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The effectivity of Garlic (*Allium sativum* L.) and Turmeric (*Curcuma longa* L.) in Tilapia (*Oreochromis niloticus*) infected by *Edwardsiella tarda*

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ABSTRACT

The aim of this study is to determine the effectivity of *Garlic* (*Allium sativum* L.) dan Turmeric (*Curcuma longa* L.) in Tilapia (*Oreochromis niloticus*) infected by *Edwardsiella tarda*. We used sensitivity test with disc diffusion Kirby-Bauer method. The 24 hours purified *E. tarda* isolate, cultivated in Mueller Hinton Agar (MHA) by swabbing it using a sterile swab on the MHA surface, next put paper disc contain variants garlic concentrations and turmeric. Incubated MHA on the room temperature (30 ^{o}C) for at least 24 hours, inhibition zone diameter is measured to see the effectivity. Serial Tube Dilution method is used to determine the Minimum Inhibitory Concentration (MIC) value for garlic and turmeric. Used as medication base on the second stage were 1 × MIC2 × MIC4 × MIC and 8 × MIC. Statistical analysis used completely randomized design, with 6 treatment and 4 repetition which density 10 ind/aquarium each. The result showed that garlic and turmeric inhibited *E. tarda* effectively, the highest survival rate was 1 × MIC at 8000 ppm for garlic and 1 × MIC at 20 ppm for turmeric. However, MIC level cannot be used as reference to determine the dose of herbal treatment, we can determine the dose by using disc volume in resistance test.

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1. Introduction

The Ministry of Marine Affairs and Fisheries has a vision to be the biggest fishery producer in 2015, so there must be an improvement in fish production especially in fish and shrimp culture. The Minister of Marine Affairs and Fisheries had been prepared nine main products, those are seaweed, catfish, tilapia, milk fish, grouper fish, shrimp, pearl and ornamental fish. Tilapia as one of the nine main products this time is being the favourite especially in South Sumatera freshwater culture. In

reaching the target there will be such a big donation needed from cultivation sector including freshwater, brackish water and seawater.

One of the target's obstacle is the appearance of fish diseases caused by the environment decrease. The diseases appeared because of the imbalanced interaction between environment, host and pathogen. The diseases can be caused by virus, bacteria, paracites and another factors from the environment, nutrients deficiency and genetics abnormality. According to KEPMEN KP RI, 2013, fish disease caused by *Edwardsiella tarda*, which one of the host is carried by tilapia, as listed in decision letter of Minister of Marine Affairs and Fisheries number: 03/MEN/2013 about Determination of Quarantine Fish Pests and Diseases, group, the carrier and its spread. *E. tarda* listed as Group II which means pests and diseases that can be treated and cured

According to Lima et al. (2008), bacteria E. tarda showed clinical symptoms in fish infections such as hemorrhage or bleeding on the body, gills, stomach, gills, and tail, as well as the appearance of ulcers due to infection. E. tarda bacteria also cause clinical symptoms in the internal organs of fish characterized by abscesses or swelling in the intestines and gas in the internal organs, as well as pale liver and kidneys (Muhanty & Sahoo, 2007).

Many efforts had been done to resolve the diseases in fish farming system, chemical application can be an environmental pollution concern for fish farmers. Using chemical and antibiotics with not appropriate dose for fish medication can cause resistance pathogen aside from that cause chemical residue accumulation in fish meat. At the moment fish farmers have been trying environtmental friendly and lowcost medication using herbal as natural antibiotics. Some researchs showed that garlic is a safely natural antibiotics for human, and has antioxiandt content. Main compound of medicine from garlic is allicin, powerful as antibiotic and anti-fungal. Turmeric is a yearly medicine bush plant (perennial) that spread all over tropics area. This plant cultured in South Asia specially in India, South China, Taiwan, Indonesia (Java) and Philipines. Main benefit turmeris is as traditional medicine, raw materials for the herbal and cosmetic industry, cooking ingredients, farms and so on. The turmeric rhizome also useful as anti-inflammation, anti-oxiandt, anti-microbe, cancer prevention, lowering fat blood level and as blood purifier (KKP, 2013). As the matter of affect, there was needed to determine the effectivity of garlic (*Allium sativum* L.) and turmeric (*Curcuma longa* L) to tilapia (*Oreochromis niloticus*) infected by *E. tarda*.

2. Material and methods

The study was held in cultivation pond at Air Batu Village, Banyuasin District, South Sumatera Province. The study was held at Laboratory of Bacteriology, Sultan Mahmud Badaruddin II Palembang Fish Quarantine. The equipment for the study is fiber container volume 8000 liter, aquarium 20 cm x 30 cm x 40 cm, blower, autoclave, oven, glass ware, ose needle, dissecting set, analitical balance, laminary air flow, incubator, vortex mixer, hand counter, shaker waterbath, microscope, and water quality checker, haemocytometer. Isolate of E. tarda from Testing Laboratory of Sultan Mahmud Badaruddin II Palembang. The medium for bacteria identification is Tryptic Soy Agar (TSA;Merck), Triple Sugar Iron Agar (TSIA;Merck), Methyl Red Voges Proskauer (MR-VP;Merck), Citrate, Glucose (Merck), Sucrose (Merck), Maltose (Merck), Urea (Merck), Nitrate (Merck), Gellatin (Merck), Sodium Chloride (Merck), Oxydative Fermentative (OF;Merck), Muller Hinton Agar (MHA;Merck), Muller Hinton Broth (MHB;Merck), the essence of garlic and turmeric and EDTA (Merck).

First stage of the study is consisted of : (1) adaptation of test fishes for seven days and E. tarda free test (2) Purity test of E. tarda which will be used by TSA media, the incubated for 24 hours at 30oC, (3) Virulent by injected 0.1 mL 105 density E. tarda to intraperritoneal Tilapia with 15-20 cm sized (4) Determination LC50 garlic and turmeric to Tilapia by dipping the fish in varians concentration of garlic and turmeric, every concentration observed its influence to the test fish life sustainability (5) Determination of Minimum Inhibitory Concentration (MIC) garlic and turmeric (6) Sensitivity test of E. tarda to garlic and turmeric using 100%, 75%, 50 % and 25 % concentration.

Sensitivity test to determine the sensitivity level of E. tarda to garlic and turmeric, with disc diffusion Kirby-Bauer method. The 24 hours purified E. tarda isolate, cultivated in Mueller Hinton Agar (MHA) by swabbing it using a sterile swab on the MHA surface, next put paper disc contain varians garlic concentrations and turmeric. Incubated MHA on the room temperature (30ºC) for at least 24 hours, inhibition zone diameter is measured to see the effectivity (Jang, 1980).

Serial Tube Dilution method is used to determine the MIC value of garlic. (MIC) is the minimum concentration which inhibited bacterial growth by making garlic serial concentrations on Mueller Hinton Broth (MHB) each 1 ppm, 2 ppm, 4 ppm, 8 ppm, 16 ppm until the lowest concentration that inhibit bacterial growth shown by transparent area. Each tube filled with MHB and 106 cfu/ml cultivated garlic extract. After 24 hours incubated in 30oC, we observe the bacterial growth on each tube. MIC determined on the lowest garlic extract which shown inhibited bacterial growth/transparent diameter. MIC value use as medication base on the second stage which are 1 x MIC, 2 x MIC, 4 x MIC, and 8 x MIC. We do the same thing for turmeric extract.

Second study to know the infuence of garlic and turmeric extract to life sustainability of Tilapia infected by E. tarda. Statistical analysis using completely randomized Design (CRD), with 6 treatment and 4 repetition with each density 10 fish/aquarium. First aquarium used as negative control (not infected and no treatment), second aquarium is positif control Tilapia infected by 104 cfu/ml. Third aquarium until sixth are Tilapia infected by 104 cfu/ml E. tarda immersion until it show clinical symptoms, then 8000 ppm garlic extract, 16000 ppm, 32000 ppm, and 64000 ppm, on each different aquarium with different treatment. Garlic extract is given by 600 second/day with immersion method, for 5 days. Turmeric is given 24 hours in a row 5 days and each day the water replaced. Blood examination, isolation and bacterial identification on 10^{th} day after infected.

Blood sampling is done from caudal blood vessel (intra vena). The preparations preserved with 10% Giemsa for 30 minutes, then washed and dried in the air. It observed under microscope start with lowest magnification. The leucosytes cfus observed using 1000× magnification, and counted until 100 cfus in percentage result. Eritrocyte counted with haemocytometer. Then the cfu counted under microscope and the result in ”n x 106 per mm^3”.

Water quality checked every 6 hours include the temperature, pH and dyssolved oxygen, ammonia, nitrate. Observed until 10^{th} day since bacterial infection and the rest of the fish checked for bacterial re-isolated. Survival rate each treatment calculated.

3. Results and Discussion

Edwardsiella tarda used in this study was coming from the collection of SMB II Palembang Fish Quarantine isolated from Tilapia *(Oreochromis niloticus)*. The isolation continued with isolate purity test and identification. The bacteial colony was gram negative. rod, motile, the fermentation result shown catalase positive, anaerobic facultative, oxydase (Table. 1).

Table 1. *E. tarda Charasteristics*

Tests	*E. tarda* (standar) *	Result
Metabolism fermentations produce:		
- Catalase	+	+
- H_2S	+	+
- Indole	+	+
- Oxydase	+	+
Methyl Red (MR)	+	+
Voges Proskauer (VP)	-	-
Gellatin	-	-
Urea	-	-
Sodium Citrate	-	-
Acid production:		
- Aesculin	-	-
- Arabinose	-	-
- Dulcitol	-	-
- Glucose	+	+
- Lactose	-	-
- Maltose	+	+
- Mannitol	-	-
- Sorbitol	-	-

*) Source : Austin and Austin, (1987)

The change of clinical symptoms from Tilapia infected by *E tarda* and given garlic and turmeric at the difference concentrations analyzed descriptively. The LC_{50} counting with Dragstedt Behrens (Carpenter, 1975) for turmeric, and Reed-Muench (1993) for garlic, life sustainability and blood analyzed statistically.

3.1. Virulent Test

Revirulence by injecting Edwardsiella tarda in tilapia size 7-12 cm with a density of 105 cfu/ml using the Mac Farland standard of 0.1 mL intraperitoneally. Revirulence (Koch's Postulate Test) aims to restore the virulence of bacteria resulting from the storage process at a certain time. After 3 days post-infection, the fish that showed symptoms were isolated. The result was *E. tarda* (Table 1). Clinical signs of fish seen haemorrhage in the tail (Figure 1A) and haemorrhage around the operculum (Figure 1B).

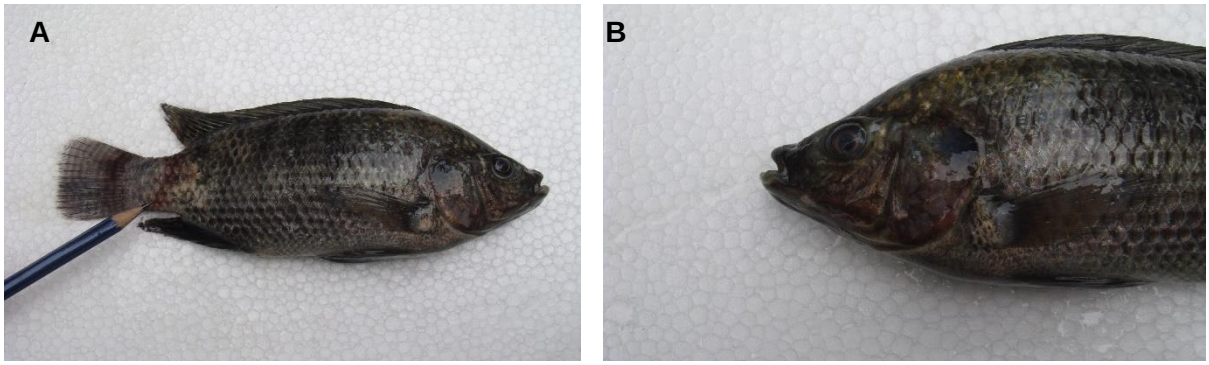

Figure 1. Clynical symptoms post-revirulence. A. tail haemorrhage B. operculum haemorrhage

3.2. Garlic LC_{50} Determination

LC_{50} garlic to Tilapia shown death rate is 0 (dose 100 ppm), 1 (300 ppm, 700 ppm and 900 ppm), 2 (dose 1.100 ppm) and 10 (dose 1300 ppm) (Tabel 5). LC_{50} garlic determinated with Reed-Muench method (1993) for 1.124,99 ppm (Table 2). The observation of clinical symptoms in garlic LC_{50} test showed that fish started to jump, gasp, getting weak at the bottom of aquarium, then die.

Table 2. LC_{50} Garlic to Tilapia

Treatment (ppm)	Death rate (fish)
100	0
300	1
700	1
900	1
1100	2
1300	10

3.3. Turmeric LC_{50} Determination

LC_{50} Toxicity test turmeric to Tilapia shown that fish mortality is 0 (5000 ppm), 1 (10.000 ppm), 2 (15.000 ppm) and 10 (20.000 ppm) (Table 4). Turmeric LC_{50} Determinated by Dragstedt and Behrens method (1993) as 1,66 x 10^4 ppm (Additional 2)

Table 3. Toxicity of Turmeric to Tilapia

Treatment (ppm)	Death Fish (amount)
5000	0
10000	1
15000	2
20000	10

Clinical observation showed that fish swim undirectly, than become less active, lost its pigmen, gasp and die. Tilapia mortality suspected was stress by existence of certain substances in the water which intolerance. Irianto (2005), declare that stress is a circumstance when an animal cannot set a normal physiological condition caused by detrimental factors which can influence its health condition, environmental challenge caused homeostatic or other balancing process beyond the normal limits of biological level, affect body defense system of the definitive host such as osmoregulation and disturb the electrolyt body balance (Na, K, Cl) cause excessive water absorption and even dehydration.

Clinical symptoms to each treatment was colour changing, inactive movement, fish stay in the aquarium bottom, hard to breath (*asphyxia)* and finally die. This described that the fish get stressed for the addition of garlic and turmeric extract changed fish osmotic pressure in the Tilapia. Duke (1992) declared that garlic include in family *Liliaceae.* Sulfur originated from unstable and degradated *Allisin*, than take the oxygen from the air and turn into sulfur (Rana *et al.*, 2011). The sulfur affect shown by the clinical symptoms of Tilapia before death, with opened operculum, we can suspect it because of the distinctive smell and sulfur content caused heat to the fish surface. Naganawa *et al* (1996) and Tung (2010) declared garlic contain *allicin, dialil disulfida* and other sulfids. Wiryowidagdo (2000), allisin is unstabel and decomposed during distillation or hydrolisation with water or natrium carbonate becomes polysulfide, dialil dissulfide, and formed distinctive smell from its essential oil. Determination of turmeric LC_{50} to Tilapia using Dragsted and Behrens (Carpenter, 1975) is 16.596 ppm.

3.4. MIC Determination of garlic and Turmeric

Determination MIC level of garlic with "*Serial Tube Dilution*" method. At 6000 ppm and 7000 ppm garlic concentration, bacterial growth still going marked with cloudy medium, at 8000 ppm to 35000 ppm the clear medium colour showed that there is *E. tarda* inhibition, start at 8000 ppm (Table 4)

Table 4. Determination of Minimum Inhibitory Concentration (MIC) of Garlic

Garlic Concentrations	Observation	Information
6000	cloudy	
7000	cloudy	
8000	clear	MIC = 8000 ppm*)
9000	clear	
10000	clear	

MIC garlic level 8000 ppm used as basic dose of treatment at second stage test, we use 1 X MIC, 2 X MIC, 4 X MIC, 8 X MIC and 16 X MIC as 8000 ppm, 16000 ppm, 32000 ppm, 64000 ppm, 128000 ppm.

3.5. Sensitivity Test of Garlic and Turmeric to E. tarda

Sensitivity test showed that for 100% concentrations of garlic and turmeric the resistance diameter to *E. tarda* is 15 mm. At 50% and 75% the resistance diameter is 12 mm for garlic disc and 0 mm and 10 mm for turmeric. At 25% concentration turmeric's resistance diameter is 0% and garlic is 8 mm. (Table 4 and Figure 2).

Table 5. Garlic and Turmeric Sensitivity Test to E. Tarda

Test Material	Diameter (mm)
100% garlic	15
75% garlic	12
50% garlic	12
25% garlic	8
100% turmeric	15
75% turmeric	10
50% turmeric	0
25% turmeric	0

The result showed resistance diameter to *E. tarda* from garlic is bigger. It is equal with about the ability of garlic as antibacteria. *Allicin* has function as fungi and bacteria inhibitor (Lingga and Rustama, 2005).

Sensitivity test showed that garlic and turmeric have ability to inhibit the growth of *E. tarda*. According with Duke (2002) that turmeric has antibacteria activity. Curcumin (*alkanon hidroksimetoksi fenilheptadiena-dion*) is antibacteria (Rao, 1997). This is the guidelines using turmeric in this study.

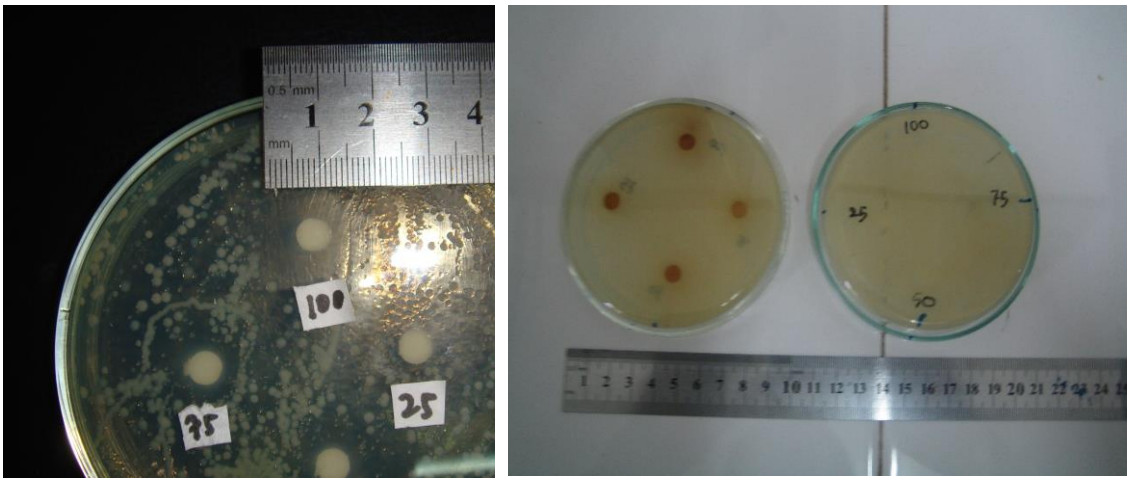

Figure 2. Sensitivity Test Result of Garlic (A) and Turmeric (B) to *E. tarda*

3.6. Garlic Treatment

Treatment started after *E. tarda* infected Tilapia show clinical symptoms, on the third day after infection characterized by the appearance of lesion on the body, less active and *anorexia.*

Survival rate observation of the treated fish after 5 days is non treatment (positive control), 1 x MIC, 2 x MIC, 4 x MIC, 8 x MIC and negative control showed 0 %, 50 %, 10 %, 0 %, 0 %, 100 % (Table 5).

Table 6. Survival Rate (SR) Garlic's Treatment of Tilapia

Treatment	Concentration (ppm)	Repetition				Total	Mean	SR (%)
		1	2	3	4			
K (0x MIC)	0	0	0	0	0	0	0	0
A (1x MIC)	8000	3	4	1	2	10	2,5	50
B (2x MIC)	16000	1	0	1	0	2	0,5	10
C (4x MIC)	32000	0	0	0	0	0	0	0
D (8X MIC)	62000	0	0	0	0	0	0	0
Negative control	0	5	5	5	5	20	5	100

The result showed the highest survival rate is 50% at 8000 ppm, treatment more than 8000 ppm caused the fish unable to survive (survival rate 0% to 10%). Analysis of variance, 1 x MIC garlic treatment totally different with positive control, from all the treatment unreal different with negative control. Treatment 16000, 32000 and 64000 ppm have a real impact to survival rate but also caused negative effect which is poison the fish as concentration increased.

Bacteria isolation sampling showed at 8000 ppm,16000 ppm and 32000 ppm *E. tarda* still detected on the fish organs. At 64000 ppm the fish die on the first day of treatment, isolation and identification negative for *E. tarda*. At that dose *E. tarda* and test fish dead because of the too high garlic concentration. (Table 6). Sulfur compound content in garlic become the cause of the dead fish on the treatment process. The low survival rate of the treated fish was thought to have occurred because the fish were unable to tolerate stress due to immersion in garlic extract. Garlic contains 2 sulfur groups which are very active and can burn and irritate tissues (Lukistyowati et al., 2008). The garlic content causes the fish to not survive, because the fish when soaked in a weak state in this case are infected with E. tarda bacteria, so this condition is exacerbated by the stress and irritation caused by garlic by showing the water in the rearing container. cloudy quickly caused by fish releasing mucus, thus worsening the quality of aquarium water. Meanwhile, in the prevention of fish soaked with garlic extract, the condition of the fish is in good health, although there are some fish

that experience stress during immersion, but the fish have high endurance and can survive, while fish that cannot stand it will die.

This shows that the added garlic content can reduce mortality due to bacterial infection E. tarda There are many substances contained in garlic as antibacterial, one of the garlic content that can prevent bacterial infection is allicin. This is reinforced by Lengka (2013), which states that allicin is one of the active substances that can kill pathogens (antibacterial) such as bacteria. While allicin contained in garlic can significantly increase leuokist cells in fish blood, so garlic can be used as an efficient immunostimulant.

Table 7. Isolation and Identification of *E. tarda* on Tilapia Treatment by Garlic (BP)

Treatment	Dose	1			2			3		
		H	G	L	H	G	L	H	G	L
BP	8000	-	-	-	-	-	-	-	-	+
	16000	-	-	-	-	-	+	-	-	+
	32000	-	+	-	+	-	+	-	-	-
	64000	-	-	-	-	-	-	-	-	-

Blood observation showed on garlic treatment fish get anemia at the end of the treatment, at positive control average value is 1.376.667 cfu/mm^3, highest eritrosit is on 16000 ppm treatment with 2.280.000 cfu/mm^3. Average heterophyl on positive control is 30.468,7 cfu/mm^3, average heterophyl for the treatment fish is at 2000-3000 cfu/mm^3 range. Blood observation post garlic treatment eritrosit and heterophyl value is as seen on Table 8.

Table 8. Blood Observation of Tilapia in Garlic's Treatment at 10th day

No	Sample	Eritrosit (cfu/mm^3)	Heterophyil Abs (cfu/mm^3)
1	Negative control	1630000	1368
2	Negative control	1290000	1221
3	Negative control	2530000	9808
4	Positive control	1260000	8600
5	Positive control	1180000	31626
6	Positive control	1690000	51180
7	BP 1-1	1850000	580
8	BP 1-2	1460000	1992
9	BP 1-3	1040000	4440
10	BP 2-1	2420000	3129
11	BP 2-2	1960000	3905
12	BP 2-3	2460000	4250
13	BP 3-1	1280000	4042
14	BP 3-2	1040000	480
15	BP 3-3	670000	4209
16	BP 4-1	1170000	3698
17	BP 4-2	990000	1725
18	BP 4-3	1580000	1674

Table 8 showed blood observation post garlic treatment, the fish mostly get anemia. This is happened while the fish still healthy (pre-infected). Presumed the fish was stressed. Leucosytosis in positive control indicated fish immune system in a good function, therefor lecocyt produced to fight

the bacteria infection. Post treatment, the leukocytes of same fish approach normal and some other decreased. (leukopenia). It happened as most leukocytes pull into the damage tissues. Infected Tilapia showed increased heterophyl (heterophylia). Heterofilia used to happen in bacterial infection. Haematopoietic tissues will produced a lot of heterophyl to fight the bacterial infection. Post garlic and turmeric treatment, heterophyl become normal. In a longtime infection, heterophyl will be pulled to the damaged tissues. The body produced limphocyt in a large amount (limphocytosis). Limphocytosis happened to chronically bacterial infection. Treatment Tilapia showed normal lymphosit, means garlic and turmeric inhibited bacteria.

3.7. Turmeric Treatment

Tilapia Survival Rate in post turmeric treatment are 0 concentration (positive control), 20 ppm, 40 ppm, 80 ppm, 160 ppm and negative control is 0, 80%, 75%, 70%, 45% and 100% negative control (Table 11).

Table 9. Survival Rate (SR) for Turmeric Treatment Tilapia

Treatment	Concentration (ppm)	Repetition				Total	Average	SR (%)
		1	2	3	4			
K (0xMIC)	0	0	0	0	0	0	0	0
A (20 ppm)	20	4	3	5	4	16	4	80
B (40 ppm)	40	2	5	4	4	15	3,75	75
C (80 ppm)	80	3	4	4	3	14	3,5	70
D (160 ppm)	160	2	1	3	3	9	2,25	45
Negative control	0	5	5	5	5	20	5	100

Highest survival rate is 80% on 20 ppm (figure 3), we assumed in the low treatment Tilapia still have the ability to adapt in environment for 24 hours turmeric immersion/treatment. Turmeric concentration disturb the metabolism balance of the fish and influenced fish endurance to fight pathogen. It is also infuenced the amount of erytrosit in blood during treatment.

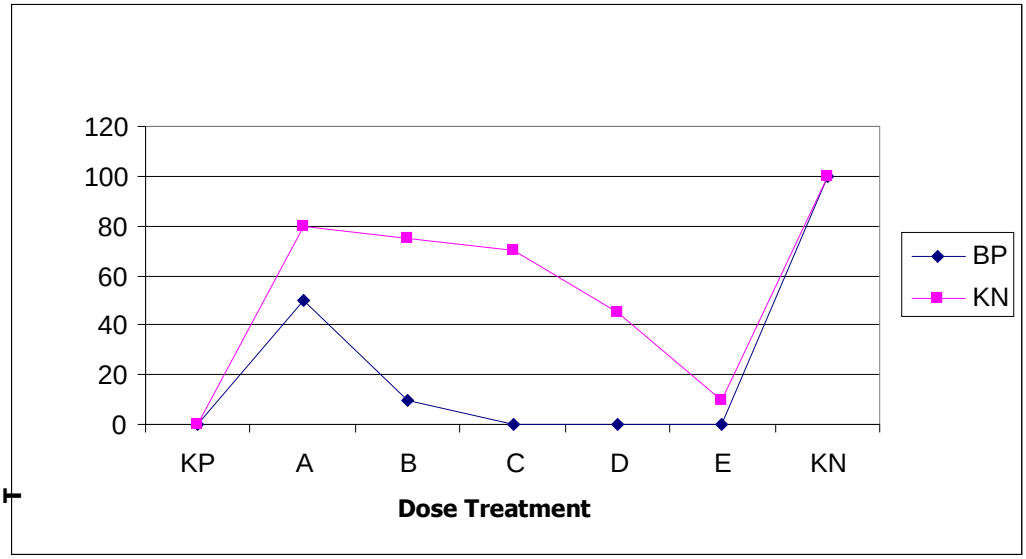

Figure 3. Survival Rate of Garlic (BP) and Turmeric (KN)

Bacterial isolation and identification in the end of treatment showed that at 20 ppm, 40 ppm, 80 ppm, and 160 ppm *E. tarda* is positive (+) detected in Tilapia's body (Table 10).

Table 10. *E. tarda* Isolation and Identification in Tilapia post Turmeric Treatment

Traetment	Dose	1			2			3		
		H	G	L	H	G	L	H	G	L
K	20	-	-	-	-	+	-	+	+	-
	40	-	-	+	+	+	+	+	-	-
	80	-	-	-	-	-	-	-	+	-
	160	-	-	-	-	-	+	-	-	-

Rao (1997) declared curcumin (*alkanon hidroksimetoksi fenilheptadiena-dion)* is antibacteria. In this study, curcumin from turmeric inhibited *E. tarda* shown by inhibition zone at sensitivity test, but it also shown that *E. tarda* still exist in Tilapia, means turmeric can only reduced the bacteria, not removed. The inhibition zone formed from turmeric extract appears due to the active ingredients from turmeric which are pulled out due to the extraction method, the active ingredients in turmeric are dominated by essential oils and curcumin. According to Harikhrishnan *et al*. (2009) and Sukrasno *et al*. (2012), the essential oil in turmeric is bactericidal, while curcumin is bacteriostatic.

Bacteria isolation and identification at the end of treatment period showed that at all concentration 20 ppm, 40 ppm, 80 ppm, 160 ppm and 320 ppm positive *E. tarda* (+) detected in the fish. Rao (1997) said curcumin (*alkanon hidroximetoxi fenilheptadiena-dion*) is antibacteria. In this study, curcumin of the turmeric inhibit *E. tarda* by forming inhibition zona on sensitivity test, however it can only reduced the bacteria, cannot totally removed it from the fish.

The antibacterial mechanism of turmeric according to Singh and Jain (2012), and Shawket (2013) is an essential oil that can damage cell biological membranes so that microbes will lyse or at least inhibit their growth. Curcuminoids in turmeric rhizome are phenolic compounds that can change the permeability of the cytoplasmic cell membrane which will cause leakage of nutrients to bacterial cells, causing bacterial death or inhibiting their growth. The presence of essential oils and curcuminoids in turmeric creates a clear zone in the in vitro test which is expected to provide the same antibacterial effect in the in vivo test. The extraction method with the dekoxy method was able to attract more active substances of essential oils and curcuminoids seen from the area of the resulting inhibition zone.

Conclusion

The study described us several facts about the effectivity of using garlic and turmeric to inhibit *E. tarda* in *Tilapia*, which are garlic and turmeric inhibit *E. tarda* effectively. The 1x MIC turmeric (20 ppm) is more effective than 1 × MIC garlic (8000 ppm) to EPDC. However, the MIC level coudnot use as reference to determine the dose of herbal treatment, we could use disc volume in resistance test to determine it. We also found that blood description for fish in the post both of garlic and turmeric treatment, approached blood description for healthy fish.

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