cells (hyaline cells and semi-granular cells) produced perform this function more. This is in line with the opinion of Sahoo et al (2008) that hemocytes play a role in phagocytosis, encapsulation, degranulation, and nodular aggregation of pathogens or foreign particles, as well as the production and release of proPO.

Differential hemocytes in shrimp consist of hyaline, semi-granular and granular cells. Differences in the types of hemocytes based on morphological criteria and their respective functions in the host (shrimp) defense reactions. Changes in the percentage of DHC, hyaline cells, semi-granular or granular in studies using algae extracts were usually not significantly different from controls or physiological saline (PS) (Chen et al 2015). Changes in the percentage of DHC in this study generally had variations in the differences in the percentage of each hemocyte cell on days 3, 5, and 7.

Increased hyaline is usually associated with increased resistance to pathogens (Rosales & Uribe-Quero 2017). Hyaline cells observed in shrimp are characterized by the absence of granules (agranular). Hyaline cells perform a function in immunity as phagocytosis (Rosales & Uribe-Quero 2017). According to Muthaiyan et al (2020), based on morphological and cytochemical properties, several functions and involvement in different defense reactions are associated with different cell types, for example, hyaline cells with coagulation (Bouallegui 2021), granular and semi-granular cells with phagocytosis and proPO system (Liu et al 2020).

The highest increase in semi-granular cells was found at a dose of 10 μg g^{-1}. Giving alginate at a dose of 10 μg g^{-1} can recognize and respond to foreign bodies or bacteria. This is in line with the statement of Li et al (2018), that semi-granular cells have several small granules. These cells are responsible for recognizing and responding

to foreign molecules or pathogenic bacteria that enter the crustacean's body. This increase is also associated with increased resistance to pathogens (Chaplin 2010).

The increase in the percentage of granular cells indicates that alginate can react in increasing the defense of the shrimp body. This is inseparable from the function of other hemocyte cells. Granular cells are characterized by the presence of a large number of granules. These cells function in storing and releasing the proPO system as well as cytotoxicity together with semi-granular cells (Johansson 2000). An increase in the percentage of granular cells is usually associated with an increase in PO activity and resistance to pathogens (Chen et al 2015). Granular cells are hemocyte cells that have the biggest role in increasing the defense of the shrimp body. The increase in the PI indicates that alginate can increase the phagocytic activity of phagocytic cells. Giving alginate can increase resistance to infectious diseases, not because of increased specific immune responses but because of increased non-specific defense mechanisms (Lee & Mooney 2012). Thus, alginate can stimulate the shrimp's body defense system. If bacteria or foreign substances enter the body of the shrimp, there will be a response in the semi-granular and granular cells by releasing an activated proPO system, which includes cell-adhesive and opsonic protein, peroxinectin. Moreover, it can stimulate phagocytosis by hyaline cells or encapsulation by semi-granular cells (Widanarni et al 2020). According to Lv et al (2014) and Liu et al (2020), granulocytes are the main phagocytic cells in shrimp, lobster, and crabs.

The enzyme phenoloxidase (PO) plays an important role in the process of melanization in crustaceans in response to foreign bodies (Liang et al 2023). PO is present in the hemolymph in the form of an inactive proenzyme called proPO (Widanarni et al 2020). In crustaceans, proPO functions in the foreign body recognition system and melanization (Amparyup et al 2013). Immunostimulants function to activate the proPO system to produce enzymes that play a role in the melanization process. The increase in PO activity indicates that sodium alginate can stimulate shrimp hemocytes so it will increase the process of phagocytosis against bacteria. Increased phagocytosis will increase the resistance of shrimp against pathogenic bacteria. If the bacteria enter the body of the shrimp, they will be immediately recognized by the plasma and bound to them. There is then a response in the semi-granular and granular cells by releasing an activated proPO system, which includes the cell-adhesive and opsonic protein, peroxinectin. Moreover, it can stimulate phagocytosis by hyaline cells or encapsulation by semi-granular cells (Liu et al 2020).

Conclusions. Sodium alginate has been shown to have antibacterial activity with different inhibition zones according to the dose used. The number of *V. harveyi* colonies formed is also different according to the dose given, this is indicated by the formation of a free zone of bacterial inhibition around the disc paper containing sodium alginate, which indicates that sodium alginate has the potential to inhibit and suppress the growth of *V. harveyi* bacteria. This study recommends that the administration of sodium alginate as an immunostimulant can increase the non-specific immune response in shrimp as measured by total hemocytes, hemocyte differential, phagocytosis index, and phenoloxidase activity. *In vivo*, studies focusing on the conversion ratio, protein efficiency ratio, and feed water stability need to be conducted to confirm the use of moringa leaf meal extract in vannamei shrimp disease management.

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Conflict of interest. The authors declare that there is no conflict of interest.

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