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Effect of Giving the Active Ingredient Jellyfish Alkaloids (Bougainvillia sp.) Through Immersion Method on Changes in the Amount of Protein Plasma of Tiger Grouper (Epinephelus fuscoguttatus) Infected with Vibrio harveyi

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ABSTRACT

The purpose of this study was to obtain information regarding the potency of the alkaloid active ingredient in jellyfish (Bougainvillia sp) on the immune response of tiger grouper fry infected with Vibrio harveyi bacteria through the immersion method by observing changes in appearance and the amount of plasma protein in tiger grouper infected with bacteria. V. harveyi. The research results obtained explained that the immunostimulant ingredients from jellyfish alkaloids (Bougainvillia sp.) given by immersion can stimulate non-specific and fish-specific immunity. This is characterized by an increase in the amount of protein found in fish blood plasma after administration of alkaloids. Besides that, it can also be seen that the administration of this alkaloid is able to inhibit the infection power of Vibrio harveyi. It is proven that in fish given alkaloid immunostimulants, the amount of protein that appears can be more and the protein content is also high. With a larger protein, it will be able to further stimulate non-specific immunity so that later various antibodies as specific immunity in fish will also be formed. This research ultimately concludes that jellyfish alkaloids (Bougainvillia sp) can be used as immunostimulants to inhibit bacterial attacks Vibrio harveyi in tiger grouper seeds with the optimal dose through immersion method that can be applied is 10.22 ppm. As a follow-up to the results of this study, it is suggested to carry out further research on the application of immunostimulants using jellyfish alkaloids (Bougainvillia sp.

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1. INTRODUCTION

Indonesia as a maritime country has a large marine fishery product potential. The Government's attention to the marine fisheries sector has increased with the establishment of the Ministry of Maritime Affairs and Fisheries. This is done in the framework of exploiting and maintaining marine fisheries potential as much as possible so that it can meet the nutritional needs of the Indonesian people and can increase the country's foreign exchange from non-oil and gas commodities. Grouper is a marine commodity that has important economic value.

The demand for grouper fish as a consumption material has been increasing lately. These commodities are marketed in fresh form or in packaged form with sales reaching an international scale. The main obstacle in grouper farming is the high mortality rate of cultivated fry which can reach 99% due to, among other things, Vibriosis, where infection with pathogenic bacteria in cultivated fish can cause fish mortality of more than 80%. Vibrio is a Gram-negative microorganism or bacterium that causes a systemic infection in fish called vibriosis (Wang and Leung, 2000).

Diseases that arise are due to interactions between the host, the disease-causing organism and the environment. Prevention is a more effective disease control measure than treatment. Control of fish diseases using antibiotics and other chemotherapy agents can cause problems, namely reducing the body's resistance of fish, affecting fish growth, and can cause bacterial resistance. In addition, the use of antibiotics can also cause environmental pollution and the accumulation of antibiotic residues in fish meat.

One of the most effective ways to prevent disease is to apply the principle of immunoprophylaxis, namely a disease prevention by increasing the body's immunity against various diseases (Ellis, 1988). The immune system in fish is generally detected through blood tests. Some blood components can be observed to detect certain infections including blood plasma. Therefore this study seeks to see the effect of using jellyfish immunostimulants through the immersion method on the immune system of tiger grouper by observing its appearance and the amount of plasma protein.

Plasma cells have a spherical nucleus, which is located accentuatedly and the chromatin is unevenly distributed. Plasma cells have cytoplasm that is basophilic and pyroninophilic, consistent with ribosome-rich cells that produce antibodies. Plasma cells are capable of making as many as 300 antibody molecules every second and sometimes these antibodies aggregate (Tizard, 1988). Plasma cells (which are also often called plasma B cells or plasmocytes) Proteins with a certain molecular weight have a certain Rf as well.

If five types of standard proteins with different molecular weights are used, five different Rf values will also be obtained. To create a molecular weight calibration curve, these five Rf values will be placed as the Y-axis and molecular weight (usually expressed as a function of the log molecular weight) is placed as the X-axis. The graph obtained is a linear graph with the equation of the line, namely $y = a + bx$. The mobility of a protein (of unknown molecular weight) can be found by plotting directly on the standard molecular weight curve or it can be calculated using the line equation of the molecular weight standard curve.

2. METHOD

2.1 Types of research

The research method used in this study is the experimental method. The experimental method is a form of observation under artificial conditions, where these conditions are created and regulated by the researcher. That is, basically conducting an experiment to see the results, and the results of the experiment will confirm how the causal position is between the variables being investigated

2.2 Research Variables

The variables in the study consisted of independent variables which were test animals (goldfish) treated with natural antibiotics from tempuyung plants.

2.3 Research design

The design used was a completely randomized design (CRD) where each treatment was carried out as a separate unit, there was no grouping relationship. According to Gasperz (1991), some of the advantages of using RAL are: The layout of the trial design is easier; Statistical analysis of experimental subjects is very simple; Flexible in the use of the number of treatments and the number of repetitions; The possibility of losing information is smaller.

2.4 Sampling location

In the research that will be carried out using the seeds of tiger grouper (Epinephelus fuscoguttatus) and the feed that will be given is trash fish, including tuna and lemuru.

2.5 Time and Place of Research.

This research was conducted at the Situbondo Brackish Water Cultivation Center (BBAP) Situbondo Regency from December 2006 to February 2007.

2.6 Tools and materials

The equipment used in this study are: 10 plastic tubs; Autoclave; Aerator; pH meter; Petridish; Thermometer; Erlenmeyer; Oxymeter; Needle Ose; Measuring Cup; Measuring Pipettes; Analytical Balances; Suction Rubber; Centrifuge; Micropipets; Shakers; Microtip; Spectrophotometer; Test Tube; Electrophoresis kit.

The materials used in this study are: Pure culture of Vibrio harveyi bacteria; TCBSA (Thiosulfate Citrate Billesait Sukrose Agar); Physiological NaCl; Nessler's reagent; NB; Chlorine; Gram dye; 70% alcohol; Sterile Aquades; Parchment Paper; Tissue; Spiritus; Materials for electrophoresis consisting of UGB, LGB, T-acryl, dd H2O, APS.

2.7 Research procedure

Preparation of the tools during the research test which begins with sterilizing the tools by putting all the tools that will be used into the autoclave water then heating it on the stove for 15 minutes then preparing the media Making Nutrient Broth Solution, Making TCBSA Media and planting bacteria on the media with the etching technique agar as well as Water Sanitation and Research Implementing Equipment.

2.8 Data analysis.

From the data obtained, statistical analysis was carried out. To find out the normality of the distribution of the data, a Barlet test was carried out, then an analysis of variance was carried out with the F test (ANOVA) in accordance with the design used, namely Completely Randomized Design (CRD). If from the results of the variance it is known that the treatments show significantly different (significant) or highly significant (highly significant) results, then a follow-up test is carried out in the form of the smallest significant difference test (LSD) to compare the values between treatments with the responses that occur with the level of comparison of treatments the best followed by the Least Significant Difference Test (LSD).

3. RESULTS AND DISCUSSION

3.1 Research result

The results of testing the protein profile using SDS PAGE electrophoresis, showed an overview of the profile and the total molecular weight of the protein in each grouper blood plasma sample. The presence of protein in each column can be seen by the appearance of a blue band in the image above. According to Sudarmadji (1996), the protein will be blue because it binds coomasie blue, and this blue color can be used in blood plasma, there are several components that function in the immune system.

According to Anonymous (2006) that plasma cells (which are often referred to as plasma B cells or plasmocytes) are one of the cells included in the immune system that are capable of secreting large amounts of antibodies. Antibodies or immunoglobulins found in blood plasma are Y-shaped proteins and are used in the immune system to identify and neutralize foreign substances such as bacteria and viruses.

3.1.1 Changes in Protein Levels in Blood Plasma of Grouper Fish Without and After infection

Protein levels in blood plasma of grouper fish without infection obtained from spectrophotometer measurements.

Table 1. Data on protein levels in fish plasma without infection

Treatment	Test		total	Average
	1	2		
A (6.4 ppm)	460	445	905	452.5
B (8.4 ppm)	740	740	1460	730
C (10.4 ppm)	760	770	1530	765
D (12.4 ppm)	620	640	1260	630
TOTAL	-	-	5155	2577,5

If there is an effect of different doses of the active ingredient of jellyfish alkaloids, then the analysis of variance is calculated. Based on orthogonal polynomial analysis (see Appendix 16) it can be seen that the best dose treatment for fish without infection is treatment at dose C (10.4 ppm) then dose B (8.4 ppm) then continued with dose D (12.4 ppm) and the last one is dose A (6.4 ppm). This shows that treatment C at a dose of 10.4 ppm was able to increase protein levels in the highest plasma when compared to other doses of the active ingredient jellyfish alkaloids (Bougainvillia sp.).

Table 2. Protein Levels in Grouper Blood Plasma After Infection.

Treatment	Test		total	Average
	1	2		
A (6.4 ppm)	300	300	600	300
B (8.4 ppm)	540	500	1040	520
C (10.4 ppm)	600	600	1200	600
D (12.4 ppm)	420	420	840	420
TOTAL	-	-	3680	-

Based on the results of the BNT test above, it can be seen that all of the four treatments have a very significant different effect. Therefore to determine the relationship between the dose of this immunostimulant against plasma protein levels. Furthermore, after the regression analysis was carried out, a quadratic relationship was obtained between the dose and plasma protein levels

3.1.2 Protein Levels in Blood Plasma of Healthy Fish and Diseased Fish

Protein levels from healthy fish and sick fish can be seen in plasma samples at dose K (0 ppm) for samples without and after infection. Protein levels obtained between healthy fish and sick fish also showed a decrease.

Table 3. Value of Protein Levels in Blood Plasma of Healthy Fishand Sick Fish

BM Results (ppm)	Treatment	
	Factor 1	Factor 2
660	K (0 ppm)	A (no infection)
580	K (0 ppm)	
260	K (0 ppm)	B (no infection)
400	K (0 ppm)	
	BNT a 0.01 = 0.39	BNT a 0.01 = 0.33

From the test results above, it can be seen that the average molecular weight value of plasma protein in healthy fish is 620 ppm, and the average molecular weight of sick fish is 330. From this value, it is known that there is a decrease in the number of molecular weights, from 620 to 330. or nearly half. With a decrease in plasma protein levels, it can be assumed that after an infection by Vibrio, the cells in the blood, both blood cells and plasma, will be damaged so that the molecular weight of the plasma proteins will also decrease.

3.1.3 Water quality analysis.

Water quality in this study is a supporting parameter. The results of observations on the quality of the media water during the study still gave a value in the range desired by tiger grouper seeds to form a pattern of defense against disease, especially infection by pathogenic bacteria such as Vibrio harveyi. In relation to the survival and immunity of tiger grouper seeds infected with bacteria, the quality of the media water plays a very important role, because the emergence of disease or infection by bacteria is caused by unbalanced aquatic environmental conditions and does not provide hygienic aspects for the fish being cultivated. Water quality parameters measured were temperature, pH and dissolved oxygen levels.

3.2 Discussion

This study ultimately concluded that jellyfish alkaloids (Bougainvillia sp) could be used as immunostimulants to inhibit Vibrio harveyi bacterial attack on tiger grouper seeds with the optimal dose via immersion method which can be applied at 10.22 ppm. As a follow-up to the results of this study, it is suggested to carry out further research on the application of immunostimulants using jellyfish alkaloids (Bougainvillia sp.) to tiger grouper larval and juvenile stages using other types of bacteria and the application of immunostimulants using jellyfish alkaloids in other fish species using Vibrio harveyi bacteria.

4. CONCLUSION

From the research that has been done, several conclusions can be drawn, namely the active ingredients of jellyfish alkaloids given by soaking can be used as immunostimulants in boosting the immune system in tiger grouper. This can be seen in fish given alkaloid immunostimulants, the amount of protein that appears can be more and the protein content is also high and the best dose of jellyfish alkaloid active ingredient which can total the highest molecular weight of blood plasma without infection is at dose C (10, 4 ppm) followed by doses B (8.4 ppm), D (12.4 ppm) and A (6.4 ppm). The quadratic equation for the relationship between immunostimulant dose and protein

content in tiger grouper seed plasma is: Y = -1898.375 + 526.562x – 25.78x2 , where the correlation coefficient (r) is 0.993; with X0optimal = 10.91 and YMaximum = 1170,

The quadratic equation for the relationship between immunostimulant dose and plasma protein levels of tiger grouper fry is: Y = -2000 + 511x - 25x2 where the correlation coefficient (r) is 0.990; with X0optimal = 10.22 and Y Maximum = 611.21.

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Based on the research results obtained, it can be suggested to use an immunostimulant dose of the jellyfish allaloid Bougainvillia sp. Through immersion is around 10.22 ppm and it is necessary to do further research on the immunostimulant effect of the alkaloid jellyfish Bougainvillia sp. against various parasites and other diseases that attack fish.

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