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POTENTIAL OF GINGER (*Zingiber Officinale*) CRUDE EXTRACT AS A CHEMOTHERAPEUTIC AGENT FOR NILE TILAPIA (*Oreochromis Niloticus*) INFECTED BY *ACHLYA* SP.

Norashikin Anjur[1*], Ruzaini Ahmad[2]

[1]*Tampin Community College, Jalan Impian Nuri 3, Taman Impian Jaya, Gemencheh, 73200, Negeri Sembilan, Malaysia.*
[2]*Department of Agrotechnology and Bio-Industry, Politeknik Sandakan Sabah, Education Hub, Jalan Sg. Batang, Batu 10, 90000 Sandakan, Sabah, Malaysia.*

[*]*Corresponding author: norashikin.anjur@kktns.edu.my*

Abstract

The outbreaks of *Achlya* sp. have resulted in enormous mortality of cultured fish and remain a critical disease problem, resulting in significant losses in aquaculture productivity. Experimental infection of Nile tilapia (*Oreochromis niloticus*) juvenile by *Achlya* sp. was conducted to examine the potential of ginger crude extract as a chemotherapeutic agent. A total of 100 juveniles were immersed in four different concentrations of crude ginger extract at 100 ppm, 500 ppm, 800 ppm and 1000 ppm while 0 ppm as the control treatment. The potential of the extract as a treatment agent in the infected fish was recorded in terms of fish survival rate and changes in physical appearance. The results show that the treated fish immersed in a lower concentration of ginger extract (100 ppm, 500 ppm and 800 ppm) possess a higher survival rate and no *Achlya* sp. found on the fish skin compared to the non-treated control. The highest concentration of ginger extract at 1000 ppm might be toxic to the fish due to a lower survival rate compared to other treatments. It shows that ginger crude extract potentially acts as a chemotherapeutic agent for fungal infection in tilapia juveniles when used in the appropriate concentration and treatment period.

Keywords: Water mould, antimicrobial activity, tilapia juvenile, ginger extract, survival rate.

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Introduction

Nile tilapia (*Oreochromis niloticus*) is reported to be among the top cultured food fish worldwide. Despite the tilapia's robustness, disease outbreaks, particularly those of bacterial origin, remain one of the major limiting factors that jeopardise tilapia productivity, particularly when cultivated under intensive circumstances (El-Sayed et al., 2019). Due to intensive rearing, many farmed fish are more susceptible to disease agents. The disease is a primary constraint to many aquatic cultured species, hindering economic development. The aquaculture sector is facing the challenge of decreasing production. Chronic stress immensely affects fish health, causing inhibition of specific immune responses and defence mechanisms, leading to the favourable condition of pathogen infections. Traditionally, synthetic chemicals and drugs have been used as preventive or prophylactic means of treating fish diseases. Until now, chemotherapy is the only option for preventing and treating aquaculture disease outbreaks. Nevertheless, chemical drugs have several inherited negative impacts on humans and the environment (Jana *et al*., 2018; Gabriel, 2019).

Oomycete (water mould) is an economically significant group of mycotic agents that causes epizootic ulcers in freshwater fish worldwide. When exposed to stress or poor environmental and water quality circumstances, the genus of fungus will infect host species. Water mould is a fungus that belongs to the order Saprolegniales and the family Saprolegniaceae, which includes 19 genera and 150 species, with Achlya, Aphanomyces, and Saprolegnia being important as fungi infectious agents in aquaculture (Afzali *et al*., 2013). The disease manifests itself on the body surface as cotton wool-like water tufts, allowing hyphal penetration to destroy skin or fins due to cellular necrosis, which is typically limited to the epidermis and dermis (Hussein *et al*., 2013). Usually formalin, copper sulfate and malachite green were used to reduce the infection of fungal disease (Choudhury *et al*., 2014).

There are some reports on the use of herbs in managing fungal infections (Syahidah *et al*., 2015). Ginger, or *Zingiber officinale,* is a plant of the Zingiberaceae family with a long history of therapeutic usage. The rhizome is readily available, universally acceptable, relatively inexpensive, and well-tolerated by most people. Non-volatile resins and volatile essential oils are the two most important types of phytochemicals found in ginger. Polyphenolic chemicals are the main non-volatile pungent elements of oleoresin, and they are responsible for their distinct pharmacological actions (Fu *et al.,* 2019). The previous study showed that the ethanolic extract of ginger has antifungal activity on *Candida albicans*, which is the most frequent fungi in the oral cavity (Atai *et al.*, 2009; Supreetha *et al.*, 2011). Amid reports of the health advantages of ginger extract, this study was carried out to examine the potential of ginger crude extract as a chemotherapeutic agent for *Achlya* sp.

Methods

Plant preparation and extraction

Rhizomes of *Zingiber officinale* were bought from the local market at Sandakan, Sabah. The ginger peel was removed, cleaned and rinsed in distilled water. The ginger rhizomes were cut into small pieces and allowed to be oven-dried at 38 °C for 48 hours. Dried ginger was weighed and ground into a fine powder in a blender. 10 g of fine grounded ginger powder was measured using an electronic balance (OHAUS: model NVL2101) and then transferred into a conical flask containing 50 ml of distilled water. It was corked with cotton wool and foil, shaken gently and allowed to stand at room temperature for 24 hours. The content was then transferred to a funnel bearing a sterile muslin cloth and further filtered using a Whatman No. 1 filter paper. The filtrate of the ginger extract was assumed to be 100% concentrated and stored in the refrigerator at five °C prior to use (Kigigha and Kalunta, 2017).

Fungal strain and culture

The *Achlya* sp. can be easily obtained from dead tilapia fish in dirty water. This collection of *Achlya* sp. was done in Fish Breeding Centre at Politeknik Sandakan, Sabah. Cotton-like mycelia on the surface of dead fish were collected and observed under a microscope to identify the species (Duc *et al.*, 2016). Then, culturing the *Achlya* sp. is by making mould water. The mould water was prepared for one week by adding *Achyla* sp. isolates and three tilapia fish infected by *Achyla* sp. in a freshwater aquarium (13L) with uneaten feed without aeration in order to fasten the multiplication of the *Achlya* sp.

Challenge test and experimental setup

A total of 100 tilapia fish with an average length of 3-4 inches were bought from Sabah Sandakan Polytechnic, Malaysia. Fish was acclimatized in a 500 L polyethylene tank for a week prior to the challenge test. Fish was fed at apparent satiation twice daily and kept in an optimum dissolved oxygen level in dechlorinated tap water. The experiment was conducted with four treatments 100 ppm (T1), 500 ppm (T2), 800 ppm (T3) 1000 ppm (T4) and control 0 ppm (TC) in duplicate where each replicate uses 10 fish. Fungal infection on tilapia juveniles was done by immersion technique in the mould water using aquarium of 13L for ten fish. The mould water prepared is equivalent to a MacFarland No. 4.0 standard solution. The effects of fungal infections are very rapid. After one to two days, the mould water was added to the tilapia aquarium, and signs of fish infected with the fungus can be observed. The infected juveniles will rub against the aquarium walls due to itching in the body, exophthalmia, cloudy eyes, growing fine-like cotton on the body, and lethargy movement.

After all the fish possessed symptoms of fungal infection, the infected juveniles were exposed to the ginger crude extract at the concentration that has been determined for each aquarium. Once the fungal-infected fish was exposed to ginger extract as an immersion treatment technique (Ming and Hatai, 2017), monitoring and data collection were carried out for four days. Experimental fish in all treatments were closely monitored thrice daily and fed at apparent satiation twice daily. Uneaten feed was removed from the tank to maintain water quality.

Data Collection and Analysis

Water temperature was taken daily in this study by using a digital thermometer. Dissolved oxygen and pH concentration measured by a dissolved oxygen meter, and pH meter respectively on daily basis (Alam *et al.*, 2014). The survival rate was determined using the formula: SR (%) = (number of fish survived/number of fish challenged) ×100. For clinical observation, fish were examined externally for any injuries, infections and diseases. The physical appearance and swimming behaviour of tilapia fish were evaluated using the number of scales modified from the Heiman-Carver colour rotor (Boonyaratpalin and Lovell, 1977). The scale consists of 5 numbers categorised as 1: Deteriorate, 2: No Changes, 3: Partially Recovered, 4: Recovered and 5: Fully Recovered.

Result and Discussion

Water quality parameters were in optimum condition at 3.0 to 6.0 ppm for dissolved oxygen, 7.2 to 8.2 for pH and temperature at 26 to 29 ^{o}C as shown in Table 1.

Table 1. Water quality parameters in challenged Oreochromis niloticus aquarium

Water Quality Parameter	**Ginger Extract Concentration (ppm)**				
	TC (0)	**T1 (100)**	**T2 (500)**	**T3 (800)**	**T4 (1000)**
Dissolved Oxygen (ppm)	4.0-6.0	4.0-6.0	4.0-6.0	3.0-6.0	3.0-6.0
pH	7.2-7.8	7.2-8.0	7.4-8.2	7.3-8.0	7.3-8.0
Temperature (^{o}C)	26-27	26-29	26-29	26-29	26-29

After all the juveniles infected by *Achlya* sp. and treated with ginger crude extract, the tilapia's final survival rate was calculated and shown in Figure 1. Tilapia treated with the ginger crude extract (T1, T2, and T3) shows 100% survival. For the T4 with a slightly higher concentration of ginger crude extract, only 80% of the fish survive, while for the control treatment without ginger extract application, no fish survived.

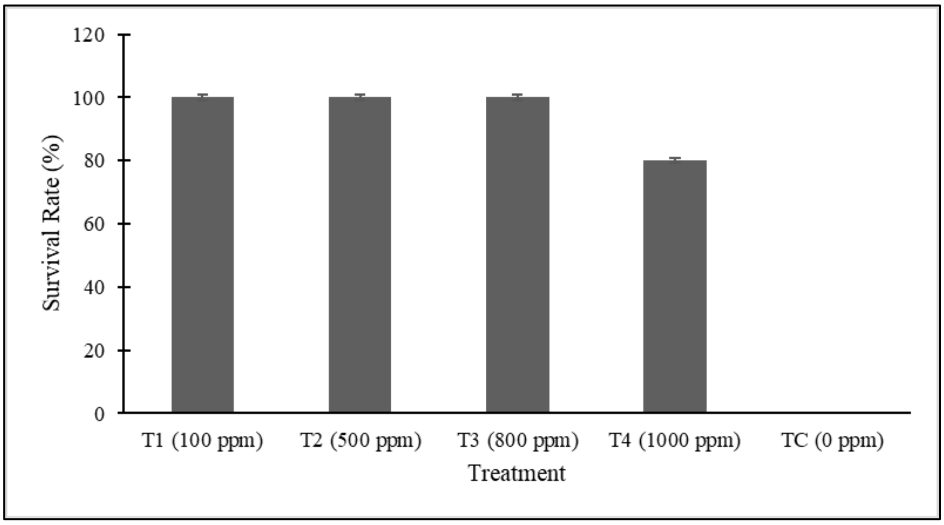

Figure 1. The challenge tilapia survival rate treated with *Z. officinale* extract.

Physical examination of Nile Tilapia body condition was daily observed. The symptom of fungal infection appeared after the second day of the juveniles infected with *Achlya* sp. (Figure 2). The most common clinical signs observed are skin discolouration of the body, descaling on the body and skin ulcers. The process usually starts from water mould attachment on fish skin, ulceration, and the skin becoming shallow or peeled off. Exophthalmia and fin rot was observed in almost all treatment aquariums. Fish become inactive after four to five hours of infection. They keep dormant at the aquarium bottom and show lethargy. After two days of infection, most fish show abnormal swimming patterns by swirling slowly and rubbing their bodies on the aquarium wall. At the beginning of the challenge test, fish started to stop consuming the feed given. Fish were fed on demand and uneaten feed was removed from the aquarium. Remarkably, after the ginger crude extract's application, detachment of the fungus was obviously observed and fish skin slowly recovered. This shows that *Achlya* sp. has no tolerance in ginger crude extract water.

Figure 2**.** Tilapia condition after exposure to water mould and ginger crude extract treatment: (a) skin lesion and fungi observed on the fish body after 2 days of fish challenge; (b) mould on the skin become darker, which affected by water quality deterioration after 4 days of treatment; (c) fully recovered fish after treated by the ginger crude extract after 4 days of treatment.

Table 2 shows the physical appearance of tilapia in this experiment. The physical appearance of infected fish was compared to the physical appearance of healthy fish, with the most significant score of 5 indicating the physical appearance of typical healthy fish and the lowest score of 1 showing the critical look of diseased fish. The normality of a fish's movement is measured by its swimming activeness, with a score of 5 indicating normal movement and a score of 1 indicating stagnant movement. Treatment with ginger crude extract aids in treating treatment of sick tilapia fish, with fish treated with ginger crude extract exhibiting improved physical appearance and swimming activity as the score examined day by day rises. Untreated fish in TC (0 ppm) perform poorly, with the same physical performance score. This study found that the higher the ginger crude extract concentration, the quicker the challenged fish recover to normal appearance and activity levels.

Table 2. Physical appearance of tilapia juvenile that being infected based on the changes of fish eyes, skin and swimming activeness.

Treatments	Fish Eye				Fish Skin				Fish Activeness			
	D1	D2	D3	D4	D1	D2	D3	D4	D1	D2	D3	D4
T1 (100 ppm)	2	3	3	4	2	3	3	4	4	5	5	5
T2 (500 ppm)	2	3	3	4	2	3	3	4	4	5	5	5
T3 (800 ppm)	2	4	4	5	2	3	4	5	4	5	5	5
T4 (1000 ppm)	2	4	4	5	2	3	4	5	4	5	5	5
TC (0 ppm)	1	1	1	1	1	1	1	1	1	1	1	1

In fish production, water quality management is vital. A healthy, balanced, and functioning aquaculture system requires optimal water quality. The water quality in this study was kept at the optimum range to reduce fish stress caused by low water quality. Water quality deterioration may cause stress in fish and lead to disease infection and microbial contamination. Fish were fed at apparent satiation to reduce organic pollution from fish feces and uneaten feed (Chong *et al.,* 2011; Makori *et al.,* 2017).

Fungal infection was identified on the tilapia juvenile's body in the form of cottony mycelium, similar to other research conducted in a culture pond on a university campus in Bhopal, India, where the juveniles also exhibit the same fungal infection symptom. One of the causes of mycotic infection is stress, which leads to mass mortality (Chauhan, 2014). Aquaculturists will suffer financial losses as a result. This fungal infection is easily spread because zoospores are linked to the fish body. Infected fish have extensive hyphae accumulation on their skin lesions and epidermal cell necrosis. The hyphae entered the epidermis and the musculature without causing a granulomatous reaction. By exposing tilapia fry to Achlya zoospores, it is feasible to infect them (Panchai *et al*., 2015). *Oreochromis niloticus* mortality was higher after exposure to Oomycetes in a water bath than after intramuscular injection (Ali *et al*., 2011).

Saprolegnia produces significant epidermal destruction and macrophage recruitment (Hussein *et al.*, 2013). According to this study, no fish survived in the control treatment. Ginger has long been utilized as a medicinal herb due to its high concentration of therapeutic phytochemicals. This research shows that ginger may be used as an antifungal to treat disease in aquaculture fish, with diseased fish treated with ginger crude extract surviving 100% of the time. Atai *et al.* (2009) confirmed this finding, while Supreetha *et al.* (2011) discovered that ginger also functions as an antifungal in their research. Since ginger is natural and organic, it contributes to creating green aquaculture unlikely that the application of inorganic chemotherapeutic agents will harm the environment and humans. Since various chemicals are applied in the current aquaculture industry, using plants in fish disease treatment is highly recommended Jana *et al.* (2018).

Conclusion

This study focuses on the survival rate of tilapia fish and the presence of fungi on the body, eyes and gills of fish. Overall, the ginger extract's reaction was rapid because the study results were obtained within 24 hours after the extract was introduced into the treatment aquarium. Fish adaptability and responses to ginger extract treatment show positive effect on physical appearance, swimming activity, and survival rate. Ginger extract treatment at 800 ppm shows a promising result according to the physical responses and survival rate. However, frequent observations using a higher concentration of ginger crude extract at a certain interval of duration for future study is recommended.

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Author Contribution

Norashikin Anjur: Writing- Conceptualization, data curation, original draft and validation. Ruzaini Ahmad: Writing- Review and editing.

Conflict of Interest
The authors declare that they have no known competing financial interests or personal relationship that could have appeared to influence the work reported in this paper.

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