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The use of ethanol extract of rose balsam *(Impatiens balsamica L)* to enhance resistance of catfish *(Clarias gariepinus Var.Sangkuriang)* against *Aeromonas hydrophila*

Syauqy Hidayah[1]*, Henky Manoppo[2], Henneke Pangkey[2]

[1]Post-graduate student of aquatic science Faculty of Fisheries and Marine Science University of Sam Ratulangi, Indonesia
[2]Lecturer Department of Aquaculture, Faculty of Fisheries and Marine Sciences, University of Sam Ratulangi Manado, Indonesia

Abstract: The objective of this study was to evaluate the potential of ethanol extract of rose **b**alsamto induce the resistance of Sangkuriang catfish *(C.gariepinus Var. Sangkuriang)* against *A. hydrophila* infection. Sangkuriang catfish measuring 10-12 cm each were obtained from Freshwater Aquaculture Board, Ministry of Marine and Fisheries at Tatelu Village North Sulawesi Province. After adaptation for one weeks, the fish were introduced into 12 aquaria at a density of 12 individuals per aquarium. In the first three aquaria (Group A), the fish were injected with 0.1 mL of rose balsam extract, seven days later the fish were infected intraperitonealy with 0.1 mL of *A. hydrophila* suspension containing $1x10^7$cfu/mL.Fish in the second three aquaria (group B) were injected first with 0.1 mL of *A. hydrophila* and after three days of infection, the fish were treated with rose balsam extract by injection of 0.1 mL extract per fish. Fish in the third three aquaria (group C) were injected with only with 0.1 mL *A. hydrophila* suspension as positive control and lastly fish in the fourth three aquaria (group D) were injected with 0.1 mL rose baslam extract only as negative control. The results showed that extract ethanol of rose balsam flower was able to increase the survival of catfish fry against*A. Hydrophila* infection.The survival of fish in group A achieved75% while in positive control group, survival of fish was only 30.55%. Survival of fish in negative control (injected only with rose balsam extract) was 91.5% indicating that the extract had no toxic effect on fish. Thus, the use of rose balsam extract was potential to improve resistance of Sangkuriang catfish against pathogen.

Keywords : catfish, *Clarias gariepinus Var. Sangkuriang*,*Impatiens balsamica* L,medicinal plant, aquaculture.

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Introduction

Sangkuriang catfish is one of the freshwater fish widely cultivated in Indonesia. This species is easy to grow in a limited area and has high demand with high economic value in the market. Yet these fish are susceptible to Motile Aeromonad Septicemia (MAS) caused by the bacterium *Aeromonas hydrophila.*Mortality due to this disease may achieve 80-100% within one weeks. The outbreak of this disease often occur during the year and had caused significant losses to the farmers.

Fish farmers usually control the disease by the use of various antibiotics such as *ampicillin, chloramphenicol, tetracycline*.Continuous use of antibiotics with inappropriate dose can lead to the development of antibiotic-resistant pathogen, accumulation of drug residues in fish bodies which are dangerous for people who eat the fish. The residue can also be accumulated in aquatic environments resulted in pollution and bioaccumulation [1,2].

In fish farming, herbs are used as growth promoter and antibacterial. Recently many researches have been conducted to evaluate the potential of herbs as an alternative for the use of antibiotics and in controlling diseases in aquaculture as well. The use of various products from medicinal plants to modulate fish immunity has been intensively investigated[3].Rose balsam (*Impatiens balsamica L*) is one of the promising medicinal plant for aquaculture. [4] Reported that rose balsam contains several compounds such as naphthoquinones, coumarins, phenolic acids, flavonoids, anthocyanin's and steroids that have the ability as an antibacterial, antimicrobial, anti-fungal, analgesic, anti-inflammation, antioxidants and anti-piuritic.[5]found that ethanol extract of rose balsam leaves has antibacterial activity against *Aeromona shydrophila.* Moreover, [6]Reported that rose balsam extract was able to increase nonspecific immune response of tilapia.This research was intended to study the prospective of rose balsam to improve resistance of Sangkuriang catfish against *Aeromonas hydrophila* infection.

Material and Method

Experimental Fish

A number of 150 individuals sangkuriang catfish *(Clarias gariepinus Var.Sangkuriang)*measuring 10-12 cm each was obtained from Freshwater Aquaculture Board, atTatelu Village . Before running the experiment, the fish were adapted in fiber tank for two weeks. During acclimatization, the fish were fed commercial pellet containing 30% protein at a feeding rate of 5%/body weight/day, twice daily.

Extraction of Rose Balsam

Rose balsam was collected from the people garden around Tatelu Village, Dimembe District, North Minahasa Regency, Indonesia. Its flowers were separated, washed, groundinto small pieces using a blender, and macerated in ethanol 70%for three days. The extract was then evaporated in an incubator at 55°C for 96 hours. Every 24 hours, the extract was stirring for 30 minutes using a magnetic stirrer.

Figure 1. Medicinal plant Rose Balsam (*(Impatiens balsamica* L*)*

Research Procedure

After adaptation, the fish were distributed into 12 aquaria (60 x 40 x 35 cm^3), each equipped with an aerator and a small submersible water pump for recirculation. The density of fish in each aquarium was 12 fish. To evaluate the efficacy of rose balsam extract in inducing resistance of fish against *A. hydrphila* infection, the fish in the first three aquaria (Group A) were injected with 0.1 mL of rose balsam extract, seven days later the fish were injected intraperitonealy with 0.1 mL of *A. hydrophila* suspension containing $1x10^7$cfu/mL.Fish in the second three aquaria (group B) were injected first with 0.1 mL of *A. hydrophila* and after three days of infection, the each individual fish were treated with rose balsam extract by injection of 0.1 mL extract solution.. Fish in the third three aquaria(group C) were injected only with 0.1 mL *A. hydrophila* suspension as positive control and lastly fish in the fourth three aquaria (group D) were injected with 0.1 mL rose baslam extract only as negative control.

During the treatments, the fish were fed commercial pellet at a dose of 5%/bb/day, twice a day at 08.00 am and 16.00 pm. Water quality was maintained stable by performing water exchange of 10-20% every day.Dissolved oxygen, pH, and water temperature were measured at the beginning, middle and end of the research. Resistance of fish was observed for 10 days after treatments. The resistance of fish (in percentage) was based on the survival rate data achieved after the research was terminated. Survival rate was defined as the ratio of the number of live fish at the end of research period and the number of fish stocked at the beginning of research multiply by 100.The data of fish resistance was analyzed descriptively.

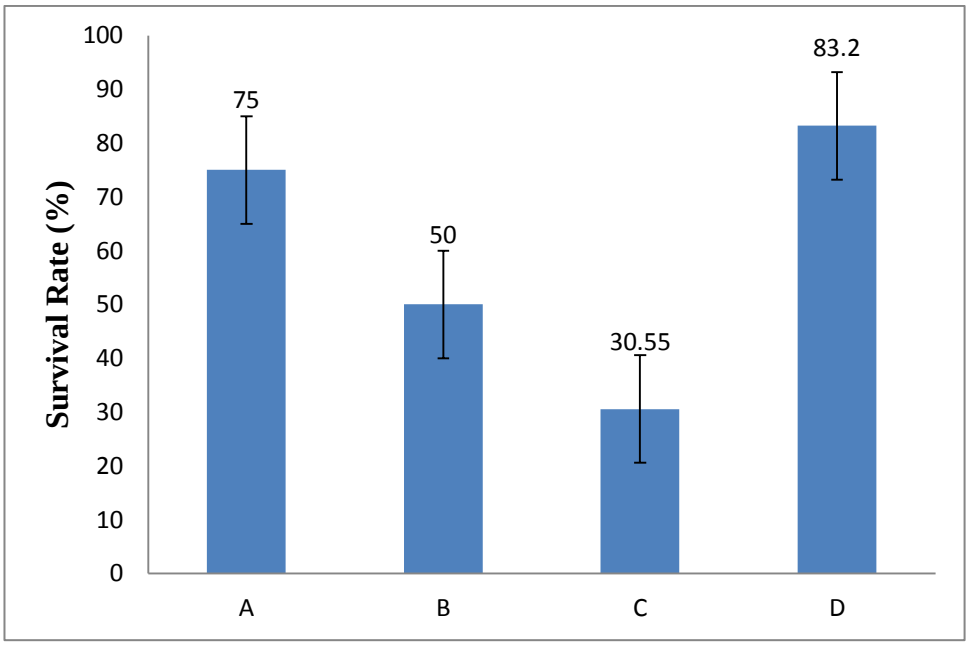
Clinical Symptoms

Clinical symptoms of fish after infected with bacteria *A. hydrophila* were observed every 3 days including inflammation, hemorrhagic, necrosis and ulcers.

Results and Discussion

Resistance of fish

The average survival rate of fish observed 14 days after treatment was presented in Figure 1.



A. The fish were injected with 0.1 mL rose balsam extract, after seven days, the fish were injected intraperitonealy with 0.1 mL of *A. hydrophila* suspension containing $1x10^7$cfu/mL
B. The fish were infected with 0.1 mL *A. hydrophila*, after three days, the fish were injected with 0.1 mL rose balsam extract
C. The fish were injected with0.1 mL *A. hydrophila* suspension(positive control)
D. The fish were injected with rose balsam extract (negative control)

The lowest survival rate of fish was obtained in positive control (groupC) while the highest were in negative control (Group D). If the fish was infected first with *A. hydrophila* and then injectedwith rose baslam extract, the survival achieved 50% (Group B). On the other hand, if the fish was first treated with rose balsam extract, the survival increased up to 75% (Group A).

The survival rate of fish in group D (negative control)achieved 91.5%. This proved that the use ethanol rose balsam extract had no toxic effect on juvenile sangkuriang catfish. The toxicity of any plant extract on the particular organism was expressed in lethal dose (LD50), which showed the dose of extract that caused 50%mortality of the population of experimental animals[7].During the experiment, the fish did not display any behavior abnormality,irregular swimming, bobbing to the surface of the water, or whirling. When the fish are exposed to toxins, they will display hyperactive movements, more often stay on the surface, floundering, and paralyzed so that the ability of fish to adapt to the environment would decrease and eventually could cause death of fish[8].

The survival rate of fish in groupAachieved 75% which was higher than survival rate of fish in group B.If the fish were not treated with rose balsam extract, the survival rate was only 30.55%. High survival in groupA was achieved due to the ethanol extract of rose balsam has the ability to enhance the immuneresponse of fish. A research report showed that the ethanol extract of rose balsam leaves was able to induce phagocytic index and total leucocytes of tilapia[9]. Immunostimulant works by increasing the activity of phagocytic cells to predate the foreign particles or pathogens that enter the body[10].Various herbs are able to enhance immune response of fish. Extract of Meniran(*Phyllanthus niruri*) had been reported to be able to modulate immune response[11]. Besides saponin, flavonoid contained in the extract was able to activate immune cells[12]. Several reports stated that rose balsam extract contained flavonoids, saponins, anthocyanin's, steroid, glycosides,anthocyanin and camperol[13,14].

A.hidrophyla is a pathogenic bacteria which can cause 80% mortality in catfish, and even may reach 100% within a period of one week[15]. The ability of *A. hydrophila* to cause mortality depending on the poisonproduced by *A. hydrophila*. Gen Aero and hlyA of the bacteria produce aerolysin and hemolysin toxins. Aerolisin is an extracellular protein produced by several strainsof *A. hydrophila*. This substance is soluble, hydrophilic and has hemolytic and cytolytic properties. Aerolysin works by binding to specific glycoprotein

receptors on the surface of eukaryotic cells before entering into the fat layer and forming a hole. The aerolysin poison that forms a hole passes into the bacterial membrane asa peptide-containing preprotoxine. The poison can attack the epithelial cells and causing gastroenteritis[16].

Clinical Symptoms

Clinical symptoms that arose as a result of the attacks *of A. hydrophila* was an emerging infection can be seen on the skin, as an internal systemic diseases (septicemia), or a combination of both.In general, clinical symptoms begins with hyperemia (redness), inflammation, necrosis (tissue damage), ulcers (a more severe tissue damage or ulcer), and ends in death.Wounds on the body surface of fish and the other part is because at the *A. hydrophila* are extracellular product in the form of an enterotoxin, cytotoxins, hemolysin, lipase and protease [17].

Inflammatory reactions are reaction to prevent the entry of microorganisms around the site of infection.In addition, the inflammatory process also forms reaction between fibrinogen and other clotting factors in the blood and forms a fibrin tissue to prevent the escape of body fluids and prevent the entry of foreign objects into the body[18].Inflammation is a basic vascular and cellular reaction of animals against bacteria that enter the body and cause tissue damage[19]. In the inflammatory reaction, a decrease in the number of leukocyte cells is possible because the cells are lysis. The release of intracellular enzymes is a consequence of lysis of phagocyte cells that would be detrimental to pathogens, even thoughthe neutrophils actively secrete their extracellular enzymes as a pathogen killing mechanism.Hyperemia caused by mobilization of white blood cells, as a form of resistance against bacterial pathogens[20].There are three main stages in inflammatory reactions *(inflammatory response).*First, there is an increase in the blood supply to the area around the wound, followed by an increase in the permeability nature of the body capillary tubes as well as the leukocyte migration out of the capillaries and into the tissues evenly.Fish in group A showed a mild clinical signs level compared with fish in group D (negative control). The clinical sign appeared only as inflammation, no hemorrhagic and ulcer observed on the body surface of fish.

Conclusion

The research results found that application of ethanol extract of rose balsam is potential to induce resistance of catfish against pathogenic infection.

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