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Effects of four Thai herbs *in vitro* on *Achlya* spp. isolated from Nile tilapia (*Oreochromis niloticus*) in Thailand and the toxicity to Nile tilapia fry

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Abstract. The anti-oomycetic activity of four Thai herbs including fruit peel of Pomegranate (*Punica granatum*), leaf of Eugenia (*Syzygium gratum*), fresh fruit of Indian gooseberry (*Phyllanthus emblica*) and Myrabolan wood (*Terminalia chebula*) were examined for their efficacy to control growth of five pathogenic *Achlya* (*Achlya klebsiana* BKKU1003, *Achlya diffusa* BKKU1012, *Achlya* sp. BKKU1117, *Achlya prolifera* BKKU1125 and *Achlya* sp. BKKU1127). The minimum inhibitory concentrations (MIC) of aqueous extraction of Pomegranate, Eugenia, Indian gooseberry and Myrabolan wood were 1,000, 4,000, 2,000 and 2,000 mg L^{-1}, respectively. It was found that an aqueous extraction of Pomegranate was the most effective against both vegetative growth and zoospore germination of all selected *Achlya*. Data showed that vegetative and zoosporic stages of all selected *Achlya* did not grow when exposed to 500 and 250 mg L^{-1} of Pomegranate crude extracts for 6 hours. Nile tilapia (*Oreochromis niloticus*) fry were tested by exposure to 63, 125, 250, 500, 1,000 and 2,000 mg L^{-1} of Pomegranate for 30 minutes, 1, 2, 6 and 24 hours each. Results showed that the fish could survive in 63-2,000 mg L^{-1} of Pomegranate crude extracts when they were exposed to the herb extract for 30 minutes. Therefore, it was concluded that aqueous extraction of fruit peel of Pomegranate may be used as an alternative treatment for water mold infection of Nile tilapia.
Key Words: anti-oomycetic activity, fresh water fish, herbal plants, aquaculture.

Introduction. *Achlya* is known as an important genus of the family Saprolegniaceae and it causes serious damage in aquaculture. Many kinds and stages of fish can be infected with this water mold (Hussein et al 2002). Treatment of this infection is very difficult and legally available drugs are also limited (Zahran & Risha 2013). The most effective chemicals used to treat infected fish are malachite green and formalin, which have been reported by many investigators. An application of antibiotics has been widely practiced by fish farmers to control water mold infectious diseases and is quite effective. However, some chemicals have harmful effects, such as malachite green, which is known as carcinogen and mutagen for human life (Sudova et al 2007; Treves-Brown 2000), and remains in aquatic animals until they reach market size (Fuangsawat et al 2011). Because of its toxicity, the use of malachite green in the aquatic animal industry has been prohibited in many countries (Khomvilai et al 2005). Formalin is potentially harmful to the user's health and also remains in the ecosystem (Schreier et al 1996). Moreover, other synthetic chemicals also cause environmental impact and hazards (Ilondu et al 2009). Fish farmers increase dosage or find new imported drugs or chemicals to use and this raises the cost of production. This evidence has driven scientists to look for a new effective antifungal chemical that is less hazardous to animals, human health and the environment. Recently, Thai natural plant products have become known for their

medicinal and antimicrobial potentials. Ethanolic extracts of fruits of Indian gooseberry (*Phyllanthus emblica*) and rhizomes of galangal (*Alpinia galanga*) have shown antimicrobial activity against the growth of food poisoning bacteria, *Staphylococcus aureus* (Mayachiew & Devahastin 2008). Prasitpuriprecha et al (2009) reported the antimicrobial activity of ethanolic extracts of four Northeastern Thai edible plants including *Tiliacora triandra*, *Cratoxylum formosum*, *Kanchanaburi gratum* and *Polygonum odoratum* and one medicinal plant, *Eupatorium odoratum*, which were active against *S. aureus. K. gratum* had the most potent activity on *S. aureus*, *Bacillus subtilis*, *Propionibacterium acnes* and *Pseudomonas aeruginosa*, in order. Kittisrisopit et al (2010) found that the ethanolic extracts of Myrabolan wood (*Terminalia chebula*) gall posed antibacterial activity effective against *B. subtilis* and *S. aureus*. Thummajitasakul et al (2014) reported both ethanolic and aqueous extracts of Eugenia (*Syzygium gratum*) had antibacterial activity against *B. subtils*, *B. cereus, S. aureus* and *S. epidermidis*. There have been a few studies conducted on Thai plants against fish pathogens, such as aqueous extract of Pomegranate (*Punica granatum*) which was shown active against *Aeromonas caviae* from infected catfish (*Clarias* spp.) (Siri et al 2008). Herbal products also had an anti-oomycetic activity against water mold of fish. Udomkusonsri et al (2007) reported that crude ethanolic extracts of betal pepper (*Piper betle*) leaves and *Kaempferia galanga* roots had high anti-oomycetic, oomycetestatic and oomycetecidal activity against fish water mold, *Saprolegnia parasitica* H2. Plant ethanolic extracts including leaves of guava (*Psidium guajava*) and betel pepper were reported as potent anti-oomycetic agents against hyphae and zoospore formation of *Saprolegnia diclina* NJM 0208, *Achlya* sp. NJM 0323 and *Aphanomyces invadans* NJM 0002 (Borisutpeth et al 2009). Moreover, alcoholic extracts from stem-bark of *Cassia fistula* were capable of inhibiting the water mold growth and of killing both hyphae and zoospores of *S. paracitica* NJM 8604 and *S. diclina* NJM 0005 (Borisutpeth et al 2014). However, few studies have been conducted on Thai plants against fish pathogens. In this study, herbal aqueous solutions of Pomegranate, Eugenia (*Syzygium gratum*), Indian gooseberry and Myrabolan wood (*Terminalia chebula*), with various concentrations, were used to determine their inhibitory capacity on both vegetative and zoosporic stages of water mold, *Achlya* spp., isolated from infected Nile tilapia (*Oreochromis niloticus*) with achlyosis. In addition, the toxicity of selected herbal solutions to Nile tilapia fry was also investigated.

Material and Method

Herbs extraction. A modification of the method described by Borisutpeth et al (2009) was used to assess the effects of crude herb extracts against *Achlya* spp. Four herbs including Pomegranate, Eugenia, Indian gooseberry and Myrabolan wood were selected based on data about their antibacterial activity (Table 1). All herbs were collected from Khon Kaen Province, northeast Thailand. Crude herb extract was prepared as follows: fruit peel of Pomegranate, leaves of Eugenia, fresh fruits of Indian gooseberry and Myrabolan wood were washed to remove debris and chopped into bits. After that, 100 g of each plant material was mixed with 1,000 mL of sterilized distilled water (SDW) and ground using an electric blender. The mixture was then kept in a plastic container and incubated at 25°C for 2 days. The liquid extraction was filtrated using gauze and then centrifuged (Heraeus Suprafuge 22, Germany) at 8,000 rpm for 10 minutes. The supernatant was air-dried in a hot air oven (Memmert BM600, Schutzart DIN 40050-IP 20, Germany) at 45°C for 2-3 days or until it was dry. The powder was weighed and stored at -20°C until used. The percentage yield of the extract was determined and calculated from equation 1 (Lagu & Frederick 2012).

$$\text{Percentage yield (\%)} = \frac{X}{Y} \quad (1)$$

X = the dry weight of crude extract (g);
Y = the weight of soaked sample material (g).

Table 1

List of Thai herbs used in this study

Family	*Scientific name*	*Common name (English and Thai)*	*Part used / Indication*
Lythraceae	*Punica granatum*	Pomegranate Tub-Tim	Fruit peel / Anti-bacterial activity (Hajoori et al 2014)
Myrtaceae	*Syzygium gratum*	Eugenia Phak-Meg, Sa-Met-Chun	Leaf / Anti-microbial activity (Prasitpuriprecha et al 2009)
Euphorbiaceae	*Phyllanthus emblica*	Indian gooseberry Ma-Kham-Pom	Fresh fruits / Anti-microbial activity (Mayachiew & Devahastin 2008)
Combretaceae	*Terminalia chebula*	Myrabolan wood Sa-Mhor-Thai	Fresh fruits / Anti-bacterial activity (Kumar et al 2009)

Oomycete isolates. Sources of oomycete isolates used in this study were selected from *Achlya* spp. isolated from cultured Nile tilapia with achlyosis (Panchai et al 2015). They were *A. klebsiana* BKKU1003, *A. diffusa* BKKU1012, *Achlya* sp. BKKU1117, *A. prolifera* BKKU1125 and *Achlya* sp. BKKU1127 and maintained on glucose yeast extract (GY) agar at 25°C. The advancing edges of each 3 days growing colony (5.5 mm in diameter) were cut and used as inoculums for all experiments.

Oomycetestatic activity of herbs on hyphal growth. Each herb was dissolved at a concentration of 100,000 mgL^{-1} in SDW and immediately prepared before use. The solution was filtered through a 0.2 µm millipore filter paper (Sartorius, Hannover, Germany) and serially diluted to concentrations of 4,000, 2,000, 1,000, 500 and 250 mg L^{-1} using 10% GY broth (Borisutpeth et al 2009). A block of the growing colony was added to each 24-well tissue culture dish (Costar®, Corning Incorporated, USA) containing 2 mL of herb solution, and for the control group they were placed in 10% GY broth without the herbs. The well tissue culture dishes were incubated at 25°C and hyphal growth was observed under the inverted microscope at 1, 2 and 5 days incubation. If no growth was observed after 5 days, the agar blocks were removed, rinsed with SDW and placed on new GY agar plates at 25°C. Then, the survival of the oomycete was observed at day 2. Three replicates of each isolate were conducted.

Oomycetecidal activity of herbs on hyphal growth. The herb solution was prepared as described above and diluted to concentrations of 2,000, 1,000, 500, 250, 125 and 63 mg L^{-1} using 10% GY broth. The agar blocks of each isolate were placed into a plastic Petri dish containing 10 mL of test solutions for 30 minutes, 1, 2, 6 and 24 hours. The blocks of the control group were placed in 10% GY broth without the herb solution for the same duration as the treatment groups. After that, the mycelia were removed, rinsed with SDW and placed on new GY agar plates at 25°C. The hyphal growth was compared with the control group to determine the viability within 48 hours.

Oomycetecidal activity of herbs on zoospores. The minimum inhibitory concentrations (MIC) of herbs that inhibited the growth of hyphae in the above experiment were also conducted to determine the proper dosage for zoospore germination. Zoospore suspension of each isolate was prepared according to Panchai et al (2015), counted and adjusted to 1.0×10^3 zoospores mL^{-1}. After that, 1 mL of each herb solution with 10 times the desired final concentration the same as the concentration of the previous experiment was added into 9 mL of zoospore suspension and zoospores of the control group were inoculated into 10% GY broth without the herb solutions. The mixture was kept at 25°C for 30 minutes, 1, 2, 6 and 24 hours. Then, 0.1 mL of the mixture was inoculated onto a GY agar plate and also incubated at 25°C. The oomycete viability of the zoospores was determined by observing the appearance of the colonies over 1, 2 and 7 day periods with the naked eye.

Effect of pomegranate on zoospore germination. Zoospores of five *Achlya* spp. were induced as described by Panchai et al (2015) and adjusted to 1.0 x 10^3 zoospores mL^{-1}. The herb solutions were prepared at final concentration of 10 times (20,000, 10,000, 5,000, 2,500, 1,250 and 630 mg L^{-1}). Three milliliters of each 10x herb solution were then added into 27 mL of zoospore suspension and incubated at 25°C for 1, 2 and 24 hours. At the end of each incubation period, the zoospores were pelleted by centrifugation at 8,000 rpm for 5 minutes. The supernatant was discarded and the zoospore pellet was rinsed 3 to 4 times with sterilized tap water (STW). The rinsed zoospores were transferred into a Petri dish containing 30 mL of 1/30 GY broth and incubated at 25°C. Zoospore germination was determined under the inverted microscope to observe the presence, absence or quantity of germinating thalli at 3 hours after incubation.

Toxicity effect of pomegranate on Nile tilapia fry. Pomegranate was selected to test the toxicity effect on Nile tilapia fry because it had low concentrations for oomycetestatic and oomycetecidal effects. Healthy Nile tilapia fry approximately 0.7-0.8 g in body weight with total length 3.0-4.0 cm were obtained from the Khon Kaen Inland Fisheries Research and Development Center, Khon Kaen, Thailand. Fish samples were acclimatized in dechlorinated water at 25°C for one week. The fish were fed daily with commercial formula food (GF Feed, Krungthai Feedmill Public Co., Ltd., Bangkok, Thailand) and starved for one day before the experiment. The herbal solutions of Pomegranate were prepared as previously described and adjusted to a concentration of 2,000, 1,000, 500, 250, 125 and 63 mg L^{-1}. Ten Nile tilapia fry were randomly selected and placed into each 500 mL of an aseptic glass tank (15 cm in diameter, 28 cm height) with aeration for 30 minutes, 1, 2, 6 and 24 hours, respectively. At each immersion time, fish of each group were removed, rinsed and placed into STW with aeration. Three replicates of each experiment were conducted. Fish survival was observed for 3 days and the percentage corrected mortality of fish was calculated from equation 2 using Abbott's formula from Barnes et al (1998):

$$Pt = \frac{Po - Pc}{100 - Pc} \times 100 \quad (2)$$

Pt = percentage of corrected mortality (%);
Po = mortality of test group;
Pc = mortality of control group.

The experiment was 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). All experiments were conducted at Department of Biology, Faculty of Science, Khon Kaen University, Thailand during October to December 2014.

Results

Total percentage yield of crude extract. Four Thai herbs including Pomegranate, Eugenia, Indian gooseberry and Myrabolan wood were extracted using water extraction and their weight percentage yield was as shown in Table 2.

Table 2

Percentage yield of all crude extracts from Thai herbs in this study

Common name	*Wet weight (g)*	*Dry weight of crude extract (g)*	*Yield (%)*
Pomegranate	100	10	10
Eugenia	100	4	4
Indian gooseberry	100	9	9
Myrabolan wood	100	11	11

Oomycetestatic activity of herbs on hyphal growth. All isolates were able to grow in 0 and 250 mg L^{-1} of all herb solutions. As presented in Table 3, the oomycetestatic

dosages of Pomegranate were found to be 1,000 mgL^{-1} for *A. klebsiana* BKKU1003, *A. diffusa* BKKU1012, *Achlya* sp. BKKU1117 and *Achlya* sp. BKKU1127, whereas, *A. prolifera* BKKU1125 was found to be 500 mgL^{-1}. The results of treatment with Indian gooseberry and Myrabolan wood showed the oomycetestatic dosages of each *Achlya* isolate were 2,000 mg L^{-1}. Eugenia showed an oomycetestatic dosage of each *Achlya* isolate at 4,000 mg L^{-1}.

Table 3
Summarized oomycetestatic effect of herbal dosages on hyphal growth of each isolate at 25°C

Isolate	*Herbal extract concentration (mg L^{-1})*			
	Pomegranate	*Eugenia*	*Indian gooseberry*	*Myrabolan wood*
A. klebsiana BKKU1003	1,000	4,000	2,000	2,000
A. diffusa BKKU1012	1,000	4,000	2,000	2,000
Achlya sp. BKKU1117	1,000	4,000	2,000	2,000
A. prolifera BKKU1125	500	4,000	2,000	2,000
Achlya sp. BKKU1127	1,000	4,000	2,000	2,000

Oomycetecidal activity of herbs on hyphal growth. The oomycetecidal dosage of Pomegranate against hyphal growth of both *A. klebsiana* BKKU1003 and *A. diffusa* BKKU1012 was 500 mg L^{-1} for 6 hours treatment and 2 hours treatment for *Achlya* sp. BKKU1117, *A. prolifera* BKKU1125 and *Achlya* sp. BKKU1127 (Table 4). Eugenia showed the oomycetecidal dosage against all isolates of more than 2,000 mg L^{-1} and treatment more than 24 hours. However, 2,000 mg L^{-1} of both Indian gooseberry and Myrabolan wood were both effective in killing both *A. klebsiana* BKKU1003 and *A. diffusa* BKKU1012 after 6 hours exposure and *Achlya* sp. BKKU1117, *A. prolifera* BKKU1125 and *Achlya* sp. BKKU1127 after 2 hours exposure, respectively.

Table 4
Summarized oomycetecidal effect of herbal dosages and exposure times on hyphal oomycete isolates at 25°C

Isolate	*Herbal extract concentration (mg L^{-1})*			
	Pomegranate	*Eugenia*	*Indian gooseberry*	*Myrabolan wood*
A. klebsiana BKKU1003	500 / 6 h	>2,000 / >24 h	2,000 / 6 h	2,000 / 6 h
A. diffusa BKKU1012	500 / 6 h	>2,000 / >24 h	2,000 / 6 h	2,000 / 6 h
Achlya sp. BKKU1117	500 / 2 h	>2,000 / >24 h	2,000 / 2 h	2,000 / 2 h
A. prolifera BKKU1125	500 / 2 h	>2,000 / >24 h	2,000 / 2 h	2,000 / 2 h
Achlya sp. BKKU1127	500 / 2 h	>2,000 / >24 h	2,000 / 2 h	2,000 / 2 h

Oomycetecidal activity of herbs on zoospores. As shown in Table 5, the oomycetecidal dosages of Pomegranate on zoosporic stage were 250 mg L^{-1} against both *A. klebsiana* BKKU1003 and *A. diffusa* BKKU1012 with 6 hours treatment and against *Achlya* sp. BKKU1117, *A. prolifera* BKKU1125 and *Achlya* sp. BKKU1127 with 2 hours treatment. The treatment for 24 hours with Eugenia at 2,000 mg L^{-1} was effective against *A. klebsiana* BKKU1003, *A. diffusa* BKKU1012 and *A. prolifera* BKKU1125, while 1,000 mg L^{-1} was effective against both *Achlya* sp. BKKU1117 and *Achlya* sp. BKKU1127. However, 1,000 mg L^{-1} of both Indian gooseberry and Myrabolan wood were effective at killing *A. klebsiana* BKKU1003 and *A. diffusa* BKKU1012 with 6 hours exposure and *Achlya* sp. BKKU1117, *A. prolifera* BKKU1125 and *Achlya* sp. BKKU1127 with 2 hours exposure, respectively. From this result, it was found that effective dosage of Pomegranate at killing *Achlya* spp. was low (250 mg L^{-1}), whereas the effective dosages of Eugenia, Indian gooseberry and Myrabolan wood were high (2,000 and 1,000 mg L^{-1}). Therefore, Pomegranate was selected as a representative to test the effect on zoospore germination and toxicity on Nile tilapia fry.

Table 5

Summarized oomycetecidal effect of herbal dosages and exposure times on zoospores of oomycete isolates at 25°C

Isolate	*Herbal extract concentration (mgL^{-1})*			
	Pomegranate	*Eugenia*	*Indian gooseberry*	*Myrabolan wood*
A. klebsiana BKKU1003	250 / 6 h	2,000 / 24 h	1,000 / 6 h	1,000 / 6 h
A. diffusa BKKU1012	250 / 6 h	2,000 / 24 h	1,000 / 6 h	1,000 / 6 h
Achlya sp. BKKU1117	250 / 2 h	1,000 / 24 h	1,000 / 2 h	1,000 / 2 h
A. prolifera BKKU1125	250 / 2 h	2,000 / 24 h	1,000 / 2 h	1,000 / 2 h
Achlya sp. BKKU1127	250 / 2 h	1,000 / 24 h	1,000 / 2 h	1,000 / 2 h

Effect of pomegranate on zoospore germination. As presented in Table 6, Pomegranate at 500 mg L^{-1} completely inhibited zoospore germination (100%) of *A. klebsiana* BKKU1003 and *Achlya* sp. BKKU1127 after 1, 2 and 24 hours, *A. diffusa* BKKU1012 and *A. prolifera* BKKU1125 after 2 and 24 hours and *Achlya* sp. BKKU1117 after 24 hours exposure time, respectively.

Table 6

Inhibition of Pomegranate on zoospore germination of *Achlya* spp.

Isolate	*Concentration ($mg\ L^{-1}$)*	*Germination rate (%) (h = hour)*		
		1 h	*2 h*	*24 h*
A. klebsiana BKKU1003	0	100	100	100
	63	55	50	47
	125	25	22	10
	250	10	5	2
	500	0	0	0
	1,000	0	0	0
	2,000	0	0	0
A. diffusa BKKU1012	0	100	100	100
	63	65	64	40
	125	53	40	10
	250	10	3	5
	500	5	0	0
	1,000	0	0	0
	2,000	0	0	0
Achlya sp. BKKU1117	0	100	100	100
	63	70	66	44
	125	56	43	12
	250	21	12	5
	500	8	3	0
	1,000	0	0	0
	2,000	0	0	0
A. prolifera BKKU1125	0	100	100	100
	63	69	53	45
	125	55	24	22
	250	32	6	2
	500	7	0	0
	1,000	0	0	0
	2,000	0	0	0
Achlya sp. BKKU1127	0	100	100	100
	63	59	45	40
	125	34	28	12
	250	21	10	0
	500	0	0	0
	1,000	0	0	0
	2,000	0	0	0

It was noted that for zoospores at concentrations of 0-250 mg L^{-1}, spherical shapes and directed germination were observed. It was also found that zoospores were able to

germinate and form a whitish colony as shown in Figure 1A-B. In addition, zoospores at concentration of 500-2,000 mg L^{-1} of Pomegranate extracts were irregularly ovoid shape, but the germination tube was not observed and there was no colonial formation as shown in Figure 1C.

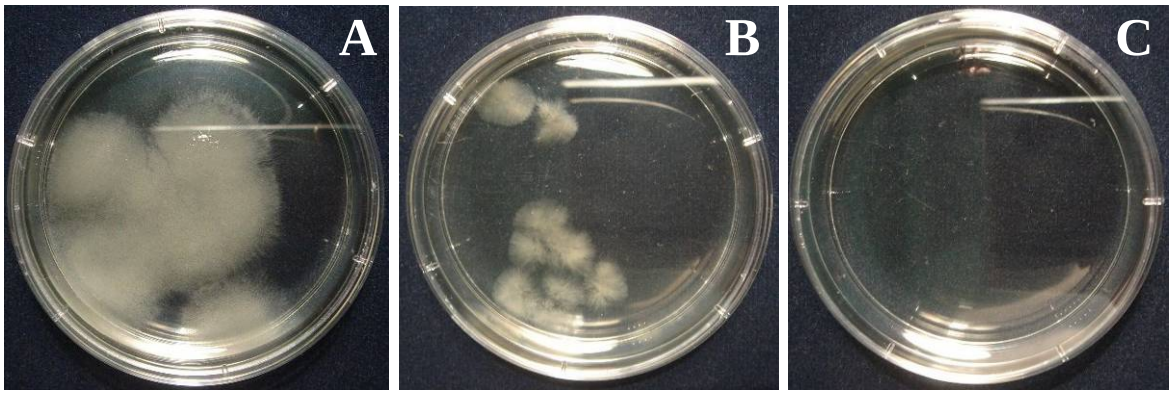

Figure 1. Colony of *A. klessiana* BKKU1003 after exposure to Pomegranate crude extract. A. At concentrations of 0 mg L^{-1}. B. At concentrations of 250 mg L^{-1}. C. At concentrations of 500-2,000 mg L^{-1}.

Toxicity effects of pomegranate on Nile tilapia fry. Due to Pomegranate being able to inhibit growth and zoospore germination of all *Achlya* isolates as previously mentioned, Pomegranate was selected to determine its toxicity effect on Nile tilapia fry. The cumulative mortality of Nile tilapia fry exposed to Pomegranate solution is shown in Table 7. The mortality rate of the fish when exposed to 63, 125, 250, 500, 1,000 and 2,000 mgL^{-1} of Pomegranate solution was 0% after 30 minutes. The highest percentage of mortality, 43% (13/30) was found in 2,000 mg L^{-1} after 24 hours exposure.

Table 7

Cumulative mortality (%) of Nile tilapia fry exposed to Pomegranate solution

Concentration (mg L^{-1})	*Total fish*	*Exposure time (h = hour)*				
		30 min	*1 h*	*2 h*	*6 h*	*24 h*
0	30	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
63	30	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
125	30	0 (0)	0 (0)	0 (0)	0 (0)	1 (3%)
250	30	0 (0)	2 (7%)	3 (10%)	3 (10%)	4 (13%)
500	30	0 (0)	5 (17%)	7 (23%)	5 (17%)	7 (23%)
1,000	30	0 (0)	8 (27%)	9 (30%)	8 (27%)	10 (33%)
2,000	30	0 (0)	10 (33%)	12 (40%)	12 (40%)	13 (43%)

Discussion. The effective chemicals have been widely used to control water mold infection of fish. The water mold infections are difficult to treat with chemicals (Borisutpeth et al 2010). Because of the toxicity of chemicals to fish and human health and that they also remain in the ecosystem, the use of chemicals is limited (Sudova et al 2007; Ilondu et al 2009; Fuangsawat et al 2011). The development of safe and effective Thai plants or herbs against water mold infection has been studied as an alternative method (Chukanhom et al 2005; Udomkusonsri et al 2007; Borisutpeth et al 2009, 2010; Panase et al 2012).

In the present study, the efficacy of aqueous extracts of Thai herbs against the growth of five pathogenic *Achlya* was in the order of Pomegranate, Myrabolan wood, Indian gooseberry and Eugenia. Because the percentage yield of all plants seemed to be low, for Myrabolan wood (11%), Pomegranate (10%), Indian gooseberry (9%) and Eugenia (4%). This may be due to the period of time since collecting and use was longer, which was supported by Cheeptham & Towers (2002). However, Pomegranate was the most effective agent against the hyphal growth and zoospore germination of all selected *Achlya.* Therefore, the effectiveness of herb extracts in inhibiting the hyphal growth may

be dependent on the toxicity of plants, which is similar to the study of Mori et al (2002). According to Dahham et al (2010), many parts of the Pomegranate were used to determine their antimicrobial activity and fruit peel was reported as having a high efficacy. There are many reports of antimicrobial activity of Pomegranate on bacteria and fungi. Siri et al (2008) found that water extract of Pomegranate was active against *Aeromonas caviae* from infected catfish (*Clarias* spp.). Khan & Hanee (2011) reported aqueous extraction of Pomegranate against *Escherachia coli*, *Pseudomonas aeruginosa* and *S. aureus*. Hajoori et al (2014) also reported aqueous extraction of Pomegranate against *B. megaterium*, *B. subtilis*, *B. cereus*, *Salmonella paratyphi*, *Proteus vulgaris* and *S. aureus*. Shaokat et al (2007) reported aqueous extract of Pomegranate against *Aspergillus fumegatus*. Malliga Elangovan et al (2015) found that petroleum ether leaf extract of Indian gooseberry has potent antibacterial effects on *S. aureus*, *B. subtillus*, *Salmonella typhi* and *E. coli* and also had antifungal effect against three different fungal strains viz. *Aspergillus niger*, *Candida albicans* and *Penicillium notatum*.

In the present study, the water mold sensitivity to the herb extracts was in the order of *A. prolifera* BKKU1125, followed by both of *Achlya* sp. BKKU1117 and BKKU1127, *A. klebsiana* BKKU1003 and *A. diffusa* BKKU1012, respectively. The oomycetecidal dosage of Pomegranate showed the highest efficacy against the hyphal growth of all *Achlya* at 500 mg L^{-1} for 6 hours treatment. Therefore, the efficacy of the herbs against the water mold may be dependent on the water mold species, hyphae, zoospore formation and the exposure time to the herbs, which was supported by Borisuthpeth et al (2009). In addition, the oomycetecidal effects of Pomegranate extracts against zoospore germination of all selected pathogenic *Achlya* appeared to be lower (250 mg L^{-1}) than the oomycetestatic effects (500 mg L^{-1}). This means that the zoospores have higher sensitivity to the herb extracts than the hyphae. This may be due to the fact that the zoosporic stage was more sensitive to chemicals than the vegetative stage (Muller-Breban et al 1995). According to Pickering & Willoughby (1982), Beakes et al (1994), Hatai & Hoshiai (1994) and Bruno & Wood (1999), zoospores play an important role in the initiation of oomycete infection and even if the hyphae and zoospores are not killed following herbal treatment, initial infection may be prohibited.

From the toxicity test of Nile tilapia fry exposed to various aqueous concentrations (63, 125, 250, 500, 1,000 and 2,000 mg L^{-1}) of Pomegranate for 30 minutes, it showed that no mortality occurred. It seemed that the fish were able to tolerate low (63 mg L^{-1}) and high (2,000 mg L^{-1}) concentrations of Pomegranate when they were bathed with the herb extracts for 30 minutes. From this result, it was able to inhibit the growth of aquatic oomycetes without harming the tilapia fry.

This study is the first report on the effects of aqueous extractions of Pomegranate, Myrabolan wood, Indian gooseberry and Eugenia to inhibit the hyphal growth and zoospore germination of pathogenic strains of *Achlya* spp. *in vitro*. As a result of these findings, it is seen that Pomegranate extract is the most potent anti-oomycete agent against the hyphae and zoospore formation in these *Achlya* spp. and it is non-toxic to Nile tilapia fry when the fry are bathed with Pomegranate extract for 30 minutes.

Conclusions. Based on the results, it is concluded that the aqueous extraction of Pomegranate used in this study may be used as an alternative treatment of water mold infection of Nile tilapia, from the results of the test regarding their toxicity to fish. In addition, the use of water as a solvent in the present study may be a good approach for fish farmers in traditional practice, due to it being a cheap and easy method for treatment by fish culturists, reducing the cost of production and increasing safety for humans.

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