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Experimental pathogenicity of *Achlya* species from cultured Nile tilapia to Nile tilapia fry in Thailand

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Abstract. Experimental infection of Nile tilapia (*Oreochromis niloticus*) fry using 6 *Achlya* isolates from cultured Nile tilapia with water mold infections was attempted. The experimental fish were exposed to 1.0×10^2 and 1.0×10^4 zoospores mL^{-1} of each *Achlya* isolate after ami-momi treatment. The cumulative mortality rates of fish exposed to 1.0×10^4 zoospores mL^{-1} of *A. klebsiana* BKKU1003, and *A. diffusa* BKKU1012 were 88.8 and 77.7%, respectively. *A. klebsiana* BKKU1003 was more pathogenic than the other isolates. Histopathological examination of the skin of Nile tilapia fry exposed to 1.0×10^2 zoospores mL^{-1} of *A. klebsiana* BKKU1003 showed numerous hyphae grew on the skin surface and some areas of skin were sloughed. The fish exposed to 1.0×10^4 zoospores mL^{-1} of *A. klebsiana* BKKU1003 showed massive accumulated hyphae on skin lesions with necrosis of the epidermal cells and the hyphae penetrated from the epidermis to the musculature without granulomatous response surrounding the hyphae. We found that it is possible to infect tilapia fry by exposing them to zoospores of *Achlya* after the ami-momi treatment.
Key Words: oomycete, ami-momi treatment, *Oreochromis niloticus*, histopathology.

Introduction. Water mold infection has usually found in Nile tilapia (*Oreochromis niloticus*) in Thailand' hatcheries under natural condition. It is caused by a member in Family Saprolegniaceae included genera *Achlya*, *Aphanomyces* and *Saprolegnia* (Yuasa et al 2000). This infection has been a serious problem and continued to occur in intensive culture during the cool season (Willoughby & Lilley 1992; Chinabut et al 1995; Chukanhom & Hatai 2004; Hanjavanit et al 2012). Water mold infection could occur throughout the year, especially when water temperatures fluctuate, handling stress occurs, fish density is extremely high, or skin parasites exist (Noga 1993). Saprolegniasis has been reported from cultured economic fish and eggs in Thailand (Willoughby & Lilley 1992; Chinabut et al 1995; Lawhavinit et al 2002; Chukanhom & Hatai 2004), and *Achlya* spp. were isolated from dead fish (Willoughby & Lilley 1992) and from eggs of common carp (*Cyprinus carpio*) (Chukanhom & Hatai 2004), eggs of Nile tilapia (Panchai et al 2007), eggs of Mekong giant catfish (*Pangasianodon gigas*) (Abking et al 2012) and eggs of African catfish (*Clarias gariepinus*) (Hanjavanit et al 2012). They were also isolated from the diseased Nile tilapia (Panchai et al 2014). Some species of Saprolegniaceae was mentioned as primary pathogen (Yuasa & Hatai 1995; Stueland et al 2005), whereas most species were mentioned as secondary pathogens (Lilley & Roberts 1997; Sosa et al 2007). Therefore, it is important to determine whether the isolated strains are able to infect fish under laboratory condition. An ami-momi (AM) treatment is a notable method to enhance the sensitivity of fish to water mold for artificial infection, which produces the

physical stress and scarification of fish skin (Hatai & Hoshiai 1993; Yuasa & Hatai 1995; Grandes et al 2001; Hussein & Hatai 2002; Kiryu et al 2002; Stueland et al 2005; Hanjavanit et al 2010; Hussein et al 2013) by shaken fish in a scoop net in the air (Hatai & Hoshiai 1993). Objectives of this study were to induce *Achlya* spp. infection to Nile tilapia fry under the AM treatment and examine the histopathological characters of the infected fish.

Material and Method

Source of experimental fish. Approximately three hundred healthy Nile tilapia fry (average 0.7–0.8 g in body weight and 3–4 cm in total length) were provided by Khon Kaen Inland Fisheries Research and Development Center and were used for the experimental infection. The fish were acclimatized at 25°C for one week. The fish were fed with commercial formula food (GF Feed, Krungthai Feedmill Public Co., Ltd., Bangkok, Thailand) daily and starved for a few days before the experiment. The experiment was carried out at Department of Biology, Faculty of Science, Khon Kaen University, Thailand during November to December 2014.

Induction of tilapia skin lesion. Two sets of fish were prepared and designed for induction of skin lesion. Group I, three fish were random selected and then shaken in a fan-shaped scoop net (10 cm in diameter) in the air for 0, 1, 2, 3, 4 and 5 minutes, respectively. This shaking process is the AM treatment (Hatai & Hoshiai 1993). The fish were then rinsed with sterilized tap water (STW) to eliminate excess mucus and then placed into each 500 mL of STW for 7 days. The experiment was carried out in a plastic tank (8 cm wide x 15 cm long x 11 cm high with water depth 4.5 cm), which placed in an experimental pond at constant temperature of 25°C. Aeration was supplied during the experiment. Cumulative mortality rates were also noted each day during the test period. Group II, the fish after the AM treatment as described above were immediately fixed in 10% phosphate buffered formalin (PBF) solution and decalcified with ethylene diamine tetra-acetic acid (EDTA) solution for histological examination. Three parts including head, trunk and caudal regions (0.5 x 0.5 cm) of the fixed fish were dissected (Figure 1) and processed into paraffin method. After that, the samples were sectioned at 5 µm with a sliding microtome. All of the section slides were stained with hematoxylin and eosin (H & E). The thickness of epidermis, which was defined as distance between apical surface of the uppermost nucleated epidermal cell and basement membrane (Wisenden & Smith 1997) and was measured by an ocular micrometer at 40x magnification (n = 5). The epidermal thickness was statistically analyzed using two-way ANOVA with Fisher's least significant difference (LSD) multiple comparison test to determine significant differences between region and treatment (Zar 2010). Three replicates of each treatment were conducted. Nine fish were used for each treatment.

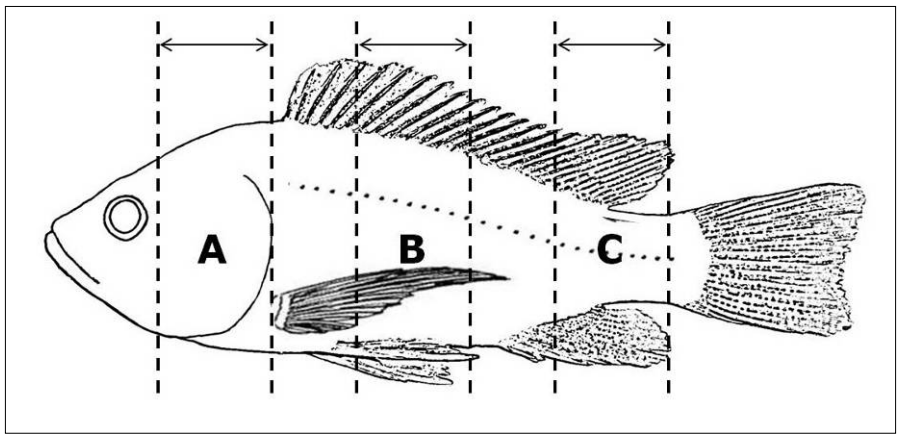

Figure 1. The positions of skin section: head (A), trunk (B), caudal regions (C) of Nile tilapia fry.

Achlya isolates used in this study. *Achlya* isolates (*A. klebsiana* BKKU1003, *A. bisexualis* BKKU1007, *A. diffusa* BKKU1012, *Achlya* sp. BKKU1117, *A. prolifera* BKKU1125 and *Achlya* sp. BKKU1127) were obtained from net cage-cultured Nile tilapia with water mold infections on the Nam Phong River in Khon Kaen Province, northeastern Thailand from September 2010 to August 2011 (Table 1) (Panchai et al 2014). The identified to species was examined by morphological structures of reproductive organs on hemp seeds (*Cannabis sativa*) cultures according to key references (Kitancharoen et al 1995; Johnson et al 2002; Chukanhom & Hatai 2004) and molecular identification (Lilley et al 2003; Phadee et al 2004; Muraosa et al 2009, 2012). They were routinely maintained on glucose yeast extract (GY) agar (Hatai & Egusa 1979) at 25°C and transferred to fresh GY agar every month and were deposited at the Department of Biology, Faculty of Science, Khon Kaen University, Thailand.

Table 1

Achlya spp. used in this study were isolated from Nile tilapia with water mold infections in cage culture in the Nam Phong River, Khon Kaen Province

Species	*Remark*	*Location*	*Date*
Achlya klebsiana	BKKU1003	Farm A, Ban Hua Sua–tent, Nam Phong district	23 September 2010
A. bisexualis	BKKU1007	Farm B, Ban Hua Sua–tent, Nam Phong district	23 September 2010
A. diffusa	BKKU1012	Farm C, Ban Hua Sua–tent, Nam Phong district	23 September 2010
Achlya sp.	BKKU1117	Farm B, Ban Hua Sua–tent, Nam Phong district	19 January 2011
A. prolifera	BKKU1125	Farm C, Ban Hua Sua–tent, Nam Phong district	19 January 2011
Achlya sp.	BKKU1127	Farm C, Ban Hua Sua–tent, Nam Phong district	20 February 2011

Preparation of zoospores. Zoospore suspensions from each isolate were prepared as follows: a small piece of the 3 days growing edge agar (5 x 5 cm) with hyphae (agar block) was cut off. Three agar blocks were placed in a plastic Petri dish containing GY broth for 3 days at 25°C. After that, only mycelia were cut and washed in successive baths of sterilized tap water (STW) and continuous incubation for 16–18 h at 25°C to get zoospores. The number of zoospores was counted using a hemocytometer (Neubauer counting chamber, Erma®) and adjusted to approximately 1.0×10^2 and 1.0×10^4 zoospores mL^{-1} for experimental infection.

Experimental design of artificial infection. Fish were randomly taken from holding tank and subdivided into four groups. Group A: AM group, fish (n = 3) were shaken in a fan-shaped scoop net in the air for 2 minutes and then rinsed with STW to eliminate excess mucus. Group B: non-AM group, both groups A and B were exposed to 1.0×10^2 and 1.0×10^4 zoospores mL^{-1}. Group C: AM group, and group D: non-AM group were control of groups A and B, respectively, which exposed to STW. The experimental fish were maintained in the same condition as described in Experiment induction of tilapia skin lesion. Numbers of fish exposed to each isolate of each concentration and the control group were nine. Both infected and controlled groups were kept observation for 7 days by naked eyes. These experiments were performed after the experimental protocol had been approved by the Institutional Animal Ethics Committee, Khon Kaen University, Thailand (Reference NO. 0514.1.12.2/33).

To confirm the presence of the *Achlya* isolates, triplicate samples of the infected hyphae from infected moribund fish were repeatedly placed onto GY agar for identification. Infection and cumulative mortality rates were also daily recorded during the assay and tested statistically using one-way ANOVA with Fisher's least significant

difference (LSD) multiple comparison test to determine significant differences among treatment (Zar 2010).

Histopathological examination. Gross pathological changes and lesions were observed and photographed. After removal of some hyphae as described above, the fish were routinely necropsied. The histopathology of hyphal infection was verified. All fish were fixed in 10% PBF solution and skin lesions were removed for preparing permanent slides by paraffin method, the same procedure as in Experiment induction of tilapia skin lesion. All slides were stained with H & E. Some selected slides were also stained with periodic acid-Schiff (PAS) reagent, Giemsa, Gram stain and Uvitex 2B (Wada et al 2003) to observe water mold hyphae in the tissues.

Results

Induction of tilapia skin lesion. Gross observation of the control and the treatment groups appeared normal (Figure 2). It was found that fish of the control and treatment groups did not die after the AM treatment for 1 and 2 minutes. Whereas, the cumulative mortality was occurred at day 1 after the AM treatment for 3 to 5 minutes (Table 2).

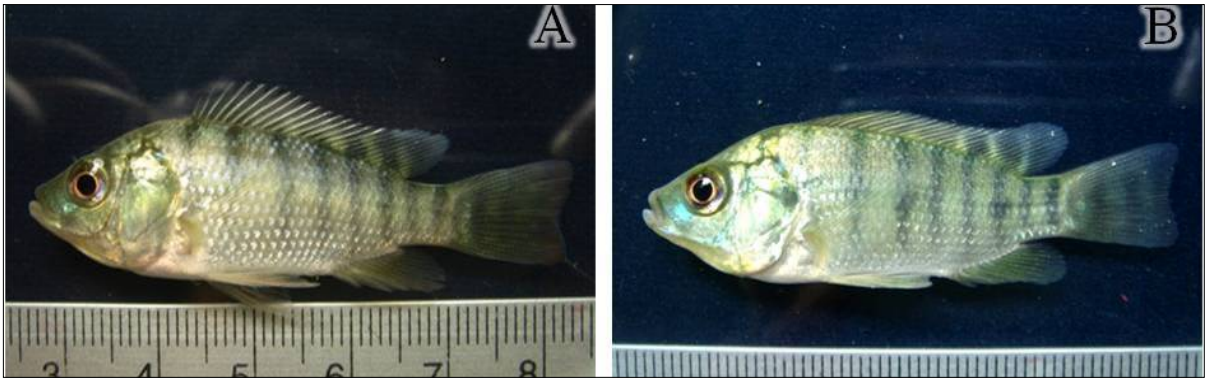

Figure 2. Gross morphology of Nile tilapia fry after net shaking. A: the control group; B: the treatment group shaking for 2 minutes.

Table 2

Cumulative mortality of Nile tilapia fry/total fish examined after the ami-momi treatment (D = day)

Durations (minute)	*No. of dead fish / No. fish examined*							*Total*
	1D	*2D*	*3D*	*4D*	*5D*	*6D*	*7D*	
Control	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0
3	1/9	1/9	0	0	0	0	0	2/9
4	2/9	0	0	0	0	0	0	2/9
5	5/9	0	0	0	0	0	0	5/9

Histological examination of induction of tilapia skin lesion. From the microscopic examination of the skin on the head, trunk and caudal regions of the control and AM groups consisted of epidermis, dermis and hypodermis covering muscular layer. The epidermis of the control group consisted of stratified squamous epithelium with scattered mucous cells (Figure 3A) and more mucous cells were found at the head region than the trunk and caudal regions (Figure 3B-C). The epidermis at the head region was thicker than those of the trunk and caudal regions with significant difference ($p < 0.05$, Table 3). The epidermis of the AM groups after 1-2 minutes treatment was composed of 3-4 layers of epidermal cells and no mucous cells appeared (Figure 3D-F). The AM group after 3 minutes treatment, the most outer cells of epidermis was necrosis and consisted of 2-3

epidermal cells layer. Whereas, the most outer cells of epidermis of the AM groups after 4 and 5 treatment were also necrosis and only 1-2 epidermal cells layer occurred (Figure 3G-I). It was also found that the epidermal thickness of all AM groups was thinner than that of the control group with significant difference ($p < 0.05$, Table 3) except the AM group after 1-2 minutes treatment showed non-significant difference from the control group ($p > 0.05$, Table 3).

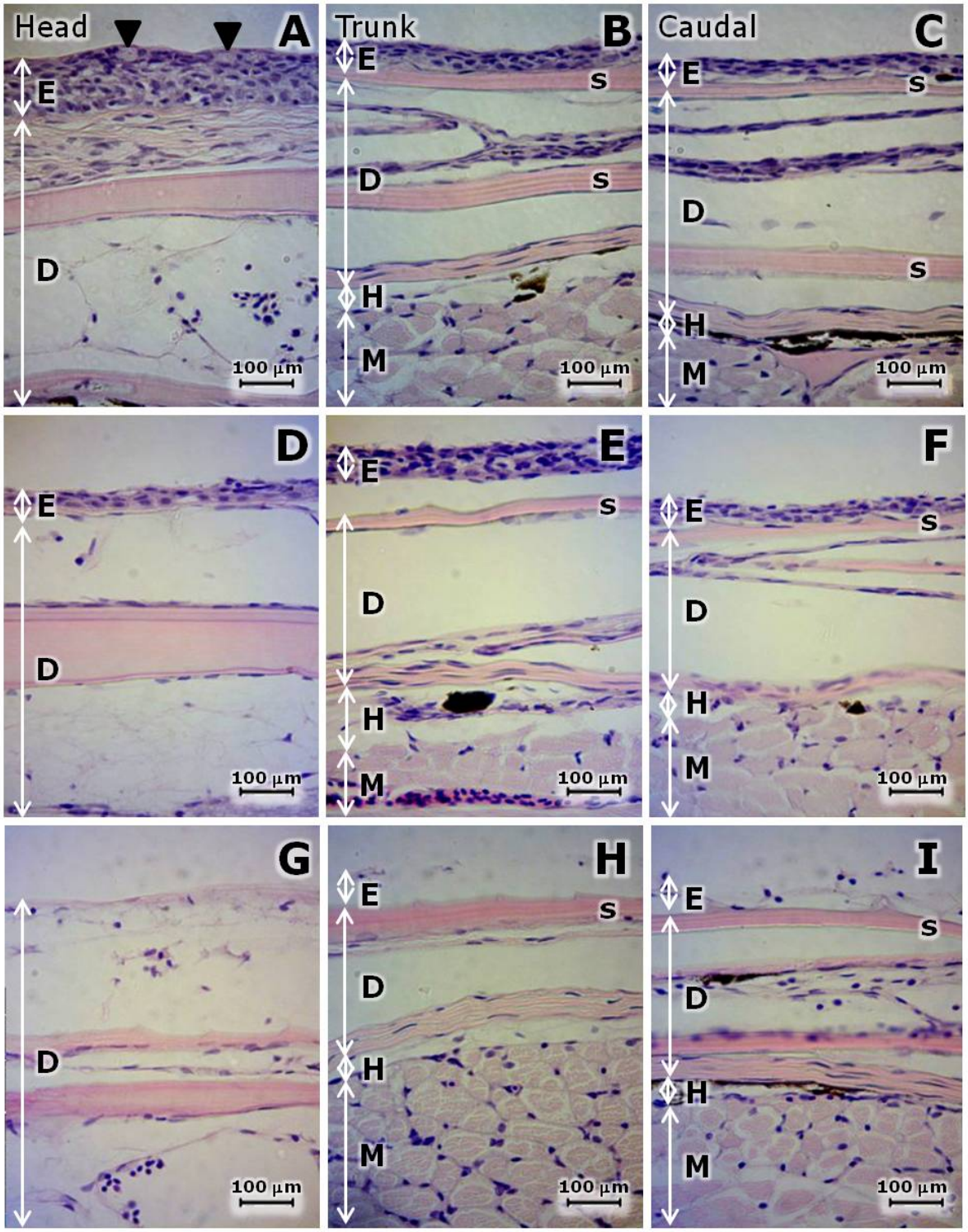

Figure 3. Cross section of skin from the head (A, D, G), trunk (B, E, H) and caudal (C, F, I) regions of Nile tilapia fry after the ami-momi treatment showing epidermis (E), scale (s), dermis (D), hypodermis (H), muscle (M) (H & E). A-C: the control group; D-F: the AM group for 2 minutes; G-I: the AM group for 5 minutes (arrow heads = mucous cells).

From the results of mortality and histological characteristics of skin of the AM groups, the 2 minutes AM treatment was selected as a representative duration time for experimental infection because no fish died and it was possible to induce the scarification of skin.

Table 3

Mean ± standard deviation (SD) of epidermal thickness of Nile tilapia fry (n = 5) of both control and treatment groups after net shaking

Durations (minute)	*Epidermal thickness (Mean ± SD) (µm)*		
	Head	*Trunk*	*Caudal*
Control	$211.0^{a} \pm 7.1$	$149.0^{b} \pm 2.1$	$113.0^{c} \pm 12.5$
1	$192.0^{a,d} \pm 5.5$	$138.0^{b,g} \pm 2.1$	$108.0^{c,j} \pm 5.5$
2	$179.0^{a,\ d} \pm 12.9$	$138.0^{b,g} \pm 2.1$	$111.0^{c,\ j} \pm 3.6$
3	$157.0^{d} \pm 21.7$	$140.0^{g} \pm 8.9$	$96.4^{j} \pm 3.6$
4	$132.0^{e} \pm 9.5$	$121.0^{h} \pm 6.2$	$77.4^{k} \pm 2.1$
5	$21.4^{f} \pm 6.2$	$13.1^{i} \pm 4.1$	$8.3^{l} \pm 2.1$

Different letters within a column indicate statistically significant differences between treatments ($p < 0.05$).

Experimental design of artificial infection. All the *Achlya* isolates used in group A (AM group) were able to infect the experimental fish. The infection was clearly visible at the site of injured areas of experimental fish and mortality were occurred at days 3 and 2 when exposed to 1.0×10^{2} and 1.0×10^{4} zoospores mL^{-1} by naked eyes, respectively. The moribund fish revealed serious oomycete infections, characteristically with hemorrhage comprising of cotton like tuft on the skin surface of forehead, dorsal fin and caudal peduncle (Figures 4A, 5A). An analysis of infection rates and cumulative mortality is summarized in Table 4. Based on the infection rate and cumulative mortalities when the fish exposed to 1.0×10^{4} zoospores mL^{-1} of each isolate, it was divided into 3 groups. Group I, high cumulative mortality 77.7–88.8% included *A. klebsiana* BKKU1003 and *A. diffusa* BKKU1012. Group II, moderately cumulative mortality 55.5–66.6% included *Achlya* sp. BKKU1117, *A. prolifera* BKKU1125 and *Achlya* sp. BKKU1127. Group III, low cumulative mortality 33.3% was *A. bisexualis* BKKU1007.

Neither sign of oomycete infection (0% infection) nor mortality (0% mortality) was recorded in group B (non-AM group) as shown in Table 4. It was also found that all fish in groups B (non-AM group), C (control of AM group) and D (control of non-AM group) were able to survive throughout the experimental period (7 days).

During the experiment, the *Achlya* spp. used in the experiments were successfully re-isolated from the lesions on the skin or muscle of some moribund fish. The morphology and growth patterns of the re-isolated *Achlya* were identical to those of the artificial isolates (Kitancharoen et al 1995; Johnson et al 2002; Chukanhom & Hatai 2004), fulfilling Koch's postulates.

Histopathological examination of experimental artificial infection. From microscopic examination of infected skin lesions with all isolates, showed hyphae covering the surface of the epidermis, the upper epidermal cell layers of skin lesions were disorganized and completely sloughed. Epidermal cells affected with hyphae were severely necrotized (Figure 4B, C). Whereas, isolate of *A. klebsiana* BKKU1003 in 1.0×10^{4} zoospores mL^{-1} caused highest infection (9/9 moribund fish, 100%). Three out of 9 moribund fish (33.3%) had massive accumulations of hyphae on the skin lesions and they penetrated the epidermis, passing through the dermis into the musculature of the trunk region (Figure 5B, C). Scattered capillaries and inflammatory cells were observed in the dermal layer. No granulomas were found surrounding the hyphae. The hyphae were not easily visible with H&E stain, but they were clearly observed with PAS and Uvitex 2B. Giemsa and Gram stains failed to reveal any bacteria within the lesions.

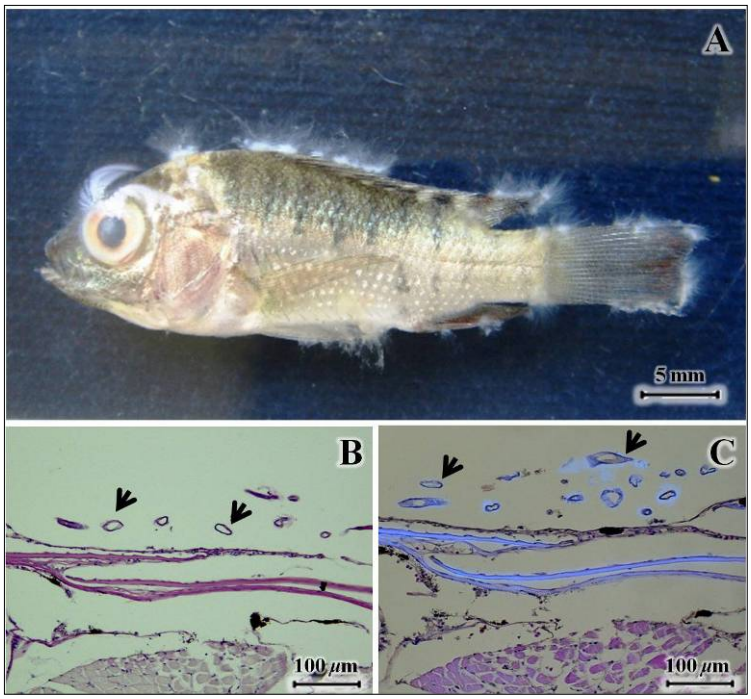

Figure 4. A: Nile tilapia fry exposed to 1.0 x 10^2 zoospores mL^{-1} of *A. klebsiana* BKKU1003 for 3 days. Note the hyphae on the head, dorsal and ventral fins and caudal peduncle. B: Histopathology of skin of moribund tilapia fry showing hyphae with pink color (arrows) attached on epidermis (PAS). C: Serial section of B showing positive Uvitex 2B-H&E stain of hyphae with blue-white fluorescence (arrows).

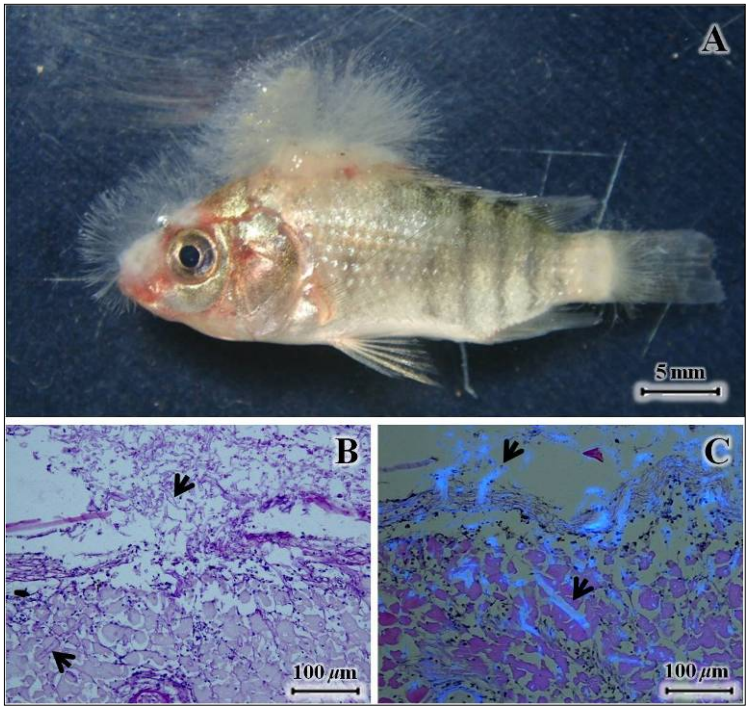

Figure 5. A: Nile tilapia fry exposed to 1.0 x 10^4 zoospores mL^{-1} of *A. klebsiana* BKKU1003 for 4 days. Note the hyphae on the head, dorsal fin and caudal peduncle. B: Histopathology of skin of moribund tilapia fry showing aseptate hyphae with pink color (arrows) invaded musculature (PAS). C: Serial section of B showing positive Uvitex 2B-H&E stain of hyphae with blue-white fluorescence (arrows).

Table 4

Infection (no. fish with infection/no. fish examined) and mortality (no. fish with mortality/no. fish examined) of Nile tilapia fry experimentally exposed to zoospores of each *Achlya* isolate for 7 days

Species	*Exposure level*							
	Ami-momi treatment				*Non-ami-momi treatment*			
	1.0x10^2 zoospores mL^{-1}		*1.0x10^4 zoospores mL^{-1}*		*1.0x10^2 zoospores mL^{-1}*		*1.0x10^4 zoospores mL^{-1}*	
	No. fish with infection/ No. fish examined (%)	*No. fish with mortality/ No. fish examined (%)*	*No. fish with infection/ No. fish examined (%)*	*No. fish with mortality/ No. fish examined (%)*	*No. fish with infection/ No. fish examined (%)*	*No. fish with mortality/ No. fish examined (%)*	*No. fish with infection/ No. fish examined (%)*	*No. fish with mortality/ No. fish examined (%)*
Control	0/9 (0)^{a}*	0/9 (0)^{a}	0/9 (0)^{a}	0/9 (0)^{a}	0/9 (0)^{a}	0/9 (0)^{a}	0/9 (0)^{a}	0/9 (0)^{a}
A. klebsiana BKKU1003	3/9 (33.3)^{b}	3/9 (33.3)^{b}	9/9 (100)^{c}	8/9 (88.8)^{d}	0/9 (0)^{a}	0/9 (0)^{a}	0/9 (0)^{a}	0/9 (0)^{a}
A. bisexualis BKKU1007	1/9 (11.1)^{a,b}	0/9 (0)^{a}	4/9 (44.4)^{b}	3/9 (33.3)^{b}	0/9 (0)^{a}	0/9 (0)^{a}	0/9 (0)^{a}	0/9 (0)^{a}
A. diffusa BKKU1012	3/9 (33.3)^{b}	2/9 (22.2)^{a,b}	8/9 (88.8)^{c}	7/9 (77.7)^{c,d}	0/9 (0)^{a}	0/9 (0)^{a}	0/9 (0)^{a}	0/9 (0)^{a}
Achlya sp. BKKU1117	2/9 (22.2)^{a,b}	1/9 (11.1)^{a,b}	7/9 (77.7)^{c}	5/9 (55.5)^{c}	0/9 (0)^{a}	0/9 (0)^{a}	0/9 (0)^{a}	0/9 (0)^{a}
A. prolifera BKKU1125	1/9 (11.1)^{a,b}	0/9 (0)^{a}	7/9 (77.7)^{c}	6/9 (66.6)^{c,d}	0/9 (0)^{a}	0/9 (0)^{a}	0/9 (0)^{a}	0/9 (0)^{a}
Achlya sp. BKKU1127	1/9 (11.1)^{a,b}	1/9 (11.1)^{a,b}	8/9 (88.8)^{c}	6/9 (66.6)^{c,d}	0/9 (0)^{a}	0/9 (0)^{a}	0/9 (0)^{a}	0/9 (0)^{a}

*Different letters within a column indicate statistically significant differences between treatments ($p < 0.05$).

Discussion. Water mold infection is a common problem in aquaculture and it is widespread in all stages of the fish life cycle (Bruno et al 2011). Experimental infections to demonstrate the pathogenicity of *Saprolegnia* or *Aphanomyces* have been attempted by some methods, such as zoospore injection into the muscle or exposure to zoospore suspension (Howe et al 1998; Khan et al 1998; Johnson et al 2004). These experiments could not reproduce the clinical signs of water mold infection, especially in salmonid fish. However, Hatai & Hoshiai (1993) succeeded in reproducing the clinical signs of saprolegniasis using the AM treatment, which enhanced the susceptibility of fish to *Saprolegnia* infection and the AM treatment predisposes fish infection because it causes stress and a decrease in epidermal thickness and in the number of mucous cells. In the present study, we selected 2 minutes of AM treatment for induced the lesion of skin of Nile tilapia fry due to there were no fish died during the experiment. This result was supported by other researches who used 2 minutes for the AM treatment in juvenile of rainbow trout (*Oncorhynchus mykiss*) (Yuasa & Hatai 1995; Grandes et al 2001), fingerlings of masu salmon (*O. masou*), sockeye salmon (*O. nerka*), brown trout (*Salmo trutta*), Japanese char (*Salvelinus leucomaenis*) (Hussein & Hatai 2002), adult of platyfish (*Xiphophorus maculates*) (Hanjavanit et al 2010) and sub-adult Nile tilapia (Hussein et al 2013).

The pathogenicity tests using 6 isolates of the genus *Achlya* from infected Nile tilapia were carried out using Nile tilapia fry. We succeeded in producing experimental infection using the AM treatment. All *Achlya* isolates demonstrated their pathogenicity toward the test fish. The levels of infection and mortality were classified into 3 groups, which were high infection (77.7–88.8% cumulative mortality), moderately infection (55.6–66.6% cumulative mortality), and low infection (33.3% cumulative mortality) to Nile tilapia fry. From this study, it may be stated that 6 isolated *Achlya* spp. from cultured Nile tilapia have pathogenicity to Nile tilapia fry. The fish exposed to *A. klebsiana* BKKU1003 showed a high infection ratio and cumulative mortality than those exposed to others, and the differences were statistically significant ($p < 0.05$). Similar results using *A. klebsiana* were reported by Chukanhom & Hatai (2004) who demonstrated pathogenicity against platy (*Xiphophorus maculates*), which showed 100% infection rate of injured fish when exposed to 1.0×10^4 zoospores mL^{-1}. In the present study, the isolate of *A. bisexualis* BKKU1007 showed the lowest pathogenicity, which was similar to the pathogenicity test against guppy (*Poecilia reticulata*) reported by Lawhavinit et al (2002), which showed 33% infection rate of injured fish when exposed to 1.0×10^2 zoospores mL^{-1}. The pathogenicity differences among *Achlya* species may be due to the water mold species or the number of zoospores (Howe et al 1998).

In the current study, the histopathology of Nile tilapia fry exposed to zoospores of *Achlya* after the AM treatment showed numerous hyphae covering the surface of the epidermis and invading into the muscle. Whereas, Hussein et al (2013) reported the experimental infection of *A. proliferoides* in sub-adult tilapia and found mycelial mats attached to the surface of the epidermis, with some penetrating only in the dermal layer. The difference of these pathological findings may be caused by the size or stage of fish. In addition, this may be related to the differences in pathogenicity of the isolates or host response (Sosa et al 2007). No granulomas were found around the hyphae in the affected musculature, which may be due to the rapid growth of the hyphae, as mentioned by Hatai & Hoshiai (1992).

The AM treatment is a kind of physical trauma, which could conduct neuroendocrine stress leading to immunosuppression in fish (Hatai & Hoshiai 1994). Moreover, the treatment decreased in epidermal thickness and in the number of mucous cells, which has been proposed to increase risk of fungal infection (Pickering 1977; Richards & Pickering 1979). Noga (1993) stated that the importance of mucous and skin as a physical barrier against external pathogens. In addition, the direct cause of death in the infected fish is most likely related to massive osmoregulatory system problems and a lethal dilution of body fluids (Grandes et al 2001; Fontenot & Neiffer 2004). The healthy fish directly exposed to a number of zoospores (1.0×10^4 zoospores mL^{-1}), it was found that no water mold infection appeared. This pointed out that the AM treatment is needed as a primary inducer for the water mold infection and these *Achlya* spp. might be

opportunistic pathogens which was supported by Lilley & Roberts (1997) and Sosa et al (2007), who stated that species of the genus *Achlya* are usually known as saprophytic or secondary, opportunistic invaders.

Environmental factor such as temperature may play an important role, as the water mold infection usually occurs in the cool season (Willoughby & Lilley 1992; Chinabut et al 1995; Chukanhom & Hatai 2004; Hanjavanit et al 2012). The present study maintained an experimental temperature at 25°C for artificial infection because this temperature is in a range that caused the water mold infection in nature.

Conclusions. This is the first report to induce *Achlya* spp. infection in Nile tilapia fry under the AM treatment in Thailand. It seems likely that the AM treatment has the same effect as handling or crowded conditions for the fish, inducing stress and disturbing their defense mechanisms. This treatment may also induce external mycotic zoospores to make contact with the skin, which serves as a highly supportive nutritional source. Our comparative study showed that 1.0×10^4 zoospores mL^{-1} of *A. klebsiana* BKKU 1003 had the highest pathogenicity. These results demonstrate the first success using the AM treatment to induce *Achlya* spp. experimental infection using the same host.

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