Journal of Fish Health Vol. 2 (2) – December 2022
https://doi.org/10.29303/jfh.v2i2.1410

ANTIBACTERIAL EFFECTIVENESS OF BLACK QUICK (*Nigella sativa* Linn) EXTRACT ON BACTERIA INHIBITORY *Aeromonas hydrophila*, AND MORPHOLOGY FISH INDIGO (*Oreochromis niloticus*) POST INFECTION

Dwi Elfa Maharani [1*], Salnida Yuniarti Lumbessy [1], Fariq Azhar[1]
[1] Aquaculture Study Program, Faculty of Agriculture, University of Mataram, Education Street No. 37 Mataram, West Nusa Tenggara.

*Correspondence:
dwielfa71@gmail.com

Received : 2022-05-23
Accepted : 2022-12-29

Keywords :
Aeromonas hydrophila, Black cumin, Tilapia

ABSTRACT

The main problem in cultivation is a disease that causes less optimal growth and even death. This study aims to analyze the effectiveness of black cumin extract on the inhibition of *A. hydrophila bacteria, analyze* its active ingredients, analyze its toxicity level to Tilapia, and analyze the effect of the extract on the survival rate and morphology of Tilapia after being infected with *A. hydrophila bacteria*. The method used was a completely randomized design (CRD) which consisted of 3 stages. Inhibition zone with 5 treatments 2 repetitions, namely P0 (aquades), P1 (25%), P2 (50%), P3 (75%), and P4 (100%). LC50 with 4 treatments 2 replications using regression analysis with the help of the probit table. In Vivo with 6 treatments, 3 replicates, namely P0 (NaCl injection) as a control-, P1 (bacterial injection) as control+, P2 (250 ppm), P3 (500 ppm), P4 (750 ppm), P5 (1000 ppm). The results showed that black cumin seed extract with various concentrations affected the inhibition of *A. hydrophila*, SR, and Tilapia morphology after infection with bacteria. The test of inhibition with concentrations of 25%, 50%, 75%, and 100% can inhibit the growth of bacteria *A. hydrophila* because it contains antibacterial activity compounds. The extract has toxic properties to Tilapia (LC50=220.23 ppm). Soaking fish for 30 minutes in the I n Vivo test P3 treatment is the best treatment because it can provide a high SR of 73.3 %. However, at high concentrations, it can reduce fish survival.

INTRODUCTION

The fishery business is currently proliferating, especially in the field of aquaculture. In cultivation activities, one must be prepared to face various disease problems that can cause cultivation failure and economic losses. Diseases that attack organisms, including fish, occur through interaction with several factors, namely environmental factors, host conditions, and the presence of pathogenic organisms and microorganisms (Kurniawan, 2012).

Diseases in cultivated fish due to bacterial infections can be treated using chemical drugs on the market or antibiotics. However, using chemical drugs for a long time can cause chemical residues to form in the body of the fish and the aquaculture water, and it can disrupt the aquaculture environment. This is because chemical drugs have persistence properties, meaning that these materials are difficult to decompose naturally. Another negative impact of using antibiotics is the accumulation of these antibiotics in tissues, especially bones, so they can harm humans who consume them (Rosidah & Wila, 2012). Therefore, using natural ingredients as a substitute for chemical drugs in treating diseases in farmed fish is needed to minimize residue levels in the environment and farmed fish.

One of the natural ingredients that can be used for controlling disease in cultivated fish is black cumin (*Nigella sativa* Linn). These natural ingredients contain antimicrobial compounds such as tannins, flavonoids, alkaloids, saponins, essential oils, and phenols. Several previous studies have shown that fish treatment with natural ingredients has been carried out on catfish (*Pangasius* sp.) infected with *A. hydrophila bacteria* using black cumin flour (Dontriska *et al.,* 2014), tilapia (*O. niloticus*) infected with *Streptococcus bacteria agalactiae* using black cumin flour (Sa'adah *et al.,* 2015) and tilapia (*O. niloticus*) infected with *Streptococcus agalactiae bacteria* using black cumin seed extract (Gustiana, 2015). The results of this study indicate that the growth of bacteria decreases with the addition of natural extracts as an antibacterial which can be seen in the clear zone in the petri dish.

Based on the description above, research on the use of natural ingredients of black cumin extract on the inhibition of *Aeromonas hydrophila bacteria* in tilapia cultivation needs to be carried out to determine the effectiveness of black cumin extract.

METHODOLOGY

Time and place

This research was conducted for 35 days at the Immunobiology Laboratory for the extraction process, the Analytical Chemistry Laboratory, the Faculty of Mathematics and Natural Sciences for the GCMS process, the Agricultural Microbiology Laboratory for the inhibition zone test, and the Fish Production and Reproduction Laboratory, Aquaculture Study Program, Faculty of Agriculture, the University of Mataram for the LC50 test and the In Vivo.

Tools and materials

The tools used in this study were aeration, stationery, autoclave, sieve, blender, petri dish, DO meter, drigalski, Erlenmeyer, hot plate, loop needle, camera, container size 54x37x29 cm, Laminar Air Flow, spirit lamp, refrigerator, micropipette, tray, ruler, pH meter, tweezers, test tube rack, rotary evaporator, siphon hose, scoop, thermometer, scale, glass jar, and lid.
The materials used in this study were freshwater, distilled water, *A. hydrophila bacteria*, black cumin seeds, tilapia, cotton, and Whatman paper no. 42, *Nutrient Broth*, *Tryptic Soy Agar*, 0.9% NaCl, Hi-profit -2 feed, 96% ethanol solvent, and plastic wrap.

Research design

This study used a completely randomized design (CRD) which consisted of 3 stages: the inhibition zone test, LC50, and In Vivo test. In the straightforward zone test, the treatment used was 5 treatments, 2 repetitions with different concentrations of black cumin seed extract. The treatment used is as follows (Table 1):

Table 1. Extract Concentrations for In Vitro Tests

Treatment	Concentration	Sterile aquadest solution	Black cumin seed extract	Repetition
P1	0%	1000µL	0µL	2 times
P2	25%	750µL	250µL	2 times
P3	50%	500µL	500µL	2 times
P4	75%	250µL	750µL	2 times
P5	100%	0µL	1000µL	2 times

On the LC50 test This consisted of 4 treatments with 2 repetitions by soaking the fish for 24 hours to see the level of toxicity to tilapia using black cumin seed extract. The treatment used is as follows:

Table 2. Extract Concentration for LC50 Test

Treatment	Extract concentration	Repetition
P1	250 ppm	2 times
P2	500 ppm	2 times
P3	750 ppm	2 times
P4	1000 ppm	2 times

In the In Vivo test the treatment used was 6 treatments with 3 repetitions with different concentrations of black cumin seed extract. The treatment used is as follows:

P0 = 0 ppm (NaCl injection) as K-
P1 = 0 ppm (*A. hydrophila injection*) as K+
P2 = 250 ppm black cumin extract + injection *A . hydrophila*
P3 = 500 ppm black cumin extract + *A injection . hydrophila*
P4 = 750 ppm black cumin extract + injection *A . hydrophila*
P5 = 1000 ppm black cumin extract + injection *A . hydrophila*

Research procedure

Sample Preparation

1,250 g of black cumin seeds used are black, have a distinctive aroma, and are not crushed. The black cumin seeds are washed until clean, then air-dried without being exposed to direct sunlight to avoid damage to the active ingredients. Then the black cumin seeds were crushed using *a blender* to become a fine powder and sieved to obtain Simplicia (Linianti *et al* ., 2017).

Extract preparation and GCMS test

The refined simplicity was then weighed, then macerated using 96% ethanol until complete and allowed to stand for 1 x 24 hours and repeated 3 times. The results of the macerate were combined, and the solvent was removed using *a rotary evaporator* with a temperature of 41°C. The yield of the extract that has been obtained is then calculated, and its compound content is tested using the GCMS method. Yield can be calculated using the following formula (Nurhastuti, 2018):

$$\% \text{ Yield} = \frac{\text{Extract weight obtained}}{Extracted\ weight\ of\ simplicia\ powder} x\ 100\%$$

***Aeromonas hydrophila* Bacterial Suspension**

Bacterial isolate *A. hydrophila* was obtained from the Jepara Brackish Water Aquaculture Center. The slanted agar isolate was taken in 1 loop, which had been fixed until it glowed and cooled, transferred to TSA media, then incubated for 24 hours. After that, yellowish-white colonies were seen, then 1 ose was retaken and transferred to a test tube containing 10 ml of sterile NB solution. After being homogeneous and allowed to stand for 18-24 hours, serial dilutions were carried out until the sixth dilution to obtain a dose of CFU/ml (Prasetio *et al*., 2017).

Testing of Black Cumin Seed Extract on *Aeromonas Bacteria hydrophila* Using the Disc Diffusion Method (Clear Zone)

The bacterial solution from the sixth dilution was taken as much as 0.5 ml (500 µL) using a micropipette and then poured into the TSA agar medium and leveled using a drigalski slowly so that the media was not prying and allowed to stand until the bacteria seep into the agar media. Then prepared, empty sterile paper discs with a diameter of 6 mm and given a solution of black cumin extract as much as 1 ml (1000 µL) with a concentration of 0% (negative control), 25%, 50%, 75%, and 100% respectively for 15- 20 minutes for it to sink in, then the discs were taken using tweezers, let stand until slightly dry and placed on the surface of a TSA media petri dish that had been treated with bacteria *A. hydrophila* and slightly pressed. The petri dish is then closed and glued with plastic wrap to prevent contamination. Then the petri dish was incubated for 24 hours at room temperature. The diameter of the clear zone produced in this test is then measured using a ruler.

Testing the Toxicity Level of Black Cumin Seed Extract on Tilapia Using the LC50 Method

Before the LC50 test, the fish were acclimatized for 3 days in a fiber tub with a volume of 350 L and fed with complete pellets. The size of the fish used is 8-10 cm. Then it was put into a treatment container in the form of a 30 L container with a stocking density of 10 heads/container containing 10 L of fresh water. Containers filled with black cumin seed extract according to treatment. The treatment in the LC50 test was carried out with 4 treatments and 2 repetitions with concentrations of 250 ppm, 500 ppm, 750 ppm, and 1000 ppm. Fish that died after 24 hours were recorded as data to process the LC50 value (Gustiana, 2015).

In Vivo Test

Preparation of Maintenance Media

Receptacle maintenance that is used, 18 pieces of 54x37x29 cm container. Before use, the container must be washed clean. Washing of these containers aims to remove unwanted organisms. The maintenance container is equipped with aeration, which will later function as an oxygen supply aid. Containers are placed randomly using a lot system (lottery) and marked. Next, the container is filled with 20 liters of fresh water, and the aeration *setting is carried out.*

Test Fish Infection

Test fish infected with bacteria *A. hydrophila* as much as 0.1 ml /head with a density of CFU/ml (Taviana *et al* ., 2015). This injection was carried out *intramuscularly* (Zaman *et al.,* 2014 Maisyaroh *et al.,* 2018). For 24 hours after the injection of the bacteria, the test fish

were not given feed, and no water changes were made (Sa'adah *et al.,* 2015). After clinical symptoms appear, treatment is carried out using black cumin seed extract (250, 500, 750, and 1000 ppm) in a container containing 10 liters of water and soaked for 30 minutes without *refreshing* (Gustiana, 2015). After soaking, the fish are transferred to the rearing container previously according to treatment using new water.
Furthermore, observations were made on the morphology and behavior of fish every day for 14 days. During maintenance, feeding is carried out with a frequency of giving as much as 3 times a day (morning, afternoon, and evening) as completely as possible and siphoning every day. Weighing of feed is done every time the fish are given feed.

Parameter measurement

The parameters tested in the study were yield, GCMS, inhibition zone, LC50, morphology, the behavior of fish, SR, and water quality. Furthermore, data such as inhibition zone, LC50, and SR were obtained from the measurements recorded using *Microsoft E x c el*. The data that has been input is then displayed in the form of a bar chart. At the same time, the LC50 calculation uses regression analysis in *Microsoft Excel*. Water quality included temperature using a thermometer, pH using a pH meter, and dissolved oxygen (DO) using a DO meter at the beginning and end of the study.

Data analysis

Survival rate data and inhibition zones were analyzed statistically using variance (ANOVA) with the *SPSS Statistics 25 software program*. If it is significantly different, Duncan's test is performed (P< 0.05) at the 95% confidence level. Data on the LC50 test were analyzed using the probit log concentration assisted by the probit table and then analyzed using regression analysis with the help of *Microsoft Excel*. The data were analyzed descriptively, such as water quality, extract yield results, GCMS test, morphology, and behavior of fish.

RESULT

Yield of Black Cumin Seed Extract

Table 3 . Yield of Black Cumin Seed Extract

Powder weight (g)	Extract weight (g)	yield	Characteristics		
			Form	Color	Smell
1230	220	17.8%	Liquid	Dark brown	Typical

GCMS Results of Black Cumin Seed Extract

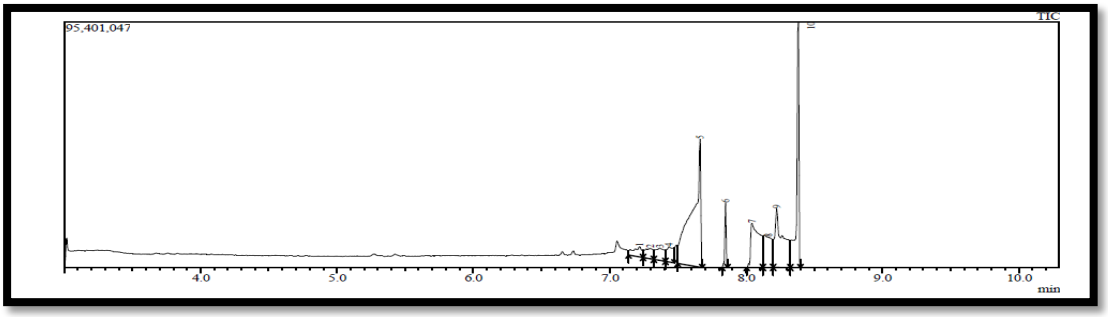

Figure 1. GCMS Analysis Results of Black Cumin Seed Extract

The results of the GCMS analysis of the ethanol extract of black cumin seeds showed that there were 10 peaks detected, but there were 6 compounds that could be identified. The compounds identified in the black cumin seed ethanol extract sample can be seen in Table 4.

Table 4 . Compounds in Black Cumin Seed Extract GCMS Test Results

peak to-	Compound name	Other name	Compound class	Chemical formula	Retention time	% Area
1	3,7-dimethyl-1,6-Octadien-3-ol acetate	Bergamiol, Linalool, Linalyl acetate	Essential oil	$C_{12}H_{20}O_2$	7,220	2.60
2	3-Hydroxy-1-Octene	Amyl Vinyl Carbinol	Essential oil	$C_8H_{16}O$	7,297	2.66
3	7-Methyl-3-Methylene-1,6-Octadiene	Beta-Myrcene	Essential oil	$C_{10}H_{16}$	7,367	3.43
4	Decane (CAS) n-Decane	Isodecane	Aldehyde	$C_{10}H_{22}$	7,435	3.34
6	1,2,3,4-Butanetetrol	tannins	Polyphenols	$C_4H_{10}O_4$	7,850	2.57
7	1,2,3-Propanetriol (CAS) Glycerol	Glycerin	Alcohol	$C_3H_8O_3$	8004	12.72

Inhibition Zone Diameter

Based on the results of the *Analysis of Variance* (ANOVA), it showed that the treatment of black cumin seed extract had an effect on the inhibition of the growth of *Aeromonas hydrophila bacteria* ($p < 0.05$) with a 95% confidence level .

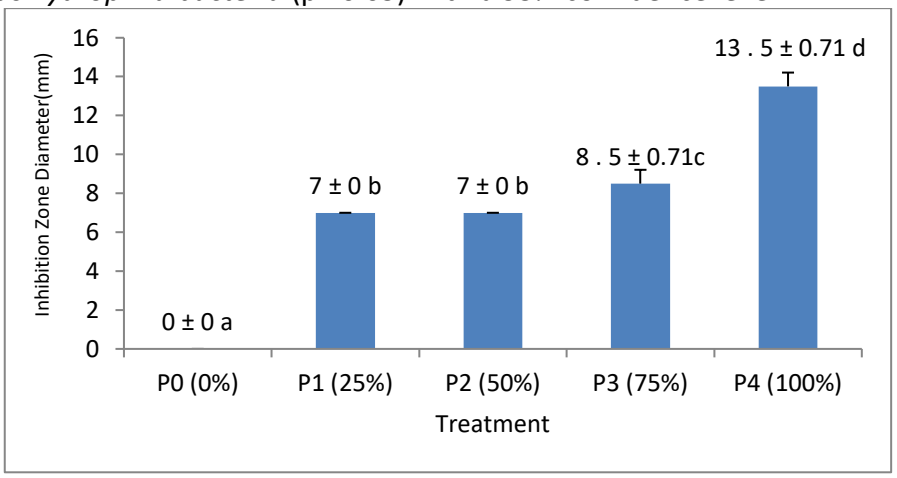

Figure 2. Results of the Diameter of Inhibitory Power of Black Cumin Extract against *Aeromonas hydrophila Bacteria*

Duncan's further test results showed that the P0 treatment was significantly different from all treatments. The P1 and P2 treatments were not significantly different but significantly different from the other treatments. The P3 treatment was significantly different from the other treatments and the P4 treatment was significantly different from the other treatments (Figure 2).

LC50 test

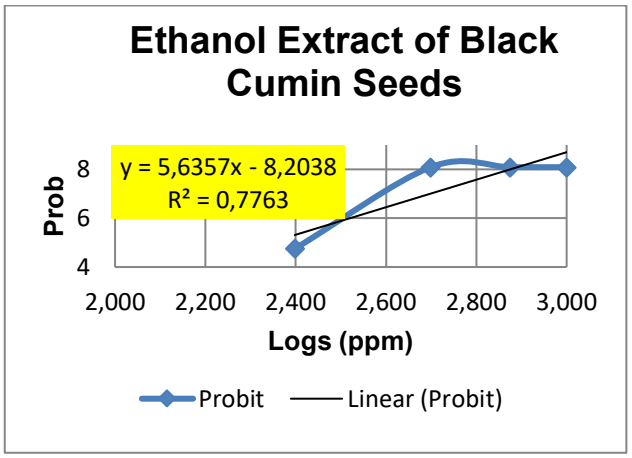

Figure 3. Graph of LC50 Analysis of Black Cumin Seed Extract on Tilapia

Each of the percent deaths in each treatment was searched for the probit value using the probit table and analyzed using the linear regression equation y=ax+b where y= probit number, x= log extract concentration, a= slope value and b= intercept value in *Microsoft Excel* so that concentrations that cause 50% mortality in tilapia are found. The results of the regression equation obtained after analysis are y=5.6357x+(-8.2038) with R^2=0.776 (Fig. 3). After calculating by entering a y value of 5 into the regression equation, an x value of 2.3428 is obtained. The LC50 value is the antilog of x, so the value is 220.23 ppm.

Morphology and Post-Infection and Submersion Behavior of Fish

Table 5. Morphology of Tilapia Post Infection and Soaking

	P0 (K-)			P1 (K+)			P2 (250 ppm)			P3 (500 ppm)			P4 (500 ppm)			P5 (1000 ppm)		
Hari ke-	Ulangan																	
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
0	*injeksi bakteri dan larutan NaCl sesuai unit perlakuan*																	
1	(++)	(++)	(++)	(+++)	(+++)	(+++)	(+++)	(+++)	(+++)	(+++)	(+++)	(+++)	(+++)	(+++)	(+++)	(+++)	(+++)	(+++)
	perendaman ekstrak jintan hitam sesuai unit perlakuan (30 menit)																	
2	(++)	(++)	(++)	(+++)	(+++)	(+++)	(+++)	(+++)	(+++)	(+++)	(+++)	(+++)	(+++)	(+++)	(+++)	(+++)	(+++)	(+++)
3	(++)	(++)	(++)	(+++)	(+++)	(+++)	(+++)	(+++)	(+++)	(+++)	(+++)	(+++)	(+++)	(+++)	(+++)	(+++)	(+++)	(+++)
4	(++)	(++)	(++)	(+++)	(+++)	(+++)	(+++)	(+++)	(+++)	(+++)	(+++)	(+++)	(+++)	(+++)	(+++)	(+++)	(+++)	(+++)
5	(++)	(++)	(++)	(+++)	(+++)	(+++)	(+++)	(+++)	(+++)	(+++)	(+++)	(+++)	(+++)	(++)	(++)	(++)	(++)	(+++)
6	(++)	(+)	(++)	(+++)	(+++)	(+++)	(+++)	(+++)	(+++)	(+++)	(+++)	(+++)	(++)	(++)	(++)	(++)	(++)	(++)
7	(+)	(+)	(+)	(+++)	(+++)	(+++)	(+++)	(+++)	(+++)	(+++)	(+++)	(++)	(++)	(++)	(++)	(++)	(++)	(++)
8	(+)	(+)	(+)	(+++)	(+++)	(+++)	(+++)	(+++)	(+++)	(++)	(++)	(++)	(++)	(++)	(+)	(+)	(++)	(+)
9	(+)	(+)	(+)	(+++)	(+++)	(+++)	(+++)	(++)	(++)	(++)	(++)	(++)	(+)	(+)	(+)	(+)	(+)	(+)
10	(+)	(+)	(-)	(+++)	(+++)	(+++)	(++)	(++)	(++)	(++)	(+)	(+)	(+)	(+)	(+)	(-)	(+)	(+)
11	(-)	(+)	(-)	(+++)	(+++)	(+++)	(++)	(++)	(++)	(+)	(+)	(+)	(-)	(+)	(-)	(-)	(-)	(-)
12	(-)	(-)	(-)	(++)	(+++)	(++)	(++)	(+)	(+)	(+)	(+)	(+)	(-)	(-)	(-)	(-)	(-)	(-)
13	(-)	(-)	(-)	(++)	(++)	(++)	(+)	(+)	(+)	(+)	(+)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
14	(-)	(-)	(-)	(+)	(++)	(++)	(+)	(+)	f(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
15	(-)	(-)	(-)	(+)	(+)	(+)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)

Description: (+++) = enlarged wound, *opacity* , *purulent* , flaky fins, *hemorrhagic* i,and*exophthalmia*
(++) = smaller wound (partially healed)
(+) = closed wound
(-) = no wound, looks normal

Table 6. Post-infection and Soaking Behavior of Tilapia

Hari ke-	P0 (K-)			P1 (K+)			P2 (250 ppm)			P3 (500 ppm)			P4 (500 ppm)			P5 (1000 ppm)		
	Ulangan																	
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
0	*Injeksi bakteri dan larutan NaCl sesuai unit perlakuan*																	
1	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
	Perendaman ekstrak jintan hitam sesuai unit perlakuan (30 menit)																	
2	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
3	(-)	(+)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
4	(+)	(+)	(+)	(-)	(-)	(-)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)
5	(+)	(+)	(+)	(-)	(-)	(-)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)
6	(++)	(++)	(+)	(-)	(+)	(-)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)
7	(++)	(++)	(++)	(+)	(+)	(-)	(+)	(+)	(+)	(+)	(+)	(++)	(+)	(+)	(+)	(+)	(+)	(+)
8	(++)	(++)	(++)	(+)	(+)	(+)	(+)	(+)	(+)	(++)	(++)	(++)	(+)	(+)	(+)	(+)	(+)	(+)
9	(++)	(++)	(++)	(+)	(+)	(+)	(+)	(+)	(++)	(++)	(++)	(++)	(+)	(+)	(+)	(+)	(+)	(+)
10	(++)	(++)	(++)	(+)	(+)	(+)	(++)	(++)	(++)	(++)	(++)	(++)	(+)	(+)	(+)	(+)	(+)	(+)
11	(++)	(++)	(++)	(+)	(+)	(+)	(++)	(++)	(++)	(++)	(++)	(++)	(+)	(++)	(++)	(+)	(+)	(+)
12	(++)	(++)	(++)	(+)	(+)	(+)	(++)	(++)	(++)	(++)	(++)	(++)	(++)	(++)	(++)	(+)	(+)	(+)
13	(++)	(++)	(++)	(+)	(++)	(+)	(++)	(++)	(++)	(++)	(++)	(++)	(++)	(++)	(++)	(+)	(++)	(++)
14	(++)	(++)	(++)	(++)	(++)	(++)	(++)	(++)	(++)	(++)	(++)	(++)	(++)	(++)	(++)	(++)	(++)	(++)
15	(++)	(++)	(++)	(++)	(++)	(++)	(++)	(++)	(++)	(++)	(++)	(++)	(++)	(++)	(++)	(++)	(++)	(++)

Description: (++) = response to normal feed and active swimming fish
(+) = slow response to feed, fish swim normally but not very active
(-) = response to feed is absent and fish swim abnormally on the bottom

Life sustainability

Based on the results of the *Analysis of Variance* (ANOVA), it showed that the black cumin seed extract treatment affected the survival rate of tilapia ($p < 0.05$) with a 95% confidence level .

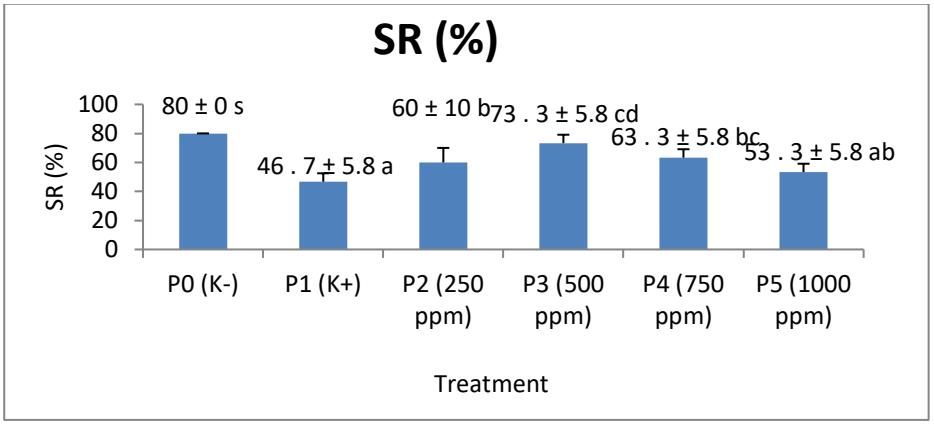

Figure 4. Graph of Tilapia Survival

Duncan's further test results showed that treatment P0 was not significantly different from P3 but significantly different from other treatments. Treatment P1 was not significantly different from treatment P5 but significantly different from treatment P0, P2, P3 and P4. Treatment of P2 was not significantly different from P4 and P5 but significantly different from P0, P1 and P3. Treatment of P3 was not significantly different from P0 and P4 but significantly different from P1, P2 and P5. Treatment of P4 was not significantly different from P2, P3, and P5 but significantly different from P0 and P1. The P5 treatment was not significantly different from the P1, P2 and P4 treatments but significantly different from the P0 and P3 treatments.

Maintenance Water Quality

Table 7. Data on the range of water quality during the study

Parameter	Treatment						
	P0	P1	P2	P3	P4	P5	References

DO	4.7 - 5.0	4.7 - 4.9	4.6 - 4.9	4.4 - 4.8	4.6- 5.0	4.7 - 4.9	>3 mg/L (Arifin, 2016)
pH	7.1 - 7.3	7.3 - 7.5	7.1 - 7.4	7.1 - 7.3	7.1 - 7.4	7.2 - 7.5	6-8 (Arifin, 2016)
Temperature	27.1 - 27.6	27.3 - 27.8	27.2 - 27.7	27.3 - 27.5	27.1 - 27.5	27.5 - 27.8	25-30°C (Aliyas *et al* ., 2016)

DISCUSSION

The evaporation results were in the form of a blackish-brown liquid extract with an extract yield of 17.8 % (Table 3). Yield is the ratio of the weight of the extract produced to the weight of the simplicia as a raw material (Cahyadi *et al*., 2018). The yield results of this study were higher when compared to research by Linianti *et al*. (2017), who obtained a yield of black cumin seed extract with the same solvent of 6.62% in the form of a thick extract. According to Wijaya *et al.* (2018), the higher the yield, the more compounds are contained therein. The factors that affect the yield results are the extraction method used, sample particle size, storage conditions and time, length of extraction time, type of solvent, and comparison of sample weight to volume of solvent used (Salamah, 2015 *in* Wijaya *et al.,* 2018).

GCMS results in data can be seen in Table 4. Based on further analysis, all detected compounds contained antibacterial compounds. The details of these compounds are as follows:

1. Peak with a retention time of 7,220

According to Indriani *et al*. (2014), linalool can inhibit microbial growth by denaturing proteins which results in bacterial cells being unable to metabolize generally so that they can inhibit growth and can result in the death of these bacterial cells.

1. Peak with a retention time of 7,297

According to Yee *et al* . (2021), this compound has anticancer and antioxidant activity.

1. Peak with a retention time of 7,367

β-myrcene belongs to the essential oil class and is reported to have biological activities, including analgesic, sedative, antidiabetic, antioxidant, anti-inflammatory, antibacterial, and anticancer effects (Surendran *et al* ., 2021)

1. Peak with a retention time of 7,435

Isodecane is an active compound that acts as an antimicrobial (Kawuri & Darmayasa, 2019)

1. Peak with a retention time of 7,850

Tannins are one of the active compounds of secondary metabolites from plants and belong to the group of polyphenols which have benefits such as *astringents*, antioxidants, and antibacterials (Marlin *et al* ., 2015 *in* Barodah *et al* ., 2017).

1. Peak with a retention time of 8,004

The glycerin compound acts as an antibacterial, killing disease-causing bacteria so that fish become healthy and maximize nutrient absorption (Pratiwi *et al* ., 2021).

The presence of inhibitory activity was indicated by the appearance of an inhibition zone around the disc paper which had previously been given black cumin seed extract. The highest inhibition was found at a concentration of 100% extract, 13.5 mm, and the lowest was found at a concentration of 0%, which was 0 mm. Inhibitory power at 0% extract concentration (aquades) was not formed, indicating the absence of bacterial inhibition activity. According to

Azis (2019), distilled water does not have antibacterial compounds. Based on the level of inhibition, the black cumin seed extract concentration of 0% did not provide inhibition. In comparison, the extract concentrations of 25%, 50%, and 75% provided medium inhibition, and the extract concentration of 100% provided potent inhibition. According to Susanto *et al* . (2012) *in* Surjowardojo *et al* . (2015) that if the diameter of the inhibition zone is ≤5 mm, then the inhibition activity is categorized as weak, an inhibition zone diameter of 6-10 mm is categorized as moderate, an inhibition zone diameter of 11-20 mm is categorized as vital, and an inhibition zone diameter of >20 mm is categorized as very strong. Thus black cumin seed extract can inhibit bacteria.

Toxicity testing aims to determine the toxic effect of black cumin seed extract on tilapia. Based on the observation of the LC50 test for 24 hours, it was found that the LC50 value was 220.23 ppm, and black cumin seed extract could kill all tilapia at concentrations ≥500 ppm. According to Gustiana (2015), it is suspected that there is a *nigellin compound* in black cumin, which causes a bitter taste and interferes with breathing and the physiological processes of fish, causing death in fish. The category of material toxicity based on the LC50 value is divided into three categories, namely very toxic (<30 ppm), toxic (30-1000 ppm), and non-toxic (>1000 ppm) (Martiningsih, 2013). Based on these categories, black cumin seed extract is included in the toxic category for tilapia. According to Bosman *et al* . (2013), the susceptibility of organisms to poisons varies based on the concentration of toxic materials, size, and type of toxic contaminated organisms.

Observation of fish morphology after bacterial infection and soaking of the black cumin seed extract can be seen in Table 5. At 24 hours after the injection of bacteria, most showed different clinical symptoms in each fish. However, most treatments showed clinical symptoms such as *hemorrhagic* and scaly fins. . Exotoxin enzymes from Aeromonas hydrophila bacteria, such as chitinase, lecithinase, protease, and hemolysin, cause the clinical symptoms. These enzymes synergize with body parts that contain lots of protein, causing damage to the infected body surface (Husna *et al* ., 2016). The P3 treatment is shown in Table 5. on the 6th day after treatment, the wound began to shrink. The clinical symptoms of the fish that survived began to lead to healing (partial recovery) gradually. The feed response gradually increased compared to the P2 treatment, which gradually improved longer on the 8th day (Table 6). It is suspected that the absorption of the compound by fish at the correct dose and duration of administration can accelerate wound healing in fish. According to Sa'adah *et al* . (2015), the adverse effects of natural ingredients may be related to toxic elements, high doses, and allergies. However, they do not cause health problems when used in the right concentration.

The decrease in feed response after 24 hours and the administration of the extract was due to the fish's digestive system disruption due to bacteria and black cumin seed extract. According to Ristianti *et al* . (2015), the weak response of fish to feed is caused by digestive disorders due to infection with the *A. hydrophila bacteria,* which attacks the part of the brain (hypothalamus) which plays a role in regulating hunger and digestion of fish, so that fish are slow to digest feed.

The highest survival rates were found in treatments P0 and P3, which were 80% and 73.3%, respectively. Meanwhile, the lowest survival rate was found in treatment P1, which was 46.7%. This is presumably due to active compounds in black cumin seed extract as anti-inflammatory, antimicrobial, antioxidant, and immunomodulator. According to Sultana *et al* . (2015), black cumin has medical benefits such as antitumor, antibacterial, antifungal, antioxidant, anti-inflammatory, antihistamine, analgesic, antidiabetic, and as immunomodulator. Survival decreased in the P4 and P5 treatments, and this was because the

compound content in the black cumin seeds exceeded the dose, so it was not suitable for fish because it was toxic. According to Marlinda *et al* . (2012) *in* Alif *et al* . (2021) that active compounds in plants at high concentrations are always toxic.

The range of water quality in this study is still feasible for the life of tilapia (Table 7.). DO results during maintenance ranged from 4.4 to 5.3 mg/L. According to Arifin (2016), tilapia generally can live in water with a dissolved oxygen content of 3-> 5 mg/L. The pH value ranges from 7.1-7.7. According to Arifin (2016), the ideal pH for tilapia cultivation ranges from 6-8. Temperature values range from 27.1-28.5°C. According to Aliyas *et al* . (2016), the optimal temperature for tilapia growth is 25-30°C. According to Dontriska *et al* . (2014), fish-rearing media must be adequately maintained so as not to trigger stress in fish so that the fish are susceptible to disease.

CONCLUSION

Based on the research that has been done, it is concluded that:

1. Black cumin seed extract (*Nigella sativa* Linn) contains compounds with antibacterial activity, such as glycerin, essential oils, tannins, and dodecane, and with concentrations of 25%, 50%, 75%, and 100% can inhibit the growth of *Aeromonas hydrophila bacteria*.
2. The extract has toxic properties to tilapia and has an LC50 value of 220.23 ppm.
3. Morphological changes in tilapia gradually improved (partial recovery) faster, namely in treatment P4 and P5 on day 4, but could reduce fish survival. The highest survival in the administration of black cumin seed extract was in the P3 treatment, which was 73.3%. This is due to active compounds in black cumin seed extract as anti-inflammatory, antimicrobial, antioxidant, and immunomodulator.

ACKNOWLEDGMENT

In this opportunity, the author would like to thank colleagues who facilitated and assisted in carrying out this research.

REFERENCES

Aliyas, SN, and Ya'la, ZR (2016). Growth and survival of tilapia (*Oreochromis* sp.) reared in saline media. *Journal of Science and Technology Tadulako, 5* (1)*,* 19-2 7*.*

Arifin, MY (2016). Growth and Survival Rate of Tilapia (*Oreochromis* sp.) Red and Black Strains Raised in Salinity Media. *Batanghari University Scientific Journal, Jambi, 16* (1), 159-166.

Cahyadi, J., Satriani, GI, Gusman, E., Encik, W., and Sabri. (2018). Phytochemical Screening of Mangrove (*Sonneratia alba*) Fruit Extract as Bioenrichment Natural Feed for *Artemia salina*. Borneo Saintek Journal, *1* (3), 33-39.

Dontriska., Ade, DS, and Yulisman. (2014) . Effectiveness of Black Cumin Flour (*Nigella sativa*) to Prevent *Aeromonas hydrophila Infection* in Catfish. *Journal of* Indonesian Swamp Aquaculture, *2* (2): 188-201.

Gustiana. (2015). *Evaluation of Black Cumin Seed Extract Nigella sativa* Linn. *Against Streptococcus agalactiae Bacterial Infection* in *Tilapia* (*Oreochromis niloticus* Linn). [Thesis]. Makassar: Hasanuddin University Postgraduate Program.

Indriani, AD, Prayitno, SB, and Sarjito. (2014). Use of Red Ginger Extract (*Zingiber officinale* var. Rubrum) As an Alternative Treatment for Tilapia (*Oreochromis niloticus*) Infected with Aeromonas hydrophila Bacteria. *Journal of Aquaculture Management and Technology*, *3* (3), 58-65.

Kurniawan, A. (2012). Aquatic Disease. Pangkal Pinang: UB Press. https://www.researchgate.net/publication/305780756_Penyakit_Akuatik

Linianti., Nur, I., Maulidiyah., and Yusnaini. (2017). Potential of Black Cumin (*Nigella sativa*) Seed Ethanol Extract for Controlling *Vibrio harveyi Bacteria* Causes Disease in Vaname Shrimp (*Litopenaeus vannamei*). *Journal of Fisheries Science and Innovation*, *1* (1):25-29. http://ojs.uho.ac.id/index.php/JSIPi

Maisyaroh, LA, Titik, S., Alfabetian, HCH, Fajar, B., and Tristiana, Y. (2018). Use of Mangosteen Peel Extract (*Garcinia mangostana*) as Antibacterial to Treat *Aeromonas hydrophila Infection* in Tilapia (*Oreochromis niloticus*). *Journal of Tropical Aquaculture Science, 2* (2): 36-43.

Nurhastuti, L. (2018). *Antibacterial Activity Test of N -Hexane Fraction, Ethyl Acetate Fraction, and Water Fraction from 96% Ethanol Extract of Black Cumin Seeds* (*Nigella sativa* L.) *against Pseudomonas aeruginosa ATCC 27853.* [Thesis]. Surakarta: Setia Budi University.

Octaviana, HN, Sasanti, AD, and Mirna, F. (2015). Prevention of *Aeromonas hydrophila Infection* in Sangkuriang Catfish Using Mahkota Dewa Flour in Feed. *Journal of Indonesian Swamp Aquaculture, 3* (2): 14-24.

Prasetio, E., Fakhrudin, M., and Hasan, H. (2017). The Effect of Aloe Vera Powder (*Aloe vera*) on Hematology of Jelawat Fish (*Leptobarbus hoevenii) Challenged by Aeromonas hydrophila* Bacteria. *Ruaya Journal*, *5* (2), 44-54.

Pratiwi, N., Lumbessy, SY, Azhar, F. (2021). The Effect of Giving Chinese Petai Leaf Extract (Leucaena leucocephala) on the Performance of Vaname Shrimp (Litopenaeus vannamei). *Journal of Fisheries Science and Innovation*, *5* (2), 72-84.

Rosidah and Wila, MA (2012). Potency of Guava Leaf Extract as an Antibacterial to Overcome *Aeromonas hydrophila Bacterial Attack* on Gurame Fish (*Osphronemus gouramy* Lacepede). *Journal of Aquatics*, *3* (1):19-27.

Sa'adah, R., Susanti, AD, and Taqwa, FH (2015). Application of Black Cumin Flour (*Nigella sativa*) for Prevention of *Streptococcus agalactiae Bacterial Infection* in Tilapia (*Oreochromis niloticus*). *Journal of Indonesian Swamp Aquaculture*, *3* (1): 58-69.

Surendran, S., Qassadi, F., Surendran, G., Lilley, D., and Heinrich, M. (2021). What Are the Potential Health Benefits of This Flavoring and Aroma Agent. *Frontiers in Nutrition*, *8* :699666, https://doi.org/10.3389/fnut.2021.699666

Wijaya, H., Novitasari., and Jubaidah, S. (2018). Comparison of Extraction Methods to the Yield of Sea Rambai Leaf Extract *(Sonneratia caseolaris* L. Engl*)*. Manuntung scientific journal, *4* (1), 79-83.

Yee, KS, Wetwitayaklung, P., Narakornwit, W. (2021). Variation in Chemical Constituents of Essential Oils of the Fresh, Dried and Fermented Leaves of *Premna serratifolia*. *Pharmaceutical Sciences Asia*, *48* (5), 481-490.