



International Journal of Fisheries and Aquatic Studies

ISSN: 2347-5129
IJFAS 2015; 2(5): 337-340
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www.fisheriesjournal.com
Received: 09-03-2015
Accepted: 07-04-2015

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Study on the antibacterial and antifungal effects of camphor oil; *Cinnamomum camphora* with emphasis on the control of overpopulation of tilapia

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Abstract

In this study, 360 *Oreochromis niloticus* were cultured in 12 aquaria for 3 months at different concentrations of camphor oil *Cinnamomum camphora* (1%, 3% and 5%). The aquaria were divided into 4 groups which were control group (CG), treatment 1 (T1), treatment 2 (T2) and treatment 3 (T3). Each treatment was replicated 3 times and each replicate contained 30 pieces of the tilapia. Water parameters consisting of temperature, pH, ammonia and dissolved oxygen were measured every 2 weeks throughout the culture period. Fish sampling were also done throughout the culture period in once per 2 weeks interval for fish weight and fish mortality. Data collected were analyzed using SAS computer software. The results showed that higher concentrations of camphor (3 and 5%) increased the immune response of the fish towards diseases that is related with bacterial infection.

Keywords: Antibacterial, antifungal, camphor oil, tilapia

1. Introduction

In this era, the world capture fisheries alone can no longer sustain the need of fish protein for the increasing world's population. Due to its nature of rapid growth, large size and palatability, tilapia is the focus of major aquaculture efforts. The world tilapia aquaculture production doubled between 1986 and 1992, and this is followed by Chinese carps and salmonids in total farm production [7]. Furthermore, tilapia is a good candidate for culture due to the fact that it can tolerate, grow, and even reproduce in saline waters, although this will not happen in a very high saline condition [1]. The value of farmed tilapia has also witnessed a great increase during the past two decades, going from US\$ 154 million in 1984 to US\$ 1800.7 million in 2002 [6]. However, diseases such as from bacteria and fungi that infect this species may lead to mortality which causes serious economic losses.

Although tilapia are relatively resistant compared to other fin fishes, many pathogenic organisms still can plague them [1]. Under stress conditions (including poor water quality and high density of fish), tilapia can easily be infected by several opportunistic pathogens belonging to *Streptococcus*, *Enterococcus*, *Aeromonas*, *Pseudomonas*, *Vibrio*, *Flexibacter* and *Edwardsiella* [9]. In order to control the proliferation of bacteria, prophylactic chemotherapeutants have been commonly used and practiced in intensive aquaculture [13]. The abuse of broad-spectrum chemo-therapeutants has resulted in an increased number of antibiotic-resistance bacteria, and, the transfer of these drug-resistance genes to bacteria or virus infecting terrestrial animals and humans [2, 11]. However, alterations of the bacterial flora both in sediments and in water column and residual act as antibiotics in seafood products. Thus it is necessary to exploit a non-chemotherapeutics method instead of the chemotherapeutic methods, such as the use of vaccine, probiotics, immunostimulants and the natural therapeutics from plants [10].

Oil extract of camphor tree using different species of camphor has been proven in experiment to have strong effects on the growth inhibition of fungi [12]. As for antibacterial, one of many chemical compound found in camphor oil which is cinnamaldehyde, is very effective in inhibiting the growth of bacteria [3]. Induction of vitellogenin, alterations in gonads, decrease in fertility and reproduction, and feminization in sex characteristics of male fish have been associated with frequently-used UV filters, including benzophenones and camphor-related substances [5]. Therefore, camphor oil from *Cinnamomum camphora* (*C. camphora*) may be used to control the overpopulation of tilapia.

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Numerous studies have been done, but most of it was done using different species of camphor. However, not much study on the antifungal and antibacterial effects of *C. camphora* has been done specifically towards its effect on overpopulation control of tilapia. Thus, the objectives of this study were to determine the antibacterial and antifungal effect of camphor oil, *C. camphora*; to determine the effect of adding camphor oil to tilapia feed on the growth parameters and immune response of *Oreochromis niloticus* (*O. niloticus*), and whether it can be used to control the overpopulation of tilapia.

2. Materials and Methods

2.1 Experimental fish, tanks and treatments

This study was conducted on 360 tilapia fries for 3 months from November 2014 until January 2015 in 12 aquaria (30 fries per tank) at the Stesen Penyelidikan Akuakultur UPM located in Puchong, Selangor. Four treatments were used with 3 replicates in each (3 treatments with 3 different concentrations of camphor oil, *C. camphora*, one treatment will be kept as a control group), namely CG (no camphor on feed), T1 (1 % camphor), T2 (3 % camphor), and T3 (5 % camphor). These aquaria were dependent on the supply of groundwater as a water source throughout the experiment. Water change was conducted twice a week. All tilapia fries were obtained from the station.

Prior to the experiment, all tilapia were conditioned in an aerated freshwater tank for one week. After the period of acclimation, tilapia fries were distributed into different tanks, fed at the rate of 10% of body weight with feed (commercial powder feed, 32 % protein) mixed with different concentrations of camphor oil, *C. camphora* (1, 3 and 5 %) for duration of 3 months.

Average fish body weight was measured every 2 weeks. Natural mortality was recorded to determine the survival rate in different treatments of camphor and in the control group. At the end of experiment, the fish were sacrificed, weighed, dissected to get the gonads and then later weighed to calculate the gonad/somatic index. Lack of gonadal development suggested the efficacy of camphor oil.

2.2 Water quality parameters

Water temperatures and pH readings of the aquaria were measured using YSI model 60-10-FT, while readings for dissolved oxygen were taken with YSI model DO200. All determinations were done once a week between 0900 and 1000 hours.

2.3 Bacterial and fungal strains

The antibacterial and antifungal effects of camphor oil were evaluated in an *in-vitro* study that conducted at Biology and Health Laboratory, Department of Aquaculture, Universiti Putra Malaysia. The bacterial and fungal strains that were used in this experiment are *A. hydrophila*, *P. fluorescens*, *P. putida*, *Vibrio alginolyticus*, *Candida albicans*, *Mucor* sp. and *Rhizopus* sp.

2.4 Antibacterial and antifungal susceptibility test

Muller's Hinton agar (MHA) was used for culture medium for both bacteria and fungi as a culture medium. The bacteria of genus *Aeromonas*, *Pseudomonas* and *Vibrio* were tested by using *C. camphora* by well diffusion method at four different volumes levels, namely 5 µl, 10 µl, 15 µl, and 20 µl.

Four holes were made into the culture medium. Sterile cotton swab was dipped into the broth culture. Excess culture was

removed by gentle squeezing the swab against the inside of the tube. The swab was then streaked over the entire culture medium. Four treatment levels of *C. camphora* were then dripped into the holes made earlier. The culture was then incubated for 18 hours at 25 °C.

The same processes were repeated, but using samples of fungi from genus *Candida*, *Mucor* and *Rhizopus*. Observation on the presence and size of inhibition zone was determined to observe any antibacterial and antifungal effects of the camphor oil.

Disc diffusion method was done using filter paper disc impregnated with *C. camphora* oil was placed on agar streaked with *P. fluorescens*. The solubility of *C. camphora* was determined by the area size of infiltration around the disc. The presence of inhibition zone around the disc ensured the antibacterial effect of *C. camphora* towards *P. fluorescens*.

2.5 Koch's Postulate

Fish from the four treatments in Puchong were used in the bacterial challenge test. The challenge bacterium was pathogen *A. hydrophila*, cultured in the Fish Biology and Health Laboratory, UPM, Serdang. The dose was determined at 0.1 ml of 10⁸ *A. hydrophila* for each fish. The tilapia used in this test was taken from each treatment (CG1, CG2, CG3, T1R1, T1R2, T1R3, T2R1, T2R2, T2R3, T3R1, T3R2 and T3R3), and divided into four aquaria, each for different treatment and stocked with 10 fish per tank. The fish was challenged by intraperitoneal injection. Results were checked day by day until mortalities subsided. The results were then recorded.

2.6 Statistical analysis

A multiple comparison (Duncan) test was conducted to examine the significant differences among treatments using the SAS computer software (SAS Institute Inc., Cary, NC, USA). A statistically significant difference was required at $p < 0.05$.

3. Results and Discussion

3.1 Results of the biological experiment of tilapia

Water temperatures and other water quality parameters of different treatments were summarized in Table 1. There were significant differences ($p < 0.05$) in water temperature, ranging from 26.8381 to 27.4667 °C. Significant differences ($p < 0.05$) were also observed in pH values, ranging from 8.93857 to 9.39238. Both dissolved oxygen and ammonia levels in the different treatment aquaria showed no significant variations. Dissolved oxygen level varied from 3.79571 to 3.86048 mg/L. The range of ammonia in the treatments aquaria was 0.30619 to 0.45143 mg/L. There were significant differences in temperature between CG to T1 and T3, T1 to CG, T2 to T3, and T3 to CG and T2. This was due to the location of the each treatment aquaria themselves which affected the temperature. CG and T2 were located above T1 and T3, respectively, thus blocking any direct sunlight towards the treatment tanks below. This was why CG and T2 were of higher temperature compared to T1 and T3. pH readings had no significant differences ($p < 0.05$) between treatments except for CG towards T1, which showed no significant difference. However, different treatments of *C. camphora* did not affect the water parameters and fish's body weight and survival rate in this study, as shown in Table 2. There was no significant difference ($p < 0.05$) between CG, T1, T2 and T3 in terms of average body weight and survival rate. This meant that the average body weight and survival rate in different treatments of camphor and in the control group were not affected by different treatment levels.

Table 1: Mean values for water parameters of different treatments

Treatment	Temperature (°C)	DO (mg/L)	pH	Ammonia (mg/L)
CG	27.4667 ^a	3.85429 ^a	9.18143 ^b	0.30619 ^a
T1	27.0571 ^{bc}	3.86048 ^a	9.10857 ^b	0.40143 ^a
T2	27.3381 ^{ab}	3.79571 ^a	9.39238 ^a	0.35429 ^a
T3	26.8381 ^c	3.82571 ^a	8.93857 ^c	0.45143 ^a

Table 2: Average body weight with standard deviation and survival rate of different treatments for three months

Treatment	Average early body weight with standard deviation (g)	Average final body weight with standard deviation (g)	Survival rate (%)
CG	0.41 ± 0.02 ^a	7.24 ± 0.22 ^a	98.6 ^a
T1	0.41 ± 0.02 ^a	7.22 ± 0.11 ^a	98.4 ^a
T2	0.41 ± 0.03 ^a	7.53 ± 0.20 ^a	98.3 ^a
T3	0.40 ± 0.03 ^a	7.10 ± 0.09 ^a	97.7 ^a

3.2 The In-vitro bacterial and fungal susceptibility test to camphor oil by well diffusion method

Well diffusion method was done to check the susceptibility of the bacteria towards different concentrations of camphor. As shown in Figure 1, there was no effect of camphor in inhibiting the bacterial strains. Experiments of susceptibility of *P. putida* and *V. alginolyticus* towards camphor also showed similar results. However, according to Chang, their experiments were proven successful with the use of *C. osmophloeum* against the bacterial strains; *Escherichia coli*, *Enterococcus faecalis*, *Klebsilia pneumoniae*, *Salmonella* sp, *Staphylococcus aureus*, methicillin-resistant *S. aureus*, *S. epidermidis*, *P. aeruginosa* and *V. parahemolyticus* in broth dilution method [3]. These were done with three replicates each.

The same exact methods were repeated for the fungal strains. Figure 2 showed the camphor oil against *C. albicans* in well diffusion method. There was no inhibition zones observed from this experiment. This was repeated but using different fungal strains, including *Mucor* sp. and *Rhizopus* sp. with no inhibition zones as well. Lee stated that *C. camphora* cannot inhibit the five fungi used in their experiment [8]. Nevertheless, in the study done by Cochrane, showed that inhibition zone was observed against *C. albicans* by using *C. camphora* although the same study showed that *C. albicans* was the least inhibited compared to other fungal strains [4].

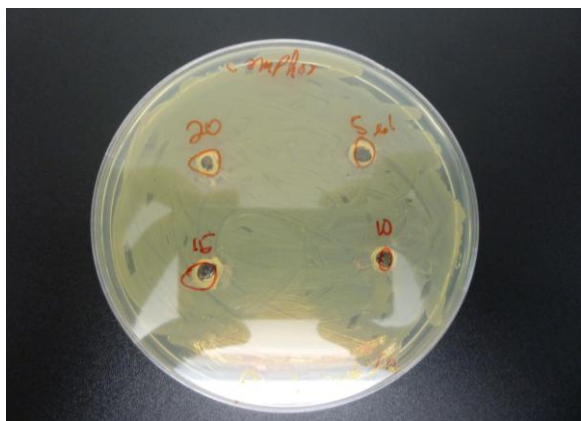


Fig 1: *A. hydrophila* on a Muller's Hinton agar in well diffusion method

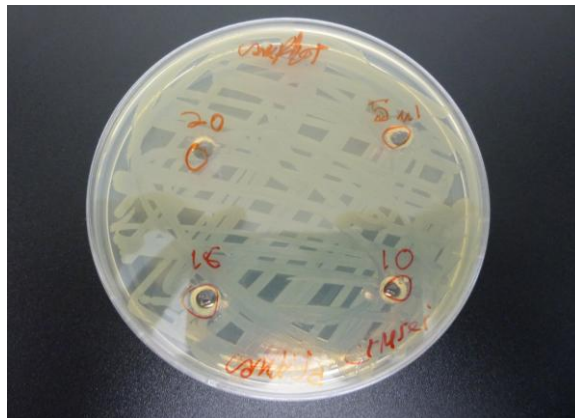


Fig 2: *C. albicans* on Muller's Hinton agar in well diffusion method

3.3 Disc diffusion method on bacterial susceptibility test to camphor oil

Disc diffusion method was done to check bacteria's susceptibility towards *C. camphora*, specifically of *P. fluorescens*. As observed from Figure 3, there was no inhibition zone. To the best of our knowledge, there have not been many studies investigating the effectiveness of camphor against fish-related fungi, therefore justifying results of the experiment.



Fig 3: *P. fluorescens* in disc diffusion method

3.4 Challenge test of experimental tilapia against A. hydrophila

The mortality rate of injected fish challenged with a hot strain of *A. hydrophila* was listed in Table 3. Death occurred after 24 hours for CG and T1 (both fed with no camphor and 1% camphor mixed feeds respectively). By the end of Day 3, all fish in T1 died. By contrast, all of the fish in T2 and T3 both survived after Day 1. At the end of the challenge test, the highest survival rates were shown in fish that received treatment of 3% and 5% camphor in their rations. It was noted that using *C. camphora* with tilapia feed has positive effect in boosting fish immune response. This was indicated by high survival rate and low challenge; both were fed with 3% and 5% of camphor oil-mixed feeds respectively.

Table 3: Results of challenge test of different treatments

Treatment	Mortality rate (pcs)			Total mortality (pcs)	Percentage (%)
	Day 1	Day 2	Day 3		
CG	2	2	1	5	50
T1	4	3	3	10	100
T2	3	0	0	3	30
T3	3	0	0	3	30

4. Conclusion

There were no significant differences, regarding growth rate and water parameters between different treatments. The addition of *C. camphora* with feed, (3%-5%) showed significant effect in increasing the immune response and resistance of tilapia against virulent bacteria, which in this study was *A. hydrophila*. From the results, it is recommended to use 3% to 5% of *C. camphora* as one of feed additive.

5. Acknowledgements

We would like to express our greatest gratitude to our supervisor, Dr Yasser Mohamed Abdelhadi for his motivation, effort and time assisting us in finishing our research. Extended thanks to Mr Mohd Fariq bin Ahmat, Mrs Nur Shafika Maulad Abd Jalil, Mr Muhamad Hafizh bin Abdul Rahim and Mr Mohd Yusuf bin Ismail for their support and generosity in helping us in this research. We also want to thank all individuals who have helped us directly or indirectly in completing this research.

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