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The Potential Toxicity of Animal Oils to Pathogenic Fungi Nila Fish (*Oreochromis niloticus*)

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ABSTRACT

Nila is a type of freshwater fish that has high economic value, as a source of animal protein for the community, and is an important commodity in the freshwater fish business. Nila is also one of the main commodities that contributes to the increase in aquaculture production. Nila farming often faces the problem of declining yields due to diseases. One of the diseases that is very detrimental to freshwater fish is diseases caused by fungi. The purpose of this study is to test the toxicity of animal oil in inhibiting the growth of Nila pathogenic fungi. Isolation of pathogenic fungi in fish is carried out by cutting the infected parts (scales, fins, and gills) with a size of about 1x1cm. After pure culture is carried out on each pathogenic fungus, then macroscopic and microscopic identification is carried out. Animal oil (lard oil) is taken in the market that is already available. Animal oil is made in four concentration series, namely 25ppm, 50ppm, 75ppm, and 100ppm and control (0ppm). The toxicity test was carried out in vitro using the toxic bait method, which was carried out by growing Nila pathogenic fungi inoculum on PDA media that had been mixed with animal oil. The results of animal fungicide toxicity testing in 5 treatments (A= control – E= 100 ppm) obtained the diameter of pathogenic fungal colonies (5 pathogenic fungi) ranging from 2.88 cm – 6.82 cm. Animal oil fungicides can affect pathogenic fungi in fish, this can be seen in pathogenic fungi Aspergillus sp. which has a relative resistance of 49% (100 ppm), the category is quite influential with a colony diameter of 3.5 cm and in the pathogenic fungus *Mucor* sp. With a relative resistance of 56% (100 ppm), the category is quite influential with a colony diameter of 2.88 cm.
Keywords: *Pathogenic fungi; Nila; toxicity; animal oil; in vitro; toxic bait*

ABSTRAK

Ikan nila merupakan salah satu jenis ikan air tawar yang mempunyai nilai ekonomis tinggi, sebagai sumber protein hewani bagi masyarakat, dan menjadi komoditas penting dalam bisnis ikan air tawar. Ikan nila juga merupakan salah satu komoditas utama yang berkontribusi dalam peningkatan produksi perikanan budidaya. Budidaya ikan nila sering menghadapi masalah menurunnya hasil karena adanya penyakit. Salah satu penyakit yang sangat merugikan ikan air tawar adalah penyakit yang disebabkan oleh jamur. Tujuan dari penelitian ini adalah untuk menguji daya toksisitas minyak hewani dalam menghambat pertumbuhan jamur patogen ikan nila. Isolasi jamur patogen pada ikan dilakukan dengan cara memotong bagian yang terinfeksi (Sisik, Sirip, dan Insang) dengan ukuran sekitar 1x1cm. Setelah dilakukan kultur murni pada setiap jamur patogen yang ada, selanjutnya dilakukan identifikasi secara makroskopik dan mikroskopik. Minyak hewani (minyak lemak babi) diambil di Pasaran yang sudah tersedia. Minyak hewani dibuat empat seri konsentrasi yaitu 25ppm, 50ppm, 75ppm, dan 100ppm serta kontrol (0ppm). Uji toksisitas dilakukan secara in vitro dengan metode umpan beracun, yang dilakukan dengan menumbuhkan inokulum jamur patogen ikan nila pada media PDA yang sudah bercampur dengan minyak hewani. Hasil pengujian toksisitas fungisida hewani pada 5 perlakuan (A= kontrol – E= 100 ppm) diperoleh diameter koloni jamur patogen (5 jamur patogen) berkisar antara 2.88 cm – 6.82 cm. Fungisida minyak hewani dapat berpengaruh terhadap jamur pathogen pada ikan, hal ini dapat dilihat pada jamur patogen Aspergillus sp. yang memiliki hambatan relatif sebesar 49% (100 ppm), kategori cukup berpengaruh dengan diameter koloni sebesar 3.5 cm dan pada jamur patogen *Mucor* sp. dengan hambatan relatifnya sebesar 56% (100 ppm), kategori cukup berpengaruh dengan diameter koloni sebesar 2.88 cm.
Kata kunci: *Jamur patogen; ikan nila; toksisitas; minyak hewani; in vitro; umpan beracun*

INTRODUCTION

The main problem that is often faced in aquaculture activities is the existence of diseases that occur due to incompatibility between aquaculture organisms, pathogens, and environmental health. According to Sarjito *et al.* (2013), disease attacks are one of the main factors that can threaten the success and continuity of a fish farming business. Likewise, according to Dewi (2011), disease is one of the obstacles in fish farming that can cause a decrease in fish production levels. Diseases in fish can be caused by pathogens such as viruses, parasites, fungi, and bacteria. Some types of fungi that are classified as pathogens because they can cause death in fish include *Ichthyophonus hoferi*, *Aphanomyces invandans*, *Branchiomyces* sp., *Achlyarosemosa*. One of the diseases that attacks farmed fish is aspergillosis caused by the fungus *A. terreus*. *Aspergillus terreus* was able to infect *Channa punctatus, Heteropneustes fossilis* and *Clarias batrachus* in the Gobindgarh tank of Rewa (Madhya Pradesh), India in 1990 (Shrivastava, 1996). *Aspergillus terreus* also produces mycotoxins which are toxins released by fungi and are harmful to health. The mycotoxins produced by *A. terreus* are citrinin and terrein (Youssef et al., 2003) and patuline (Hashem, 2011). Fungal infections (mycosis-mycotic infection) are a type of disease that often occurs in freshwater farmed fish. This disease can attack or infect eggs, larvae, seeds and adult fish. Parasitic infections in fish are generally caused by injured body parts, so other diseases such as bacteria, fungi, and viruses will more easily attack and aggravate infections in fish that have been infected with parasites (Lom, 1995 in Yulianto, 2014). The occurrence of fungal infections is caused by poor quality of aquaculture water, poor level of container cleanliness, fish injured by other diseases, dead fish or decomposition of organic matter (Soesanto, 2013). The death of fungus-infected fish occurs due to poor water quality, such as high organic matter, fluctuations, temperature, and pH. This condition can trigger the growth of fungi. These fungi attack freshwater fish such as Chef's Goldfish, Nila, Gourami Fish, Catfish, Catfish, and Eel (Winarsih and Syafrudin, 2001).

Fungi in fish are dangerous because they produce mycotoxins as a result of their metabolites. Some fungi are capable of producing mycotoxins, and some of these mycotoxins can cause some degree of acute toxicity when given in high amounts and are potential carcinogens (Zain, 2011). *Aspergillus*, *Fusarium*, and *Penicillium* are the three most important genera of toxingenic fungi in the tropics (Bankole *et al*., 2006). *Fusarium* mycotoxins can cause both acute and chronic toxic effects. Research from Marijani *et al.* (2019), has shown that this effect depends on the dose, duration of exposure, and species of fish to which it is exposed. The presence of toxicogenic fungi, some producing mycotoxins in farmed fish has increased in recent years due to the increasing use of plant materials as components for fish feed (Anater *et al*., 2016). Contamination of fish feed by mycotoxins and the possible transfer of these toxins into farmed fish and fish derivative products for human consumption remain a serious food safety issue (Chávez-Sánchez *et al.*, 1994). About 300–400 types of mycotoxins are known to date (Berthiller *et al.*, 2007), but the most important in tropical countries are aflatoxin (AF) (AFB 1, B 2, G 1, and G 2) and fumonicin (FB) (FB 1, FB 2, and FB 3) (Pitt, 2000). In addition to AF and FB, okratoxin A (OTA) and tricotathene (TH) are also important (Bryden, 2012).

Prevention and treatment efforts that are usually carried out on fish affected by fungal diseases are the use of chemical drugs. The use of chemicals causes negative

effects, namely the resistance of microorganisms, the danger posed to the surrounding environment, the fish concerned and the humans who consume them (Suggestive, 2005). The use of animal oils is a safe way to inhibit and kill microbial growth and is environmentally friendly. One of them uses pork fat oil. Therefore, it is necessary to test animal oils as fatty acid oils that have the potential to control pathogenic fungi in freshwater fish, more specifically pathogenic fungi in Nila.

METHODS

Preparation of Animal Oil (Lard Oil)

Animal oil (lard oil) is taken in the market that is already available. Animal oil is made in four concentration series, namely 25ppm, 50ppm, 75ppm, and 100ppm and control (0ppm) with the formula:

$$V1 \times M1 = V1 \times M2$$

Information:
V1 = volume before dilution,
M1 = concentration before dilution,
V2 = volume after dilution,
M2 = concentration after dilution

In Vitro Stage of Animal Oil (Lard Oil) Toxicity Testing

To perform the toxicity test of each animal oil follow the method used by Humaidi *et al*. (1999), which is invitro testing using the toxic bait method, which is carried out by growing Nila pathogenic fungal inoculum on PDA media that has been mixed with animal oil. This method is done by mixing a solution of animal oil with different concentrations (0 ppm, 25 ppm, 50 ppm, 75 ppm, and 100 ppm) before the PDA media in the petri dish solidifies. After the PDA medium solidified, the obtained fungal inoculum is grown by placing it in the middle of the PDA medium. This treatment was compared to control treatments that were not given treatment using animal oil. Observations were made by measuring the diameter of the growing mushroom colony to the mushroom colony at full control for seven days.

According to Linda (2011), to calculate the diameter of a growing mushroom colony by creating vertical and horizontal lines where the cutoff point of both lines is right in the middle of the mushroom colony (**Figure 1**). How to measure the diameter of Nila pathogenic fungal colonies in a petri dish based on the formula:

$$D = \frac{d1 + d2}{2}$$

Information:
D = diameter of the mushroom colony
d1 = diameter vertical jamur
d2 = horizontal diameter of the mushroom

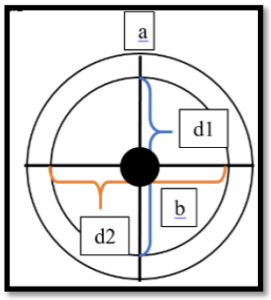


Figure 1. How to Measure the Diameter of a Mushroom Colony (a. petri dish; b. a mushroom colony; d1. diameter vertical jamur; d2. diameter horizontal) (Linda, 2011)

The calculation of the relative resistance of the vegetable fungicide to fungal growth is calculated until the fungus grows. The percentage of obstacles is calculated (Noveriza and Miftakhurohmah, 2010) as follows:

$$HR = \frac{dk - dp}{dk} \times 100\%$$

Information:
HR= relative barrier
dk = diameter control
dp = treatment diameter

The effect of a fungicide is assessed from the categories put forward by Irasakti and Sukatsa (1987) namely:

0	= Have no effect
0 – 20 %	= Very less influential
20 – 40 %	= Less influential
41 – 60 %	= Quite influential
61 – 80 %	= Influential
> 80 %	= Highly influential

RESULTS AND DISCUSSION

Results of Animal Oil Toxicity Test Against Pathogenic Fungi

Based on the toxicity testing of animal fungicides in 5 treatments (A= control – E= 100 ppm), the diameter of pathogenic fungal colonies (3 pathogenic fungi) was obtained which ranged from 2.88 cm – 6.82 cm (**Table 1**). From **Table 1**, it can be seen that animal oil fungicides can affect pathogenic fungi in fish, this can be seen in the pathogenic fungus *of the fish Geotrichum* sp. which has a relative resistance of 12% (100 ppm), the category is very less influential with a colony diameter of 6.25 cm and in the pathogenic fungus *Mucor* sp. with a relative resistance of 56% (100 ppm), category is quite influential with a colony diameter of 2.88 cm (**Figure 2**).

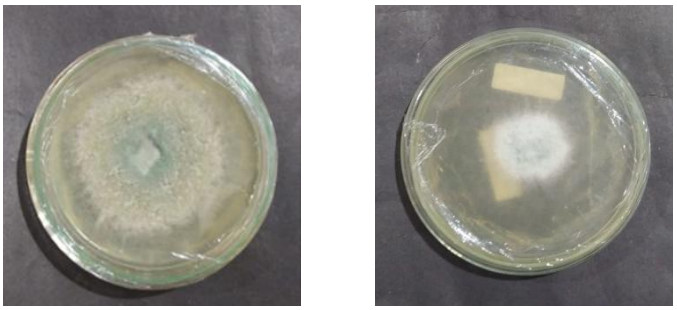
Table 1. Colony Diameter and Relative Resistance Nila Pathogenic Fungus

Fungi	Treatment Concentration	Diameter (cm)		HR (%)	Category
		DP	DK		
Mucor sp.	A	6.45	6.45	0	Has no effect
	B	6.19	6.45	4	Very less influential
	C	5.71	6.45	12	Very less influential
	D	2.93	6.45	55	Quite Influential
	E	2.88	6.45	56	Quite influential
Aspergillus sp.	A	6.74	6.74	0	Has no effect
	B	6.1	6.74	10	Very less influential
	C	5.76	6.74	15	Very less influential
	D	3.66	6.74	46	Quite influential
	E	3.5	6.74	49	Quite influential
Geotrichum sp.	A	6.82	6.82	0	Has no effect
	B	5.61	6.82	18	Very less influential
	C	5.77	6.82	15	Very less influential
	D	5.8	6.82	15	Very less influential
	E	6.25	6.82	9	Very less influential

DP: Treatment Diameter
DK: Control Diameter
HR: Relative Barriers

A: 0 ppm
B: 25 ppm
C: 50 ppm
D: 75 ppm
E: 100 ppm

Based on the results of this study in **Table 1** above, pork fat oil can inhibit the pathogenic fungus *Aspergillus* sp. in Nila although the inhibition is small with a fairly influential category (small activity) (**Figure 2**), nevertheless lard oil can be chosen because it has active ingredients as antimicrobials, such as formic acid and propionate whose acidic properties can help lower the pH, which is an environment that is not favored by fungi. In addition, these acids can interact with fungal cell membranes, interfering with cellular function and growth, although further research is needed on which type of active ingredient content is able to inhibit pathogenic fungi in Nila.



A **B**

Figure 2. Diameter of Animal Oil Barrier (Day 7)
(A: Fungal Colony *Geotrichum* sp.; B: *Mucor* Mushroom Colony sp.)

Some studies have found that formic acid and propionic acid that can be found in lard oil have the ability to inhibit fungal growth. The formic acid and propionic acid present in lard oil have the ability to inhibit fungal growth, which works by disrupting the metabolism of fungal cells and/or blocking their systems. The antimicrobial properties of formic acid and its salts have been studied in a small number of in vitro studies (Knarreborg *et al.,* 2002; Naughton and Jensen, 2001; Östling and Lindgren, 1993). The results of the study from Talanca and Mas'ud (2009), stated that phenolic compounds, propionic acid, and ammonia are chemical compounds that can be used as inhibitors of fungal growth. These organic acids, depending on concentration and pH, exhibit extensive antimicrobial activity against a variety of microorganisms, including fungi and yeasts.

The active ingredient content in lard oil that has been studied to fight microbes is medium-chain fatty acids (MCFAs) which have antimicrobial properties and long-chain fatty acids (LFAs), which have been proven against various microorganisms. MCFAs are lipophilic (fat-soluble), allowing them to easily penetrate microbial cell membranes. Once inside the cell, MCFA can disrupt the integrity of the cell membrane, causing the leakage of cellular components and eventually microbial death. MCFAs can also lower the intracellular pH of microbes, inhibiting the activity of essential enzymes and metabolic processes.

CONCLUSION

The results of animal fungicide toxicity testing in 5 treatments (A= control – E= 100 ppm) obtained the diameter of pathogenic fungal colonies (5 pathogenic fungi) ranging from 2.88–6.82 cm. Animal oil fungicides can have an effect on pathogenic fungi in fish, this can be seen in the pathogenic fungus *Aspergillus* sp. which has a relative resistance of 49% (100 ppm), the category is quite influential with a colony diameter of 3.5 cm and in the pathogenic fungus *Mucor* sp. With a relative resistance of 56% (100 ppm), the category is quite influential with a colony diameter of 2.88 cm.

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