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# Utilization of Jatropha Leaf (*Jatropa curcas*) Extract as Immune System Enhancer of Vannamei Shrimp (*Litopenaeus vannamei*) Infected with *Vibrio parahaemolyticus*

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**Abstract**

The problem that arises in vannamei shrimp cultivation is the emergence of diseases, such as vibriosis caused by *Vibrio parahaemolyticus*. Jatropha leaf extract can be used as an immunostimulant because it contains bioactive compounds namely flavonoids, tannins, saponins, and alkaloids. This research used a completely randomized design (CRD) with 5 treatments with 3 replications. The treatments used were; P1 (feed without extract and bacterial infection); P2 (feed without extract and NaCl injection); P3 : Feed + 1% + bacterial infection; P4 : Feed + 2% extract from feed and bacterial infection, P5 : Feed + 3% extract of feed and bacterial infection. Based on the results of the study, the administration of Jatropha leaf extract was able to increase the highest SR value of P2 by 88.3%, the highest THC value of P5 at 24.46 x $10^6$ cells/ml, DHC values such as hyaline cells ranged from 36.67-71%, granular cells ranged from 17-38.57%, and semi granular cells ranged from 12-24.67%, the highest AF value in P5 was 77.25%, and the lowest TBC value was at P2 of 6 x $10^8$ CFU/ml, the lowest TVC value was at P2 of 0.56 x $10^6$ CFU/ml. The higher the dose of extract given, the higher the THC and AF, besides that, Jatropha leaves were also able to increase the SR value and suppress the growth of total bacteria and total *Vibrio* in shrimp intestines.

## INTRODUCTION

Vannamei shrimp is a leading commodity for fishery exports today. Indonesia produces vannamei shrimp of 1,290,000 tons and exports 208,000 tons of shrimp. As a result, shrimp contributed to the export volume of fishery products by 18,95% of the total volume of fishery exports reached 1,260,000 tons. Indonesia is currently targeting a national production of vannamei shrimp of 2,000,000 tons or an increase in national shrimp exports by 250% until 2024. With the increasing demand for vannamei shrimp, vannamei shrimp cultivation is increasing.

The problem that is often faced by shrimp cultivation is the emergence of disease. Vibriosis is a disease that often attacks vannamei shrimp. This disease is capable of causing shrimp mortality up to 100% in a short time, for 1-2 after infection (Briggs *et al*., 2004). Vibriosis disease is caused by the bacteria *Vibrio* sp. one of which is the bacterium *V. parahaemolyticus* which can lyse the blood of shrimp blood cells, and causes the

shrimp to change color in the body to become red and even cause mass death (Jannah *et al*., 2018).

*Vibrio* sp. is a normal flora that is opportunistic which means it can attack shrimp when environmental conditions are not good. With unfavorable environmental conditions, it can reduce the shrimp immune system in vannamei shrimp. The vannamei shrimp immune system is a non-specific immune system that only controls hemocytes to attack foreign objects that enter the body (Kurniawan *et al.*, 2018).

However, to improve the vannamei shrimp's immune system is to use immunostimulants. Junaidi *et al*. (2020) The non-specific immune system is very susceptible to bacterial diseases, this immune system can be more active if given immunostimulants. Immunostimulants that can be used are plant extracts, animal extracts, yeast extracts, bacteria and complex carbohydrates. Plant extracts that can be used are Jatropha leaf extract, Jatropha leaves contain flavonoid compounds, tannins, saponins, and alkaloids. This content is believed to have the ability to boost the immune system (Nwokocha *et al*., 2011). Jatropha leaf extract contains tannins in the leaves at 7.43%, alkaloids at 4.54%, and flavonoids at 2.76%, meanwhile, the concentration of saponins in the leaves is 4.89% and phenols are 0.59% which can inhibit antimicrobial.

The content of saponins can stimulate immune cells so that they can act as immunostimulators, flavonoids can increase phagocytic activity. In addition, the availability of Jatropha plants is quite lot in Indonesia, especially West Nusa Tenggara so it is very easy to obtain. Based on data from the Department of Agriculture and Plantation of the Province of NTB, the area of planting Jatropha leaves in 2020 reached 1,382.85 Ha. Therefore, it is necessary research the use of jatropha leaf extract as an immune system enhancer of vannamei shrimp infected with *V. parahaemolyticus*.

## METHODOLOGY

### Place and Time

This research was conducted for 60 days from June to August 2021 at the Laboratory of Aquaculture Study Program, Faculty of Agriculture, University of Mataram.

### Research Materials

The tools used in this study were a container with size 28 x 30 x 40 cm, autoclave, Bunsen, digital scale, millimeter block, microscope, hemocytometer, syringe, driglaski spatula, hot plate, and serology pipette. The materials used in this study included the bacteria *V. parahaemolyticus*, seawater, TCBS (thiosulfate citrate bile salts sucrose), TSA, NaCl, Alcohol, Jatropha leaves, ethanol, methanol, and Giemsa.

### Research Design

The research design used was an experimental method with five treatments and 3 replications. The study aimed to analyze the effect of jatropha leaf extract on improving the immune system of vannamei shrimp infected with *Vibrio parahaemolyticus* bacteria originating from BPBAP Jepara.

P1 : Feed without extract and bacterial infection (positive control)
P2 : Feed without extract and NaCl infection (negative control)
P3 : Feed + 1% extract of feed and bacterial infection
P4 : Feed + 2% extract of feed and bacterial infection
P5 : Feed + 3% extract of feed and bacterial infection

### Work Procedure

### Preparation of Jatropha Leaf Extract

Jatropha leaves (*J. curcas* L) used came from the plantations of Taman Sari Village, Mataram City. Jatropha leaves used are young leaves, then the young leaves are washed until clean by removing the leaf sap, then the leaves are drained. The leaves are then dried in the sun until they are completely dry for 3 days and

then dried again using an oven at 60 °C for 2 hours until the resulting dry leaves are easily broken. After that, the leaves are blended until they become powder and sifted to produce a finer powder. Then, the fine powder was soaked in 96% ethanol in a ratio of 1 : 3 (1 kg of Jatropha leaves : 3 liters of ethanol) for 3 days. After the immersion is complete, the next step is to filter the results of the immersion using filter paper to obtain an extract in the form of a solution that does not contain pulp. Then the solution is put into a rotary vacuum evaporator at a temperature of 50 °C to produce a concentrated extract (Luliana *et al*., 2016).

## Preparation of Containers and Cultivation

The research used 15 containers with a size of 45 cm x 20 cm x 25 cm. The containers used were cleaned with detergent and dried before use and dried for 24 hours. The dried containers were arranged according to the experimental design that had been prepared previously. Then it is filled with seawater and closed the container using a container lid to prevent the shrimp from jumping. The existence of cannibalism between individual shrimp can be minimized by the use of shelters in containers. Furthermore, the container is fitted with aeration to increase the oxygen supply to the shrimp.

## Preparation of Test Animals and Test Feed

The research used post-larva (PL) 20 vannamei shrimp biota obtained from PT. Bibit Unggul. The shrimp adapted for 7 days. Furthermore, shrimp are kept in containers with a stocking density of 20 individuals per container. During the research, the shrimp were fed pellets with 40% protein. The feed was added with Jatropha leaf extract using the treatment dose used. The feed that has been added is stirred evenly, then air-dried and stored at room temperature and not humid to avoid the growth of mold in the test feed.

## Preparation of Test Bacteria

The *V. parahaemolyticus* bacteria used were first cultured using TCBS media for 24 hours. Furthermore, the bacterial colonies were taken with the ose and serial dilutions were carried out using liquid SWC (sea water complete) media to $10^6$. The bacteria were ready to be used for the challenge test.

## Challenge Test

The challenge test was carried out after the treatment was given, namely on day 51 to know the effect of giving Jatropha leaf extract to vannamei shrimp on *V. harveyi* bacterial infection. The containers used were 15 units. The treated shrimp were fasted for one day, then infected with *V. harveyi*. The bacterial injection was carried out on the back of the shrimp, which was between the second and third segments as much as 100 L/head. The density of *V. parahaemolyticus* bacteria used was $10^6$ CFU/ml (Fuandila *et al*., 2020). The challenge test was carried out for 10 days, the immune response parameter data was taken and the number of shrimp that died was counted as shrimp survival data at the end of the challenge test (Azhar, 2018).

## Research Parameters

The research parameters used to determine the level of the shrimp immune system were survival rate, total hemocyte count, differential hemocyte count, phagocytic activity, total bacteria count, and total Vibrio count.

The survival rate was calculated using the formula:

$$SR = \frac{\text{final number of cultivation}}{\text{initial number of cultivation}} \times 100\%$$

Total hemocyte count was calculated using the formula from Ismawati *et al*. (2019):

$$THC = \frac{\Sigma \text{ cells observed}}{\Sigma \text{ box observed}} \times 25 \times \frac{1}{HV} \times df$$

Where:
THC = total hemocyte count
HV = hemocytometer volume
df = diluent factor

The differential hemocyte count is calculated by the formula based on Ekawati *et al*. (2012):

$$HCT = \frac{\Sigma \text{ of each hemocyte}}{\Sigma \text{ hemocytes}} \text{ x } 100\ \%$$

Where:
HCT = percentage of hemocyte cell types

Phagocytic activity (PA) can be calculated by the formula based on Fuandila *et al*. (2020):

$$PA = \frac{\Sigma \text{ cells carry out phagocytosis}}{\Sigma \text{ phagocytic cells}} \times 100\%$$

The number of bacteria that appear is calculated using a colony counter which is then recorded and multiplied by the amount of dilution that has been carried out. The number of bacteria is expressed in CFU/ml (colony-forming units/ml) and the number of bacteria is calculated based on Fuandila *et al*. (2020).

## Data Analysis

Research data were analyzed descriptively and statistically using SPSS (Version 16.0). ANOVA test was performed at the 95% confidence level ($P<0.05$). If the results were significantly different, further tests were carried out using Duncan.

## RESULTS AND DISCUSSION

### Survival Rate

The results of the survival rate were presented in Figure 1.

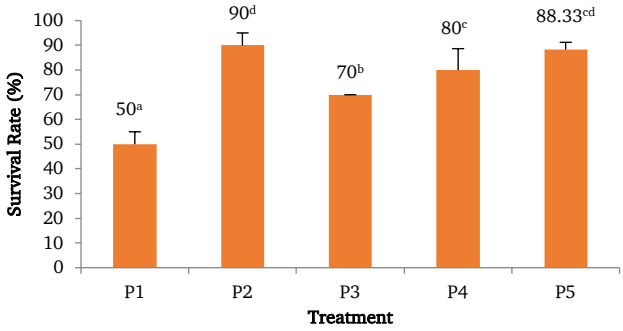


Figure 1. Survival rate graph. P1 (control +), P2 (control -), P3 (1%), P4 (2%), P3 (3%).

Based on the graph above, the highest survival rate is in P2 at 90% and the lowest is in P1 at 50%, while the highest survival rate for jatropha leaf extract is in P5 at 88.33%. Based on the results of statistical tests, jatropha leaf extract was significantly different from P1 (control +) ($P<0.05$), but P5 was not significantly different from P2 (control -) ($P>0.05$). Jatropha leaves were able to increase the survival of vannamei shrimp infected with vibriosis. The survival rate (SR) was categorized as good if the SR value was >70%, 50-60% moderate SR, and <50% low SR.

The value obtained from the results of this research is classified as good. The research indicates that the addition of jatropha leaf extract has been able to increase the resistance of vannamei shrimp to *V. parahaemolyticus* infection. The high mortality of shrimp in P1 was because it was a sick shrimp, where the shrimp was infected with *V. parahaemolyticus* and without jatropha leaf extract (Putri *et al*., 2015). The addition of a higher dose of extract containing compounds in the form of flavonoids, alkaloids, tannins, saponins, and terpenoids could increase survival. In addition, the high survival rate of white vannamei shrimp indicates that jatropha leaf extract as an immunostimulator can be accepted by white vannamei shrimp (Ni'mah *et al*., 2021). The immunostimulator that enters the body can protect or is protected against external factors such as pathogens that enter the shrimp body so that it causes the same effect as the treatment and control.

## Total Hemocyte Count

The results of total hemocyte count were presented in Figure 2.

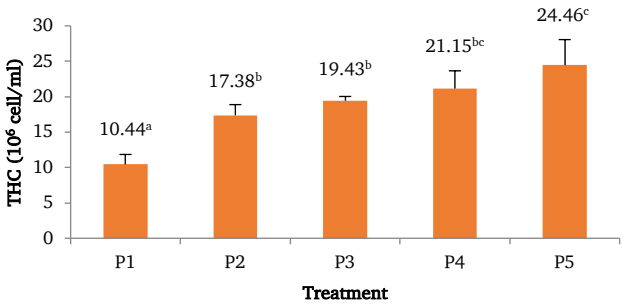


Figure 2. Total hemocyte count. P1 (control +), P2 (control -), P3 (1%), P4 (2%), P3 (3%).

Hemocytes have an important role in crustaceans including vannamei shrimp which functions to destroy foreign particles that enter the shrimp body, including the stages of recognition, phagocytosis, melanization, cytotoxicity and cell communication (Johansson *et al*., 2000). The ability of a material used to stimulate the non-specific immune system of shrimp can be seen in the increase in the number of hemocytes produced. Based on the graph above, the highest total hemocyte value was found at P5 at 24.46 x $10^6$ cells/ml and the lowest at P1 at 10.44 x $10^6$ cells/ml. The results of statistical tests showed that the treatment given Jatropha leaf extract was significantly different from the P1 treatment.

The high value of total hemocyte count obtained is thought to be influenced by the addition of Jatropha leaf extract which can stimulate shrimp blood. Rosmawaty *et al*. (2016) stated that the content of phytochemical compounds contained in the leaves such as tannins, flavonoids, and alkaloids and saponins work in activating cellular defense cells by increasing cells that play a role in hemocyte immune cells. If the dose given is excessive, it will be toxic to test animals (Mahyuddin *et al*., 2020). The content of alkaloids can increase the number of hemocytes.

## Differential Hemocyte Count

The results of differential hemocyte count (DHC) were presented in Figure 3.

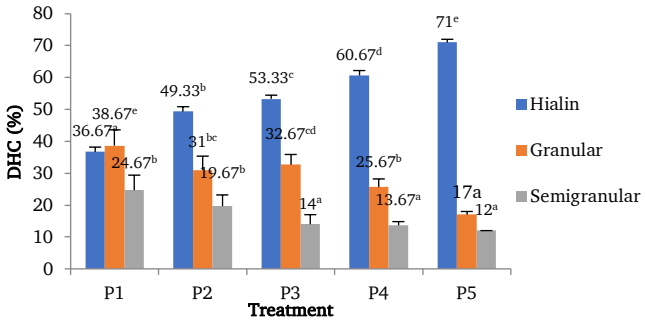


Figure 3. Differential hemocyte count. P1 (control +), P2 (control -), P3 (1%), P4 (2%), P3 (3%).

In the differential hemocyte count observation, There were three types of hemocyte cells in crustaceans (including shrimp) were observed. This type is divided based on the presence of cytoplasmic granules, namely hyaline, semi-granular, and granular. Based on the graph, hyaline values represent the

highest presentation ranging from 36.67-71%, followed by granular in the range of 17-38.57%, while the lowest is semi-granular ranging from 12-24.67%. The increase in total hemocyte count along with the addition of this dose was thought to be because the addition of jatropha leaf extract was able to increase the total hemocyte count value.

Jatropha leaves are immunostimulants that can prevent *Vibrio* spp. infection by increasing phagocytic activity. Kurniawan *et al*. (2018) stated that immunostimulants stimulate hemocytes to release proPO and protein-binding enzyme PPA. This causes hemocyte cells to increase their activity as body defense cells. The high hyaline cells obtained are closely related to the phagocytic activity. Where phagocytosis is the first line of defense to repel pathogens. These hyaline cells are activated by oponic factors resulting from the activation of ProPO to PO in granular cells so that they can phagocytose foreign material, both bacteria and viruses. Jatropha leaves are immunostimulants that can prevent Vibrio spp. infection by increasing phagocytic activity. This indicates that jatropha leaves can increase hyaline cells. Ermantianingrum *et al*. (2012) stated that each cell type has a different role in the body's defense, hyaline and granular cells are the main defense in the process of phagocytosis. Different types of hemocytes have different functions in the body's defense system. Hyaline cells and semi-granular cells have an important role in the shrimp defense system, especially in the phagocytosis process.

## Phagocytic Activity

The results of phagocytic activity were presented in Figure 4.

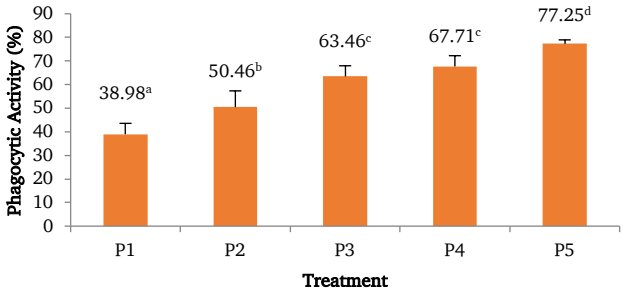


Figure 4. Phagocytic activity. P1 (control +), P2 (control -), P3 (1%), P4 (2%), P3 (3%).

Measurement of phagocytic activity was carried out to see the phagocytic process that took place during the observation by observing the level of phagocytosis in several treatments used. Based on the graph above, the highest phagocytic activity value is found in P5 at 77.25% and the lowest is in P1 at 38.98%. Statistical test results showed that the treatment given Jatropha leaf extract was different from P1 and P2. The high value of the phagocytic activity is thought to be given by jatropha leaf extract. Jatropha leaf extract contains flavonoid compounds that play a role in stimulating phagocytic activity carried out by hyaline cells. The ability of phagocytic activity can occur at the time of infection, the presence of this infection will stimulate the shrimp's immune system which is expected to be able to ward off disease attacks.

## Total Bacteria Count and Total Vibrio Count

The results of total bacteria count and total vibrio count were presented in Table 1.

Table 1. Total bacteria count and total vibrio count.

| Treatment | Total Bacteria Count (TBC) ($10^8$ CFU/ml) | Total Vibrio Count (TVC) ($10^6$ CFU/ml) |
|---|---|---|
| P1 | $84.33^d$ | $63.67^d$ |
| P2 | $6^a$ | $0.67^a$ |
| P3 | $51.67^c$ | $14.67^c$ |
| P4 | $29^b$ | $8.67^b$ |
| P5 | $14.67^a$ | $6^b$ |

Total bacteria and total vibrio are closely related to shrimp health. Based on the results of the research that has been carried out, the highest total bacteria found in shrimp intestines is in P1 of 84.33 x $10^8$ CFU/ml and the lowest is found in P2 of 6 x $10^8$ CFU/ml. While the total vibrio in the shrimp intestine was found the highest value was found in P1 of 63.67 x $10^6$ CFU/ml and the lowest was found in P2 of 0.67 x $10^6$ CFU/ml. The treatment given castor leaf extract was able to suppress the total growth of bacteria and vibrio given. Based on the results of statistical tests, the total values of P2 and P5 bacteria were not significantly different ($P>0.05$), while the total values of P2 were significantly different between all treatments.

Giving jatropha leaf extract through feed with increasing doses the total value of bacteria and total vibrio decreased. It is suspected that jatropha leaves contain phenolic compounds which have antibacterial activity. Based on the results of research conducted by Arifin *et al*. (2017) that jatropha leaf extract can inhibit the growth of *Vibrio* sp. In addition, Wicaksono *et al*. (2020) mentioned that the alkaloid content has the ability to inhibit the activity of the *Vibrio* sp. enzyme which causes the rate of biochemical and metabolic reactions to be disrupted, the material causes the death of *Vibrio* sp. bacterial cells.

## CONCLUSION

The results of the research can be concluding, giving jatropha leaf extract, the results of statistical tests were able to increase the immune system of white shrimp, the highest survival rate value of P2 was 88.3%, the highest total hemocyte count value of P5 was 24.46 x 106 cells/ml, the differential hemocyte count value such as hyaline cells was 36.67 - 71%, granular cells ranged from 17-38.57%, and semi granular cells ranged from 12-24.67%, the highest AF value was P5 at 77.25%, and the lowest total bacteria count value of P2 of 6 x $10^8$ CFU/ml, The lowest total vibrio count of P2 was 0.56 x $10^6$ CFU/ml.

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