

RESPONSE OF *Citrus aurantifolia* TO THE INHIBITION ZONE OF THE *Aeromonas salmonicida* BACTERIA IN TILAPIA (*Oreochromis niloticus*)**Respon *Citrus aurantifolia* Terhadap Zona Hambat Bakteri *Aeromonas salmonicida* Pada Ikan Nila (*Oreochromis niloticus*)**Adellia Rindi Putri Utami^{1*}, Sri Oetami Madyowati¹, Nurul Hayati¹, Maria Agustini¹¹Aquaculture, Faculty of Agriculture, Dr. Soetomo University

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*Corresponding author: adelliarindipu@gmail.com(Received May 21th 2024; Accepted June 22th 2024)**ABSTRACT**

The impact of using synthetic antibacterials creates a number of new cultivation problems, including: Bacterial resistance, retention of toxic substances and residues in the consumer's body. You need antimicrobial ingredients that can kill bacteria, one of which is the use of lime juice. The aim is to find out how strongly *Citrus aurantifolia* inhibits the bacteria *Aeromonas salmonicida* from *Oreochromis niloticus*. Using a completely randomized design (CRD) with five treatments. The results showed that the diameter of the inhibition zone for each Treatment A was 10.8 mm, Treatment B 12.8 mm, Treatment C 14.7 mm, Treatment D 16.7 mm, and Treatment E 19.1 mm. The relationship between administration of lime juice and the average diameter of the inhibition zone produced in inhibiting the growth of *Aeromonas salmonicida* bacteria shows a response that increases with increasing dose. Based on the Anova test using $F(0.000) > F$ table at the 5% level of significance, lime juice (*Citrus aurantifolia*) has an effect on inhibiting the growth of *Aeromonas salmonicida* in each treatment.

Keywords: *Aeromonas salmonicida*, Antibacterial, Bacterial Inhibition Zone, *Citrus aurantifolia*, Tilapia

ABSTRAK

Dampak penggunaan antibakteri sintetik menimbulkan sejumlah permasalahan baru budidaya antara lain: Resistensi bakteri, retensi zat dan residu beracun dalam tubuh konsumen. Perlu bahan antimikroba yang dapat membunuh bakteri, salah satunya adalah penggunaan air jeruk nipis. Tujuan mengetahui seberapa kuat *Citrus aurantifolia* menghambat bakteri *Aeromonas salmonicida* dari *Oreochromis niloticus*. Menggunakan rancangan acak lengkap (RAL) dengan lima perlakuan. Hasil menunjukkan diameter zona hambat setiap Perlakuan A 10,8 mm, Perlakuan B 12,8 mm, Perlakuan C 14,7 mm, Perlakuan D 16,7 mm, dan Perlakuan E 19,1 mm. Hubungan antara pemberian perasan jeruk nipis dengan rata-rata diameter zona hambat yang dihasilkan dalam menghambat pertumbuhan bakteri *Aeromonas salmonicida* menunjukkan respon yang semakin meningkat dengan bertambahnya dosis. Berdasarkan uji Anova dengan menggunakan $F(0,000) > F$ tabel pada taraf nyata 5%, air jeruk nipis (*Citrus*

aurantifolia) berpengaruh dalam menghambat pertumbuhan *Aeromonas salmonicida* pada setiap perlakuan.

Kata Kunci: *Aeromonas salmonicida*, Antibakteri, *Citrus aurantifolia*, Ikan Nila, Zona Hambat Bakteri

INTRODUCTION

Oreochromis niloticus is commercial important in the global freshwater business and has high economic value. Tilapia is easy to cultivate, liked by many people, the price is relatively cheap and grow fast. One of the problems that often hampers the development of fisheries is disease outbreaks which always cause the most serious impact, namely loss of production (Wirawan et al., 2018). National tilapia production continues to increase and is very good. However, in 2018 it experienced a decline due to various obstacles, especially fish diseases such as those caused by the *Aeromonas salmonicida* bacteria which can cause economic losses for farmers.

A. salmonicida bacteria is a bacteria that targets salmonid and non-salmonid fish and can spread quickly through the environment, farming equipment and direct contact. This bacterium causes furunculosis which causes bleeding in the fish, thinning of the fins, loss of appetite, slow swimming, ulcers and death within two to three days without visible clinical symptoms. *A. salmonicida* infection has previously been recorded infecting Blitar gourami (*Osphronemus gourami*) and Yogyakarta goldfish (*Cyprinus carpio*) causing death. *Aeromonas salmonicida* can cause damage and various histopathological changes to the kidneys, spleen, intestines and liver (Fajrin, 2020).

Overcoming disease problems fish require handling and treatment *A. salmonicida* disease, among others, by using environmentally friendly alternative medicines. Today's society developing natural ingredients obtained from medicinal plants. Use of traditional medicine safer. Because it has fewer side effects than industrial drugs. Apart from that, the use of natural medicinal plants to maintain health and prevent disease is cheap and easily available to everyone (Salim & Ernawati, 2017). Several natural medicinal plants such as lime (*Citrus aurantifolia*) are used to treat bacterial diseases in fish.

Lime is a shrub measuring 3.5 meters high. This plant has a round shape and a whitish yellow taproot. This plant has a woody stem, is round and has white-green spines, has round, blunt compound leaves with serrated edges and pinnate leaf spines. Round limes with a diameter of 3.5-5 cm. Limes are light green when they are young, but turn yellow as the fruit ages (Herbie, 2015).

Lime (*Citrus aurantifolia*) contains several compounds such as amino acids, iron, citrate, vitamin C, essential oils and vitamin B1 (thiamine). Essential oil is a component of lime which has anti-bacterial properties and flavonoids also inhibit bacteria. Citric acid gives lime a sour taste and can protect our body. A study examining the anti-bacterial effects of (*Citrus aurantifolia*) a dangerous bacterial species found that the citric acid contained in the juice can reduce the spread of bacteria (Suryana, 2018).

The aim was to determine the effect of giving *Citrus aurantifolia* on the growth of *A. salmonicida* bacteria in tilapia (*Oreochromis niloticus*) and to determine the optimal focus of lime juice (*Citrus aurantifolia*) on the growth of *A. salmonicida* bacteria in tilapia.

METHODS

Place and Time

The research was carried out from Monday, March 18 to Friday, March 22 2024, at the Surabaya 11 Fish Quarantine and Quality Control Center Test Laboratory in Sambikep Jemundo Village, Taman, Sidoarjo, East Java.

Tools and Materials

Ingredients include sterile distilled water, alcohol, media general TSA (Tryptic Soy Agar), MHA (Muller Hinton Agar) media, *Aeromonas salmonicida* bacterial culture, lime juice. The tool used is a sterile needle tube, petri dish, tube reaction, incubator, BSC, autoclave, oven, micropipette, cotton bud, ruler, disc paper, mask, gloves, label paper, tissue.

Research Methods

Experimental research methods. Method Experimental research is a type of quantitative research where the researcher controls other related variables and observe the effect of the manipulation on the dependent variable. Experimentation involves intentionally and systematically introducing changes and observing the results of those changes. Just through research questions researchers can manipulate conditions suitable for experimental research (Rukminingsih *et al.*, 2020). Using a completely randomized design (CRD) with five different treatments that is:

1. Treatment A with 20% lime juice (*Citrus aurantifolia*)
2. Treatment B with 40% lime juice (*Citrus aurantifolia*)
3. Treatment C with 60% lime juice (*Citrus aurantifolia*)
4. Treatment D with 80% lime juice (*Citrus aurantifolia*)
5. Treatment E with 100% lime juice (*Citrus aurantifolia*)

In accordance with result previously, Trisbiantara (2008) showed that oranges thin contain essential oil inhibits the growth of *Staphylococcus aureus* by 20%, 40%, and 80%.

Preparation Stage

1. Sterilization of Tools and Materials

Cahyani (2014) states that microbiological sterilization is a method of killing and eliminating all microorganisms attached to an object. Media sterilization use autoclave temperature 121°C and pressure 1 ATM for 15 minutes. The tools are sterilized in an oven at 180°C for 2-3 hours.

2. Making Trypticase Soy Agar (TSA) Media

Material TSA powder and distilled water. Then, weigh the TSA and place it in a triangular container, add distilled water and homogenize it, put it in the autoclave and sterilize at 121°C for 15 minutes and distribute it in a petri dish. store in the refrigerator.

3. Making Lime Juice (*Citrus aurantifolia*)

Lime fruit is a native plant of Semowal Surabaya. Selection of oranges must be in good condition, with smooth skin, free of defects, and green in color. Making process: clean the lime, cut it in half and squeeze it.

4. *Aeromonas salmonicida* Bacteria

Test bacteria from samples of tilapia infected with *Aeromonas salmonicida* collected from the Fish Quarantine Test Center, Quality Control and Safety Standards. Target bacteria isolated in TSA medium using a loop needle were incubated for 24 hours in an incubator at 27°C to 30°C. To obtain pure cultures, individually grown colonies are taken, isolated and incubated for 24 hours in an incubator at 27°C to 30°C.

5. *Aeromonas salmonicida* Bacterial Culture

Pure cultures of test bacteria are grown in TSA medium (*Tryptic Soy Agar*) for 24 hours temperature 27–30°C. Pure bacterial cultures were collected with a sterile tube needle, transferred to BHI B (*Brain Heart Infusion Broth*) media. and incubated at 27–30°C for 24 hours.

6. Making Bacterial Suspensions

A bacterial suspension was made from a number of test tube bacteria containing 10 ml of 0.9% physiological NaCl solution along with pure culture in a test tube, shaken until

homogeneous, then mixed with Mc-Farland standards (Misna & Khusnul, 2016). To prepare a suspension, first pick up one or two colonies with a sterile tube needle, add them to 10 ml of sterile distilled water, and homogenize using a vortex. Turbidity corresponds to the McFarland standard value of 0.5.

Making Lime Juice (*Citrus aurantifolia*) with Concentration

First prepare 25 cups of TSA media which has been added with *A. salmonicida* bacteria. Juice Lime is put into a petri dish containing sterile paper discs and soaked until the paper discs absorb lime juice with varying concentrations, namely treatment A 20%, treatment B 40%, treatment C 60%, treatment D 80% and treatment E 100%. After the disc paper slightly dry, transfer the disc paper to Petri dish containing TSA medium and *A. salmonicida* bacteria. Incubation was then carried out for 24 hours at a temperature of 27°C to 30°C.

Determination of Bacterial Inhibition Zones

Aeromonas salmonicida bacteria is determined by observing the light zone of the water after 24 hours incubation temperature 27–30°C. The larger the inhibition zone (light zone) the greater it is its ability to grow *Aeromonas salmonicida*. The zone of inhibition was measured with a ruler (mm).

Table 1. Classification of Responses to Bacterial Growth Inhibition

Inhibition Zone Diameter	Growth Barrier Response
<5 mm	Weak
5-10 mm	Currently
10-19 mm	Strong
20-30 mm	Very Strong

Source: Sartika *et al.* (2015)

Data Analysis

Analysis variation (ANOVA) done for know influence treatment. If the treatment is effective, continue Duncan's test in step 5 comparing F with F calculated table.

- F If F calculation table 5%, then treatment very different results.
- If F-table < 5% then F-value < F-table is 1%, then different treatments will give real results.

Data research using tables and graph depicting zones lime juice (*Citrus aurantifolia*).

RESULT

Results shows that treatment E (100%) yield highest zone of inhibition. Shown below.

Table 2. Bacterial growth for each treatment during the study. Average data for *A. salmonicida*, range value, and standard deviation

Treatment	Growth Range of <i>A. salmonicida</i> Bacteria (mm)	Average Growth of <i>A. salmonicida</i> Bacteria (mm)	Standard Deviation (elementary)
A (20%)	10.0 – 11.5	10,800	0.6708
B (40%)	12.0 – 13.5	12,800	0.6708
C (60%)	14.0 – 15.5	14,700	0.5701
D (80%)	16.0 – 17.5	16,700	0.5701
E (100%)	18.5 – 20.0	19,100	0.6519

Source: Primary Data (2024)

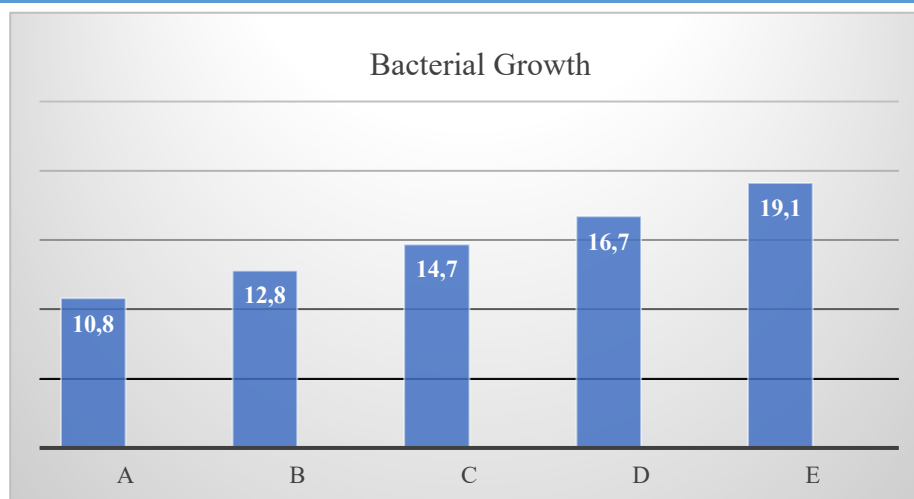


Figure 1. Growth of *A. salmonicida* Bacteria

To find out the response of *Citrus aurantifolia* inhibition zone of *Aeromonas salmonicida* bacteria was carried out by ANOVA test.

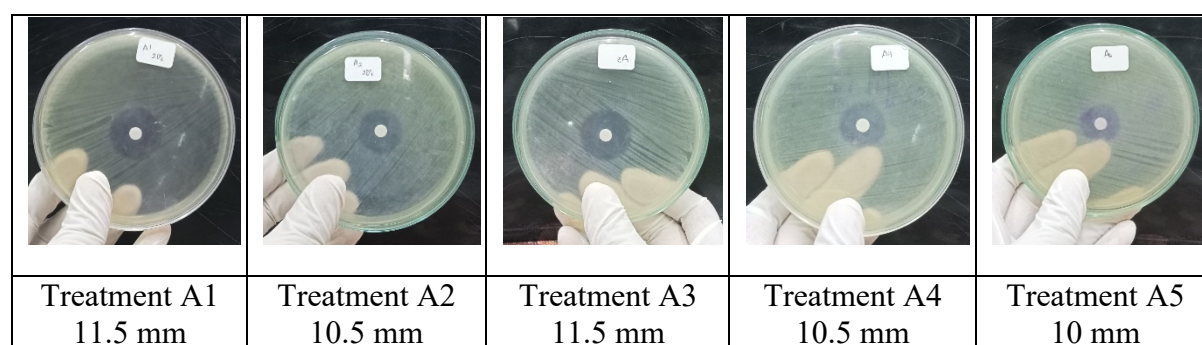
Table 3. ANOVA Test Results for The Growth of *A. salmonicida* Bacteria

ANOVA					
	Sum of Squares	Df	Mean Square	F	Sig
Between Groups	210,540	4	52,635	133,253	0,000
Within Groups	7,900	20	0.395		
TOTAL	218,440	24			

Based on the ANOVA test, F count > F table it is necessary to carry out another test, namely a multiple comparison test using the Duncan test.

Table 4. Differences in Notation of Duncan Test Results Regarding the Average Growth of *A. salmonicida* Bacteria

Treatment	N	Subset for alpha = 0.05				
		1	2	3	4	5
A (20%)	5	10,800				
B (40%)	5		12,800			
C (60%)	5			14,700		
D (80%)	5				16,700	
E (100%)	5					19,100
Sig.		1,000	1,000	1,000	1,000	1,000



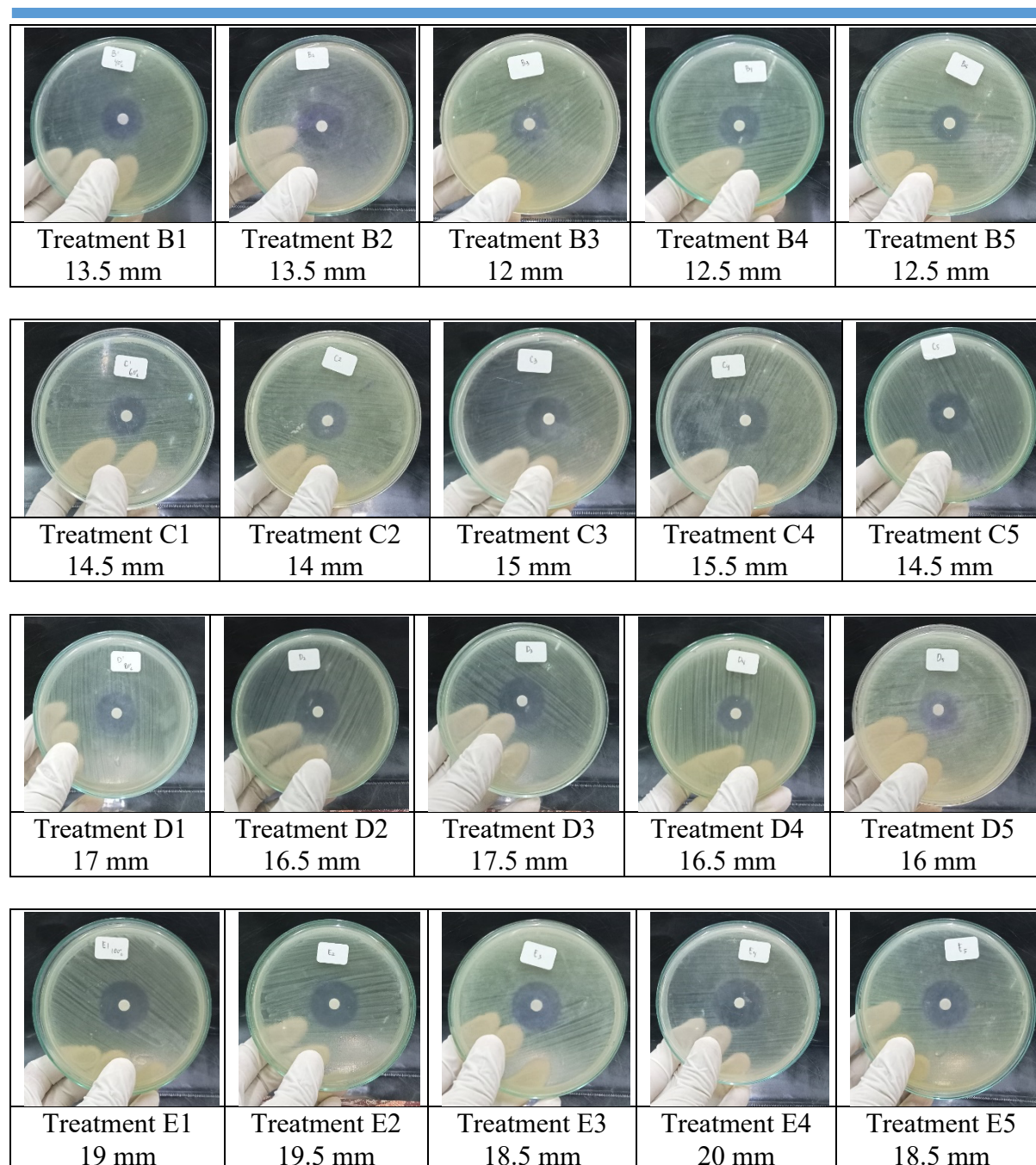


Figure 2. Clear Zone Results of the Antibacterial Effect of Lime Juice (*Citrus aurantifolia*) on the Growth of *A. salmonicida* Bacteria

DISCUSSION

The calculation results show that the calculated F value > the table F value is a significant value of 5%, so it is concluded that the *Citrus aurantifolia* treatment actually inhibits growth coming from the bacteria *A. salmonicida*.

Test really different the smallest (BNT) determines the treatment which produces different average responses. Based on the test results, different notations appeared for all treatments, but in one group no treatment was found. Treatment E has the largest zone of inhibition with an average value of 19.1 mm followed by treatment D 16.7 mm, treatment C 14.7 mm, treatment B 12.8 mm and treatment A 10.8 mm.

Treatment results noticed lime (*Citrus aurantifolia*) with paper disk contain *Aeromonas salmonicida* bacteria with different concentrations using the paper disk diffusion method.

Testing the lime inhibition zone on *Aeromonas salmonicida* bacteria shows that the higher the concentration, the higher the value obtained, the higher the clear zone, so the largest clear zone is a concentration of 100 with an average of 19.1 mm. Clear zone all around paper disc show vulnerability microorganism antimicrobial compound. Astutiningrum (2016), transparent zone around the paper disc showed that lime juice has antibacterial properties against the growth of *Aeromonas salmonicida*. The clear zone is an area where bacteria do not grow in the surrounding area. The ability of lime juice to inhibit *Aeromonas salmonicida* bacteria is due to the presence of active antibacterial compounds in lime juice.

Average diameter grouped according to categories resistance. Antimicrobial inhibition zone activity categories are divided into 4 categories: Inhibition zone for weak activity < 5 > 19–20 mm, and for very strong activity > 20–30 mm (Sartika *et al.*, 2013). Based on this category, research shows the activity of lime juice (*Citrus aurantifolia*) for each treatment. Treatment A 20% produces 10,800 mm, treatment B 40% produces 12,800 mm, treatment C 60% produces 14,700 mm, and treatment D 80% produces 16,700 mm and treatment E 100% produces 16,700 mm. Average zone of inhibition 19,100 mm. Results study response *Citrus aurantifolia* on the growth of *Aeromonas salmonicida* in treatments A, B, C, D and E (strong).

Yahya (2016) found that lime juice contains the antibacterial substances citric acid, flavonoids and limonene. The citric acid in lime juice lowers the pH of lime juice. The low pH of lime juice changes the pH of bacterial cells. When the pH of the bacterial cell changes, the release of amino acids from RNA is inhibited thereby inhibiting bacterial growth. According to Yunikawati (2013), flavonoids this could play a role as an agent antibacteria bother function of microorganisms and prevent growth. They have active processes that can damage cell wall permeability. Flavonoids inhibit bacterial growth by damaging cell walls, inactivating them Enzymes, binding to adhesins damage cell membranes. Yahya (2016), limonene in lime juice has antibacterial properties which can damage cell membranes. The talpen group in limonene interferes with the ability of the cytoplasmic membrane to function as a selectively permeable membrane, a barrier to substances entering and leaving the cell.

Based on further testing, the actual minimum difference in the table is that treatment A (20%) is different from treatment B (40%), C (60%), D (80%), and E (100%) it can be seen that they are different. Treatment B (40%) was significantly different from treatments A (20%), C (60%), D (80%), and E (100%). Treatment C (60%) was significantly different from treatments A (20%), B (40%), D (80%), and E (100%). Treatment D (80%) is different from treatment A (20%), B (40%), C (60%), and E (100%). Treatment E (100%) was significantly different from treatments A (20%), B (40%), C (60%), and D (80%).

The results showed that hypothesis H0 was not possible and hypothesis H1 was accepted, indicating that the lime juice activity test had a real effect in inhibiting the growth of *Aeromonas salmonicida*.

CONCLUSION

Results of research on the response of *Citrus aurantifolia* to areas inhibiting the growth of *Aeromonas salmonicida* bacteria from tilapia fish (*Oreochromis niloticus*).

1. Providing lime juice (*Citrus aurantifolia*) causes *Aeromonas salmonicida* to be inhibited.
2. 100% juice concentration (Treatment E) gave the best results in inhibiting the growth of *Aeromonas salmonicida* bacteria (19.10 mm, followed by Treatment D (80%), namely 16.70 mm, Treatment C (60%) 14.70 mm, Treatment B (40%) 12.80 mm, and treatment A (20%) 10.80 mm. Increasing *Citrus aurantifolia* resulted in greater growth of *Aeromonas salmonicida* bacteria.

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