Effect of *Plumeri acuminate* Leaf Extract on Survival of *Cyprinus carpio* Seedlings infected with Bacteria *Aeromonas hydrophyla*

[2]Mita Alvionita, [2]Ade Muharam, [1.2]Syamsuddin

[1]syamsuddin@ung.ac.id
[2]Department of Aquaculture, Faculty of Fishery and Marine Sciences
Universitas Negeri Gorontalo

Abstract

This study aimed to determine the effect of frangipani leaf extract (*Plumeri acuminate*) with different doses on the survival of carp fish (*Cyprinus carpio*) infected with *Aeromonas hydrophyla* bacteria. This research was carried out at the Wet Laboratory of the Fish Quarantine Station, Quality and Safety Control of Fishery Products Gorontalo. This study used the RAL method with 4 treatments and 3 replications. The test method was carried out by soaking the infected carp seedlings with different doses of frangipani leaf extract to observe the survival of the seedlings. Treatment A (46.70%), Treatment B (60.00%), Treatment C (93.33%) and Treatment D (33.33%). Analysis of Variance (ANOVA) showed that the administration of frangipani leaf extract with different doses had a significant effect on the survival of carp seedlings between treatments. Water quality parameters during the study were in normal conditions for the life of carp seedlings.

Keywords: Carp; *Cyprinus carpio*; *Plumeri acuminate*; *Aeromonas hydrophyla*; frangipani; leaf extract; survival.

Introduction

Fish disease can be defined as anything that can cause disruption of a function or structure of a body organ or part of a body organ, either directly or indirectly. In principle, diseases occur under three factors: environmental (water), host (fish), and the presence of pathogenic (bacteria) (Kordi, 2008).

Aeromonas hydrophyla, a pathogen bacterium, causes the "Motile Aeromonas Septicemia" (MAS) disease especially for freshwater fish species in tropical waters. These bacteria are opportunistic pathogens that are almost always present in water and are ready to cause disease if the fish are in unfavorable conditions (Afrianto and Liviawaty, 2000).

The frangipani plant (Plumeria sp.) is an example of the Apocynaceae family grow in tropical and sub-tropical areas (Rolliana, 2010).

Research Methods

This research was carried out for 3 months, from March to May 2017 at the Wet Laboratory and Bacterial Laboratory of the Fish Quarantine Station Gorontalo, and the manufacture of frangipani leaf extract was carried out at the Pharmacy Laboratory of the State University of Gorontalo.

The procedure in making frangipani leaf extract includes: frangipani fresh leaves that will be used as extracts are not too old and not too young, where old leaves tend to dry out so that the chemical content needed dismiss, while leaves that are too young the chemical content needed is less. The fresh and young leaves are washed with clean water, cut into small pieces to facilitate the drying process, dried indoor without exposure to sunlight, and weighed to determine the dry weight. Obtained 753 g dry weight of frangipani leaves. Put the dried frangipani leaves in a glass jar, then add 3 x 3000 ml of 96% ethanol. Left it to stand for 24 hours. The filtrate was separated using a rotary evaporator until no more solvent dripped, this viscous extract was then used to control MAS disease that attacks carp by immersion method.

The carp to be tested is approximately 8 – 9.5 cm in size from the Central Fish Seed Center of Bendungan Village, North Bulango District, first adapted for 3 days, during adaptation the fish

are fed FF-999 pellets with a feeding frequency of 3 times daily (morning, afternoon and evening) as much as 5% of body weight. Fish were randomly added to the aquarium according to the treatment.

Bacterial inoculant growth medium was TSA (Triptic Soya Agar) with a ratio of 40 g of TSA dissolved in 1 liter of distilled water. The solution is put in a magnetic stirrer which functions to homogenize the media, heated on a hot plate, after boiling it is transferred and put into an auto clave to be sterilized at 121^{O}C with a pressure of 2 atm for 30 minutes, then transferred to a laminary flow and left to a temperature of ± 37^{O}C, Then the TSA media was poured into a petri dish. If the media is not used directly, it can be stored in the refrigerator (Lukistyowati, 2005 in Sari et al., 2012).

The carp seeds to be infected are checked first to ensure that the seeds do not contain *Aeromonas hydrophyla*. Bacterial examination was carried out on the liver and kidneys of two carp seed by dissection. The test material is 60 carp (*Cyprinus carpio*) seedlings with an average length of 9 cm which is divided into 12 containers, while the treatment material (the frangipani leaves extract) divided according to the predetermined dose. Each container has as many as 5 goldfish.

The treatment is carried out 48 hours after bacterial injection into the back of the fish (Mangunwardoyo et al, 2010) or after fish infected with *Aeromonas hydrophila* when the fishes have shown the disease symptoms. The bacterial density used in carp seeds infection was 5 x 106 cfu/ml with a bacterial volume of 0.1 ml/head.

The administration of frangipani leaf extract was carried out in different doses: Treatment A (5 g extract / 200 ml aquadest); Treatment B (10 g extract / 200 ml aquadest); Treatment C (15 g extract / 200 ml); Treatment D without the leaf extract. The containers was filled with 8 liters of water, equipped with aeration, then placed in a predetermined place based on the layout of the experiment. During the rearing process the fish were fed twice a day (morning and afternoon) and siphoning was carried out.

After the period of administration completed, each treatment was replaced with 100% water and the experimental fish were kept with normal water (without frangipani leaf extract) and observed for 14 days. Maintenance is carried out in separate containers and given aeration, siphoning is carried out every 2 days in the afternoon.

Results and Discussion

Bacterial infection

Bacterial examination on the liver and kidneys of two sample of carp seeds before bacterial injection shows the result that *Aeromonas hydrophyla* was found but in small quantities. Then 48 hours after the bacterial injection the fish shows clinical symptoms such as no appetite, swimming position is at the bottom, fish movement becomes sluggish, pale color, internal bleeding occurs, and death.

The process of infection of pathogenic bacteria Aeromonas hydrophyla into the fish body is initiated by attaching bacteria to the surface of the skin by using pili, flagella, and hooks to move and firmly attach to the outermost layer of the body, namely the scales protected by chitin. During the process, Aeromonas hydrophyla bacteria produce chitinase enzymes and play a role in degrading the chitin layer so that bacteria can easily enter the fish body. In addition to utilizing chitinase, Aeromonas hydrophyla bacteria also secretes other enzymes such as lestinase in an effort to enter the bloodstream (Mangunwardoyo, 2010).

Bacteria move very quickly in the blood vessels, and easily reach important organs of fish such as the sinusoids of the liver and kidneys. This location will be used by bacteria as a medium for living and multiplying, as well as using nutrients that are around for metabolic processes (Rahmaningsih, 2012. The entry of bacteria in activating the immune response by producing polymorphonuclear leukocytes, such as melano macrophages, monocytes and neutrophils that act as phagocytic cell.

Survival of the carp seedlings

After the treatment period was completed, the surviving goldfish seeds were re-examined to determine the presence or absence of Aeromonas hydrophyla bacteria in the body of the carp fry. Based on the results of the examination that, carp seeds that were reared for 7 days still contained Aeromonas hydrophyla but in small quantities, if the maintenance was carried out for approximately 14 days in the carp's body there was no longer any Aeromonas hydrophyla bacteria, which means that frangipani leaf extract was able to kills Aeromonas hydrophyla bacteria in the body of goldfish seeds.

Frangipani leaf extract contains a lot of flavonoids, where flavonoids are one of the secondary metabolites, the possibility of their presence in the leaves is influenced by the photosynthesis process so that young leaves do not contain too many flavonoids, while the function of flavonoids itself is used as body defense (Ardiansyah, 2007). The active substances contained in frangipani leaf extract can inhibit the growth of microbes such as Aeromonas hydrophyla bacteria (Ikrom, 2013).

Based on this thought, a trial of frangipani leaf extract was carried out on carp seeds infected with Aeromonas hydrophyla bacteria with different doses. The survival results of carp seeds that have been soaked using frangipani leaf extract can be seen in Table 1.

Table 1. Survival rate of carp seedlings

Treatments	SR (%)
A (5gr)	46,70
B (10 gr)	60,00
C (15 gr)	93,33
D (control)	33,33

The results of the one-way of variance (ANOVA) analysis showed that there was a significant difference between the treatments. The result of the calculation of the calculated F value of 10.09 is greater than the F table of 4.07 at the 0.05 level. If F count > F table 0.05 then accept H0 and reject H1 which means that there is a difference in the effect of treatment on the survival of carp (*Cyprinus carpio*) fry with different doses using frangipani leaf extract.

The results of the study on survival in each treatment were different, namely in treatment A (5gram/200ml Aquades dose), survival rate was 46.70%, Treatment B (10gram/200ml Aquades dose) survival rate was 60%, Treatment C (Dose 15gram/200ml) as seen in Figure 1.

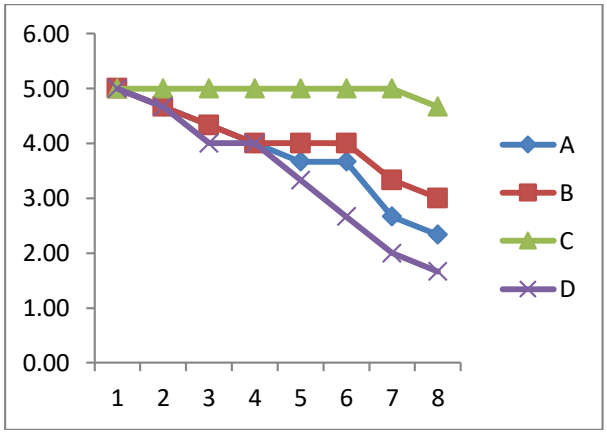

Figure 1 Graphic of Survival rate of carp seedling after treatments

Water qualities

The results of water quality measurements during the maintenance of carp seedlings obtained are in a good range for the life of the seedlings. Measurement of water quality parameters in the study was carried out 3 times, namely before immersion, during immersion, and after immersion. The measurement results can be seen in Table 2.

Table 2. Water quality parameters

Parameters	Before immersion			
	A	B	C	D
Temp. (°C)	26	26	26	26
pH	7,57	7,70	7,40	7,30
DO	7,69	7,63	7,85	7,64
	During immersion			
	A	B	C	D
Temp. (°C)	28	28	28	26,67
pH	6.97	6.5	6.33	7.22
DO	7.47	6.73	6.8	7.67
	After immersion			
	A	B	C	D
Temp. (°C)	27	27	27	27
pH	7,53	7.6	7.4	7,43
DO	5,25	6,50	7,47	7,46

According to Kordi and Kancung (2007) in Mas'ud (2014), the optimal temperature range for fish life is 28^{O}C – 32^{O}C, carp can live in the range of 6.8 - 8.5 ppm, while for dissolved oxygen it must be greater than 2 mg/l minimum content of 6 mg/l and should not occur from 8 consecutive hours (Khairuman, et al 2002).

Conclusion and Suggestion

Provision of frangipani leaf extract with different doses has an effect on the survival of carp fry infected with A. hydrophyla bacteria.

The highest survival was obtained in treatment C, treatment B obtained the second survival rate, for treatment A and treatment D obtained the lowest survival.

Based on the conclusions above, the suggestions that can be put forward are that further research needs to be carried out to examine the appropriate and best dose of frangipani leaf extract to be used as an anti-bacterial *A. hydrophyla* in carp.

References

Afrianto dan Liviawaty, 2000, *Pengendalian Hama Dan Penyakitikan.* Yogyakarta: Kanasius

Ardiansyah A. 2007. *Antimikroba Dari Tumbuhan.* Tohuku.Universitas Sendai, Jepang

Ikrom, Denok Asih T.R , Perkasa B, Tiara R, dan Wasito 2014. Studi *In Vitro* Ekstrak Etanol Daun Kamboja (*Plumeria alba)* Sebagai Anty *Aeromonas hydrophyla.* Fakultas Kedoktera Hewan UGM. Semarang

Khairuman, dkk.2002. *Budidaya Ikan Mas Secara Intensif*. Agromedia Pustaka.Subang

Kordi, M. G.H. 2008. *Budidaya Perairan*. PT. Citra Aditya Bakti. Bandung

Rahmaningsih Y. 2012, Penyakit Akibat *Aeromonas hydropyla*. PT Penebar Swadaya, Jakarta