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Immunomodulatory Effect of *Paederia* (*Paederia foetida* Linn.) based on Phagocytic Activity of *Litopenaeus vannameii*

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Abstract. Vannamei shrimp farming usually encounters bacterial invasion. The bacteria's attacks make the vannamei shrimps unable to be treated. Therefore, farmers must prevent the attacks by increasing their immunity. In this research, the researchers observed the immune response-ability of the shrimp from the phagocytic activity of *Litopenaeus vannameii* as the indicator. The researchers prepared paederia flour, a mixture of commercial shrimp feed, and various formulations, starting from 0%; 10%; 20%, 40%, and 80%. The researchers determined the phagocytic activity from the mixture of *Vibrio alginoliticus* bacteria cells in shrimp blood. The researchers observed the phagocytosis of vannamei shrimp on 0, 10, 20, and 30 days. The results showed did not significant effects of the applied treatment to phagocytosis activity. The sig-value is 0.067, with significant confidence: 5. The highest phagocytic activity on the treatment of paederia addition was 40%, with the number of macrophage cells found in phagocyte bacterial cell *Vibrio alginoliticus* 41.29%.

1 Introduction

The aquaculture Industry perceives vannamei shrimp cultivation as an important matter in Indonesia. Shrimp cultivation brings significant profits for farmers. However, this cultivation is not free from viral and bacterial attacks. These attacks may cause the vannamei shrimps to die within three phases [1]. Farmers anticipate the viral and bacterial attacks by increasing the immunity system of the shrimp. One of the applied efforts is – using immunomodulators to improve immune responses [2]. Immunomodulatory compounds are useful for the dynamic activity of immune cells while being exposed to a foreign substance, both specific and non-specific substances.

The immune response of mammals is observable from the macrophage cells. These cells belong to phagocytic activity and bring an immune response to initiate the clearing mechanism process. The cells also defend the body from harmful pathogens. Phagocytosis is a major activity of the immune system mechanism and activates the innate immune response. Phagocytosis also initiates the adaptive immune response [3].

The research determined the phagocytic activity of *Litopenaeus vannamei* with paederia treatment while feeding. In this research, paederia became the immunomodulatory agent for vannamei shrimps' immune cells in this research. Then, the researchers measured the phagocytic activity. Then, the researchers verified the potency of paederia leaf flour as an immunomodulatory agent. The researchers chose paederia because paederia was a natural plant for immunomodulatory agents. Paederia is also safe for vannamei cultivation.

Traditional medicines from plants become interesting for creating immunomodulators of complex immune systems. Some studies investigated the use of natural ingredients, such as *M. oliefera*. One of them is [4]. The researchers found the effects toward the growth, immunity, and antistress response. Another study found bioactive compounds of plants with a traditional medic method of India, such as alkaloids, terpene, flavonoids, and other compounds for immunomodulators against immune cells [5].

Native citizens of Sumenep regency Madura, believe that paederia, *Paederia foetida* Linn, is excellent to treat stomachache, cold flatulence, and diarrhea. The native citizens also believe paederia could increase appetite. The phytochemicals of paederia contain iridoid, paederone, paederolone, paederine, and paederonine. Paederia is popular in Bangladesh to treat dysentery and diarrhea [6,7]. The evaluation of paederia methanol has a cytotoxic activity based on the brine shrimp lethality test (BSLT) [8].

2 Material and Methods

Preparation of sample treatment. The researchers did the phagocytic activity research in a microbiology laboratory at the Faculty of Agriculture, University of Wiraraja. The researchers used a microscope, centrifuge, microtubes, micro pipet, Petri, and UV sterilizer. The researchers made the paederia flour from dried and smoothed paederia leaves. Then, the researchers used the paederia flour, mixed with vaname feeding, to feed vannameii shrimp. The researchers took 8 grams of vannamei shrimp and cultivated the shrimp in an aquarium. In this research, the researchers isolated and purified *Vibrio alginoliticus* from the seawater with the aseptic procedure. The applied aseptic procedure was TCBS (Thiosulfate Citrate Bile Sucrose). Then, the researchers incubated the bacteria at room temperature for 24 hours.

Treatment. The researchers took the blood of vannamei shrimp to determine the phagocytosis activity. This treatment referred to a control treatment. The researchers took the fresh paederia from plantation areas in Andulang Village, Gapura District, Sumenep Regency. The applied drying method in this research for paederia leaves was wind-drying. In this case, the researchers used the wind to dry the leaf for 4-6 days and lessen the water content between 9% - 10% [9]. Then, the researchers milled the dried leaf with a disk mill machine. This research dealt with paederia flour added to commercial shrimp feed. The treatment varieties were 0%, 10%, 20%, 40% and 80% of paederia flours. Then, the researchers used 75 cultivated shrimps from an aquarium that had circulation and aeration. Then, the researchers put the shrimps in some aquariums that contained 15 shrimps for each aquarium. The researchers kept the shrimps in these aquariums for 30 days and fed them with a percentage of 3% of the shrimps' weights per day. The researchers observed the effect of paederia flour on phagocytic activity on the tenth, twentieth, and thirteenth days. The researchers used *V. alginoliticus* cells mixed with formalin. Then, the researchers let the precipitate suspension settle for 24 hours. Then, the researchers centrifuged the solution at 100 rpm for five minutes to separate the filtrate. The results were 250 μ L of hemocyte and

500 μ L of suspension. This process killed the bacteria at incubated room temperature for 50 minutes.

Data Analysis. The researchers used the phagocytic ratio and phagocytosis index. The phagocytic ratio refers to the percentage of active macrophage cells from 100 macrophages. The researchers determined the phagocytosis index with the percentage of phagocytosed beads per phagocytosing cells [10,11]. The researchers observed the data and analyzed the data with ANOVA (p: 0,05) to determine the effect of treatment differences. The ANOVA analysis showed the effect differences. Then, the researchers continued with the Duncon test to determine the effect of the treatment concentration of paderia on phagocytic activity based on the phagocytosis ratio of vannamei shrimp blood cells.

3 Result and Discussion

Phagocytosis Activity. The mechanism of phagocytic activity must be on a clear platelet, on essential thrombocythemia based on in-vitro treatment [12]. Phagocytosis activity refers to the percentage of macrophage cells consumed latex by the active macrophage. The phagocytosis mechanism is dynamic, and its phagocyte latex ability forms toxins. Thus, immunostimulants can stimulate bacterial infection [13,14]. In this research, the researchers found the fish improved the non-specific immune responses against *V alginoliticus* after administering herbal supplementation with *Sauropus androgynous* [15]. Figure 1 shows the macrophage activity in activated macrophages with 400x zoom under a microscope.



Figure 1. (a) Active and (b) inactive Macrophages

The percentage of macrophage cells with various paederia flour concentrations added to vannamei shrimp feeds. Figure 2 shows the phagocytic ratio and the percentages of macrophage cells with various concentrations paederia flours added to vannamei shrimp feed as an immunostimulant agent.

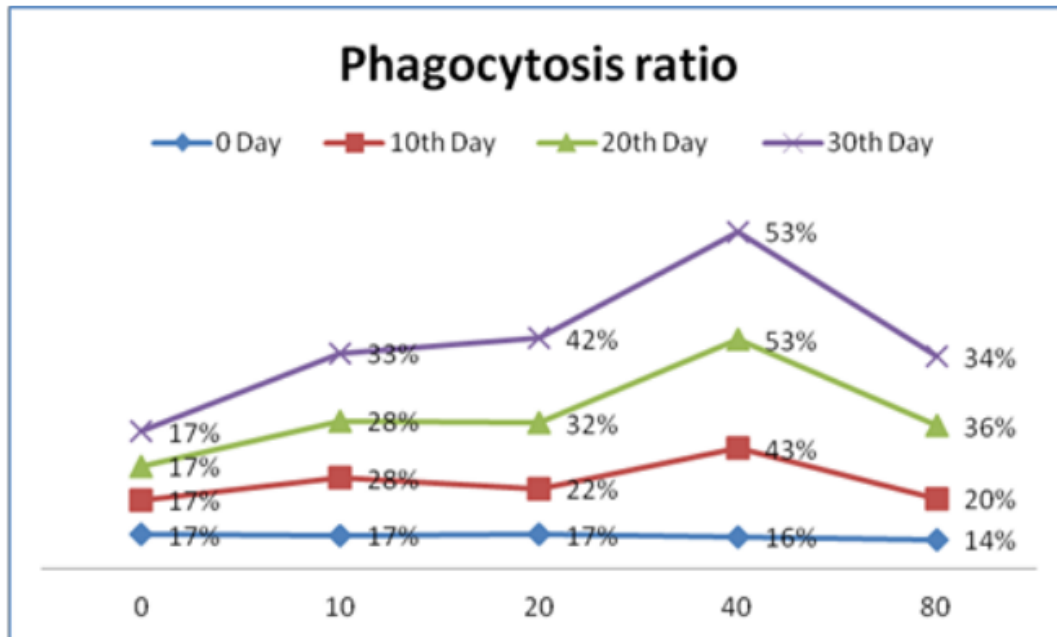


Figure 2. Percentage of macrophage cells

The Immunostimulant effect and antioxidant effect of plants can produce macrophages. These macrophages kill microbes [16]. On the other hand, the researchers found that aromatic plants with vitro and in-vivo experiments had antioxidant, antimicrobial, and anesthetic abilities [17]. Paederia is an aromatic plant with complex chemical compounds. The plant is also useful in traditional medicine [18]. In this research, the phagocytic ratio in a 0% paederia flour treatment showed the same phagocytic, 17%, on 0-30 days. Then, the ratio decreased on the 30th day, 16%. This result indicates that paederia flour influences the macrophage cellular consumption of latex. The researchers found 100 macrophage cells that defended vannamei shrimp immune system in this research.

The one-way ANOVA analysis showed variance homogeneity. The test obtained a significant value of 0.067, lower than 0.05. Thus, the data were valid for further ANOVA tests. The test result found that the concentration of paederia did not have a significant influence, although the results showed phagocytic ratio differences. The treatment with 40% paederia also provided the same phagocytosis effect ratio on the 20th and 30th days, 53%. In this research, the administration with 30-40% paederia for feeding purposes had an increased effect in terms of non-specific immune responses of the shrimps. This finding was also in line with [15]. The researchers used *Sauropus androgynous L.Merr* in the grouper of *Epinephelus bcoioides* with a concentration of 102.5g/kg found different effects on the growth and non-specific immune response groupers. Then, the concentration of *Sauropus androgynous L. Merr* leaf with 1g/kg weight led to mortality due to contact with *Vibrio alginoliticus*. [19] used herbal plants, *Phyllanthus niruru L*, and garlic. They found the different effects based on the given amount for broilers.

4 Conclusion

Paederia provides immunomodulatory effects on vannameii shrimp based on phagocytic, although the effect is insignificant. Paederia 30-40% for feeding the shrimp has the highest phagocytic activity, 53%.

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