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AQUATIC FUNGI DEVELOPING ON EGGS OF TILAPIA, *OREOCHROMIS NILOTICUS* LINN. AND PREVENTION

MR. KWANPRASERT PANCHAI

A THESIS FOR THE DEGREE OF MASTER OF SCIENCE

KHON KAEN UNIVERSITY

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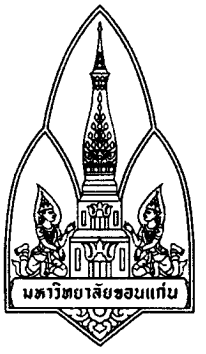

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A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
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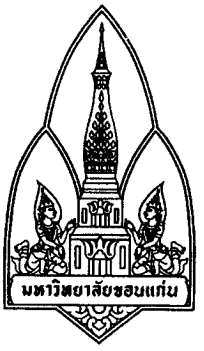

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Thesis Title: Aquatic Fungi Developing on Eggs of Tilapia, *Oreochromis niloticus* Linn. and Prevention

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ขวัญประเสริฐ พันธุ์ชัย. 2550. **ราน้ำที่พบบนไข่ปลานิล (*Oreochromis niloticus* Linn.) และการป้องกัน.** วิทยานิพนธ์ปริญญาวิทยาศาสตรมหาบัณฑิต สาขาวิชาชีววิทยา บัณฑิตวิทยาลัย มหาวิทยาลัยขอนแก่น

อาจารย์ที่ปรึกษาวิทยานิพนธ์: รองศาสตราจารย์ ดร. ชุติมา หาญจวณิช,
ผู้ช่วยศาสตราจารย์ ดร. นิลุบล กิจอันเจริญ

บทคัดย่อ

ตัวอย่างไข่ปลานิล (*Oreochromis niloticus* Linn.) ที่ติดเชื้อราเก็บจากโรงเพาะฟักจังหวัดกาฬสินธุ์ ขอนแก่น มหาสารคาม และสกลนคร ระหว่างเดือนมิถุนายนถึงกรกฎาคม เดือนตุลาคมถึงเดือนพฤศจิกายน พ.ศ. 2548 และเดือนมีนาคมถึงเดือนเมษายน พ.ศ. 2549 ผลการตรวจสอบลักษณะสัณฐานวิทยาสามารถจำแนกเชื้อราได้ 2 วงศ์ 4 สกุล และมากกว่า 6 สายพันธุ์ ได้แก่ วงศ์ Saprolegniaceae สกุล *Achlya* คือ *Achlya* spp. 3 สายพันธุ์ *A. ambisexualis* 2 สายพันธุ์ และ *A. bisexualis* 7 สายพันธุ์ สกุล *Aphanomyces Aphanomyces* spp. 2 สายพันธุ์ สกุล *Saprolegnia S. diclina* 1 สายพันธุ์ และวงศ์ Pythiaceae สกุล *Pythium Pythium* sp. 1 สายพันธุ์

ผลการศึกษาคุณสมบัติทางชีววิทยาบางประการของเชื้อรา พบว่าอุณหภูมิที่เหมาะสมต่อการเจริญของเส้นใยราสกุล *Achlya* และ *Aphanomyces* อยู่ในช่วง 25-35 องศาเซลเซียส สกุล *Pythium* และ *Saprolegnia* อยู่ในช่วง 20-35 องศาเซลเซียส ส่วนอุณหภูมิที่เหมาะสมต่อการสร้างสปอร์ของเชื้อราสกุล *Achlya*, *Aphanomyces* และ *Saprolegnia* คือ 25 องศาเซลเซียส ยกเว้น *Pythium* sp. ไม่สร้างสปอร์ พีเอชที่เหมาะสมต่อการเจริญของเส้นใยราสกุล *Achlya*, *Aphanomyces*, *Saprolegnia* และ *Pythium* อยู่ในช่วง 6.0-8.0, 6.0-9.0, 7.0-9.0 และ 6.0-7.0 ตามลำดับ ส่วนพีเอชที่สปอร์ของเชื้อราสกุล *Achlya*, *Aphanomyces* และ *Saprolegnia* สามารถงอกได้อยู่ในช่วง 4.0-11.0 นอกจากนี้ยังพบว่าระยะเส้นใยสกุล *Achlya*, *Aphanomyces*, *Saprolegnia* และ *Pythium* เจริญได้ในอาหารเลี้ยงเชื้อแข็งที่ระดับความเค็มโซเดียมคลอไรด์ 1.5, 1.5-2.0, 3.0 และ 2.0 % ตามลำดับ

ผลการตรวจสอบลักษณะทางจุลพยาธิสภาพของไข่ปลานิลที่ติดเชื้อราจากตัวอย่างที่เก็บได้ พบมีเส้นใยราจำนวนมากเกาะที่ผิวเยื่อหุ้มไข่และแทรกผ่านเยื่อหุ้มไข่สะสมที่ไซโทพลาซึมและไข่แดงที่สลายตัว

ผลการทดสอบประสิทธิภาพของสารละลายโซเดียมคลอไรด์และด่างทับทิมต้านการเจริญระยะสปอร์และระยะเส้นใยของเชื้อราที่อุณหภูมิ 25 องศาเซลเซียส พบว่าโซเดียมคลอไรด์ที่ระดับ

ความเข้มข้น 2.5 และ 3.0 % มีผลฆ่าเชื้อราระยะสปอร์และระยะเส้นใยตามลำดับ เมื่อบ่มนาน 2 และ 24 ชั่วโมง ตามลำดับ ส่วนด่างทับทิมที่ความเข้มข้น 25 และ 200 ไมโครกรัมต่อมิลลิลิตร มีผลฆ่าระยะสปอร์และระยะเส้นใยของเชื้อราเมื่อบ่มนาน 30 นาที และ 24 ชั่วโมง ตามลำดับ

สารละลายโซเดียมคลอไรด์ความเข้มข้น 2.0, 2.5 และ 3.0 % ที่มีประสิทธิภาพควบคุมการเจริญของเชื้อรานำมาทดสอบความเป็นพิษต่อไข่ปลานิลระยะมีจุดตาโดยแช่ไข่นาน 1 และ 24 ชั่วโมง พบว่าความเข้มข้นของสารละลายโซเดียมคลอไรด์ 2.0 % หรือเพิ่มขึ้นมีผลทำให้ร้อยละของอัตราการมีชีวิตรอดของไข่ปลาลดลง ซึ่งมีความแตกต่างกันอย่างมีนัยสำคัญ ($P<0.05$) ระหว่างกลุ่มทดลองและกลุ่มควบคุม โดยเฉพาะกลุ่มทดลองที่แช่ในสารละลายโซเดียมคลอไรด์ 3.0 % นาน 24 ชั่วโมง ทำให้ร้อยละอัตราการมีชีวิตรอดเป็น 0

ส่วนผลของสารละลายด่างทับทิมที่มีประสิทธิภาพควบคุมการเจริญของเชื้อราที่ระดับความเข้มข้น 25, 50, 100, 150 และ 200 ไมโครกรัมต่อมิลลิลิตร มีความเป็นพิษสูงต่อไข่ปลานิล ทำให้ไข่ปลามีร้อยละอัตราการมีชีวิตรอดเป็น 0 เมื่อแช่นาน 1 และ 24 ชั่วโมง

ดังนั้น อาจเป็นไปได้ที่จะนำมาสารละลายโซเดียมคลอไรด์ความเข้มข้น 3.0 % หรือเพิ่มขึ้นมาใช้ป้องกันการเจริญของเชื้อราบนไข่ปลานิล โดยแช่ในระยะเวลาน้อยกว่า 1 ชั่วโมง

Kwanprasert Panchai. 2007. **Aquatic fungi developing on eggs of tilapia, *Oreochromis niloticus* Linn. and prevention.** Master of Science Thesis in Biology, Graduate School, Khon Kaen University.

Thesis Advisors: Assoc. Prof. Dr. Chutima Hanjavanit,
Assist. Prof. Dr. Nilubol Kitancharoen

ABSTRACT

Samples of fungal infected eggs of tilapia (*Oreochromis niloticus* Linn.) were collected from hatcheries at Kalasin, Khon Kaen, Maha Sarakham and Sakon Nakhon Provinces during June-July, October-November 2005 and March-April 2006. From morphological characterized, they were identified to 2 families, 4 genera and more than 6 isolates. Family Saproegniaceae was composed of genus *Achlya* (3 isolates of *Achlya* spp., 2 isolates of *A. ambisexualis* and 7 isolates of *A. bisexualis*), genus *Aphanomyces* (2 isolates of *Aphanomyces* spp.) and genus *Saprolegnia* (1 isolate of *S. diclina*). Family Pythiaceae was composed of genus *Pythium*, 1 isolate of *Pythium* sp.

Some biological characteristic studies, the optimal temperature for vegetative growth of *Achlya* and *Aphanomyces* were 20-35 °C, *Pythium* and *Saprolegnia* were 25-35 °C, whereas the optimal temperature for zoospore production was 25 °C, except *Pythium* sp. did not produce zoospores. The optimum pH for vegetative growth of *Achlya*, *Aphanomyces, Saprolegnia* and *Pythium* were 6.0-8.0, 6.0-9.0, 7.0-9.0 and 6.0-7.0, respectively. The optimum pH for zoospore germination of *Achlya*, *Aphanomyces* and *Saprolegnia* were in a wide range of 4.0-11.0. Effect of various salinity of sodium chloride on vegetative growth showed that *Achlya*, *Aphanomyces, Saprolegnia* and *Pythium* grew up to 1.5, 1.5-2.0, 3.0 and 2.0 % sodium chloride, respectively.

The histopathological examination of fungal infected eggs from the samplings showed numerous hyphae spread over outer layer of egg envelope, penetrated the egg envelope and accumulated in cytoplasm and degenerated yolk granules.

The anti-fungal effect of sodium chloride and potassium permanganate on zoosporic and vegetative stages was conducted at 25 °C. It showed that 2.5 and 3.0 % sodium chloride exposed for 2 and 24 hours were toxic to zoosporic and vegetative

stages, respectively. Whereas 25 and 200 μg/mL potassium permanganate was effective in killing the zoosporic stage at 30 minutes and the vegetative stage at 24 hours, respectively.

Toxicity of 2.0, 2.5 and 3.0 % sodium chloride controlling fungal activity were tested on tilapia eyed eggs for 1 and 24 hours treatment. The result showed that 2.0 % sodium chloride or higher can reduce percent hatching rate of the treatment groups which were significantly different ($P<0.05$) between the treatment groups and the untreated control group. Especially 3.0 % sodium chloride treatment within 24 hours caused 0 % hatch rate.

Treatment of eyed eggs with 25, 50, 100, 150 and 200 μg/mL potassium permanganate against fungal activity had high toxic effect on eyed eggs. The result showed 0 % hatching rate of all treatment groups within 1 and 24 hours exposure.

Therefore, it may be possible to use 3.0 % sodium chloride or higher concentrations to prevent fungal infection on tilapia eggs by bath less than 1 hour exposure.

To My Parents and Teachers

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Finally, I am indebted to my parents and younger brother for all their love, cheerfulness and moral support throughout my study.

Kwanprasert Panchai

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CHAPTER I
GENERAL INTRODUCTION

Aquatic fungi were broadly known as water moulds can be grouped into class Oomycetes, family Saprolegniaceae by their mainly zoosporic stages (Roberts, 2001). They play an important role in the decomposition and degradation of dead plant material, leaves and wood and animal materials such as insect exoskeletons and fish scales, whereas some fungi are pathogens of plants and animals in freshwater habitat (Sivichai and Boonyene, 2004).

It is known that water moulds genera *Achlya, Allomyces, Aphanomyces, Dictyuchus, Leptolegnia, Pythium* and *Saprolegnia* were commonly reported in aquatic systems or on infected fish and their eggs (Mer *et al.*, 1980; Srivastava, 1980; Post, 1983; Kitancharoen *et al.*, 1995; Kitancharoen *et al.*, 1997; Nejadsattari, 2000; Steciow, 2001; Czeczuga *et al.*, 2002). Saprolegniaceae has been reported from all parts of the world because of its capability to spread and universally distributed (Alexopoulos, 1962). Little known of Saprolegniaceae is reported from Thailand. Willoughby and Lilley (1992) isolated *Achlya, Aphanomyces* and *Saprolegnia* from dead fish left in freshwater at Udon Thani in the Northeast of Thailand. Yuasa *et al.* (2000) were able to isolate genera *Achlya, Aphanomyces, Saprolegnia* and *Pythium* from various economically freshwater fish and their eggs in the Northeast of Thailand. Munchan (2003) isolated *Achlya* and *Saprolegnia* from ornamental fish i.e. platyfish (*Xiphophorus helleri*), guppy (*Poecilia reticulate*) and ballon (*Poecilia shenops*) from Khon Kaen Province, while Rakmanee *et al.* (2004) reported *Achlya* and *Saprolegnia* from eggs of African catfish (*Clarias garepinus*) and common carp (*Cyprinus carpio*) from Maha Sarakham Province. Chukanhom and Hatai (2004) isolated *Achlya* sp., *Saprolegnia diclina* and *Allomyces* from infected common carp eggs from Khon Kaen Province.

The purposes of this research were to study (1) morphological characteristics and diversity of fungi isolated from tilapia eggs with fungal infection from 7 sampling sites during 3 periods: June-July, October-November 2005 and March-April 2006 (Chapter II), (2) some biological characteristics of the fungi (Chapter III), (3) histopathology of tilapia eggs with fungal infection comparing with healthy eggs (Chapter IV), and (4) anti-fungal effect of sodium chloride (NaCl) and potassium permanganate ($KMnO_4$) and the toxicity to tilapia eggs (Chapter V).

CHAPTER II
ISOLATION AND MORPHOLOGICAL CHARACTERISTICS OF FUNGI FROM EGGS OF TILAPIA (*OREOCHROMIS NILOTICUS* LINN.) WITH FUNGAL INFECTION

1. Introduction

Tilapia (*Oreochromis niloticus* Linn.), family Cichlidae, is one of the most economically important cultured fish species and more resistant to disease than many other species and has high yield protein potential (Wohlfarth and Hulata, 1983). It has been introduced to many countries worldwide with a global distribution second to common carp (Popma and Masser, 1999). The global tilapia aquaculture production is relatively high during the past decade and exceeding 1.5 million tons in the year 2002 (Silva *et al.*, 2004). At present, tilapia culture is very popular in freshwater aquaculture in Thailand (Tawaratmaneekul and Tungtrongpiroj, 1993; Wiwattanachaiseat, 1996; Srisakuntiew, 2000). Its typical spawning behavior is laying their eggs in a pit called nest and then the female incubates fertilized eggs in buccal cavity for several days even after the eggs hatch (Pillay, 1993). To increase productivity, fish farmers must remove the fertilized eggs shortly from female buccal cavity to artificial incubation with circulating water as same as natural (Suresh, 2003).

In general, infectious diseases due to viruses, bacteria and fungi are still increasing in the intensive cultured fish (Woo and Bruno, 1999). At present, tilapia cultures are often in semi-intensive and intensive system (Lio-Po and Lim, 2002) and always have problems both of stress and diseases (Bittencourt *et al.*, 2003). The annual production of tilapia is low when the disease becomes a big problem in fish culture.

A variety of pathogenic agents on tilapia has been reported such as *Saprolegnia, Achlya, Ahanomyces* and *Dictyuchus* (Wiwattanachaiseat, 1996), ciliated protozoans *Ichthyopthirius multifilis, Trichodina* sp. and *Epistylis* sp. (Popma and Masser, 1999), myxosporean parasites *Myxobolus* sp. and *M. zillii* (Gbankoto *et al.*, 2001), monogenetic fluke *Gyrodactylus* sp., fish louse *Argulus* sp. and anchor worm

Lernaea sp. (Klingker and Francis-Floyd, 2002), iridovirus (Lio-Po and Lim, 2002) and *Edwardsiella tarda* (Benli and Yildiz, 2004).

Fungal disease is a problem in tilapia broodstock husbandry that occurred in hatcheries, affecting eggs and reducing hatching rates (Luzur, 2002; Suresh, 2003). When some eggs were infected by fungus, the mycelia will grow and spread to surrounding eggs (Suresh, 2003). Aquatic fungi belonging to class Oomycetes, commonly known as water moulds, family Saprolegniaceae affected on fish cultured and their eggs were widely distribution (Mer *et al.*, 1980; Srivastava, 1980; Post, 1983; Kitancharoen *et al.*, 1995; Kitancharoen *et al.*, 1997; Nejadsattari, 2000; Steciow, 2001; Czeczuga *et al.*, 2002). Griffin (1994) reported that some species of family Saprolegniaceae were saprophytes and some were parasites. This family consists of many genera i.e. *Saprolegnia*, *Achlya* and *Aphanomyces*. They have been reported as fungal infection on fish and their eggs (Srivastava, 1980; Chinabut *et al.*, 1995; Kitancharoen and Hatai, 1997; Nejadsattari, 2000; Kiryu, 2002; Chukanhom and Hatai, 2004). According to Bruno and Wood (1993), Oomycetes has a complex life cycle involving sexual stage which produces antheridial and oogonial gametangia. Asexual stage produces motile zoospores from zoosporangia, and then zoospores encysted and may germinate and sometimes produce chlamydospores or gemmae. Both of them may germinate and produce a vegetative growth that called hyphae or mycelia. Identification of this fungal group is traditionally based on morphological characteristics of sexual and asexual reproduction features according to the identification keys of Johnson (1956), Scott (1961), Seymour (1970) and Van Der Plaats-Niterink (1981).

Though fungal infection has widely been well known, the documentation of fungal diseases on tropical fishes and their eggs including taxonomic works in Thailand is little known (Lawhavanit *et al.*, 2002; Chukanhom, 2004; Laoprasert *et al.*, 2005).

The purposes of this chapter were (1) to examine the morphology of fungi that isolated from fungal infected tilapia eggs; (2) to report the occurrence and diversity of fungi, and (3) to examine some incubation water qualities in order to associate with the occurrence of fungi.

2. Materials and Methods

2.1 Study sites

Samples of tilapia eggs with fungal infection were collected from hatcheries in Kalasin, Khon Kaen and Sakon Nakhon Provinces, including hatcheries in Kalasin Inland Fisheries Station, Khon Kaen Inland Fisheries Research and Development Center, Maha Sarakham Inland Fisheries Research and Development Center and Sakon Nakhon Inland Fisheries Research and Development Center northeastern, Thailand (Figures 2.1 and 2.2).

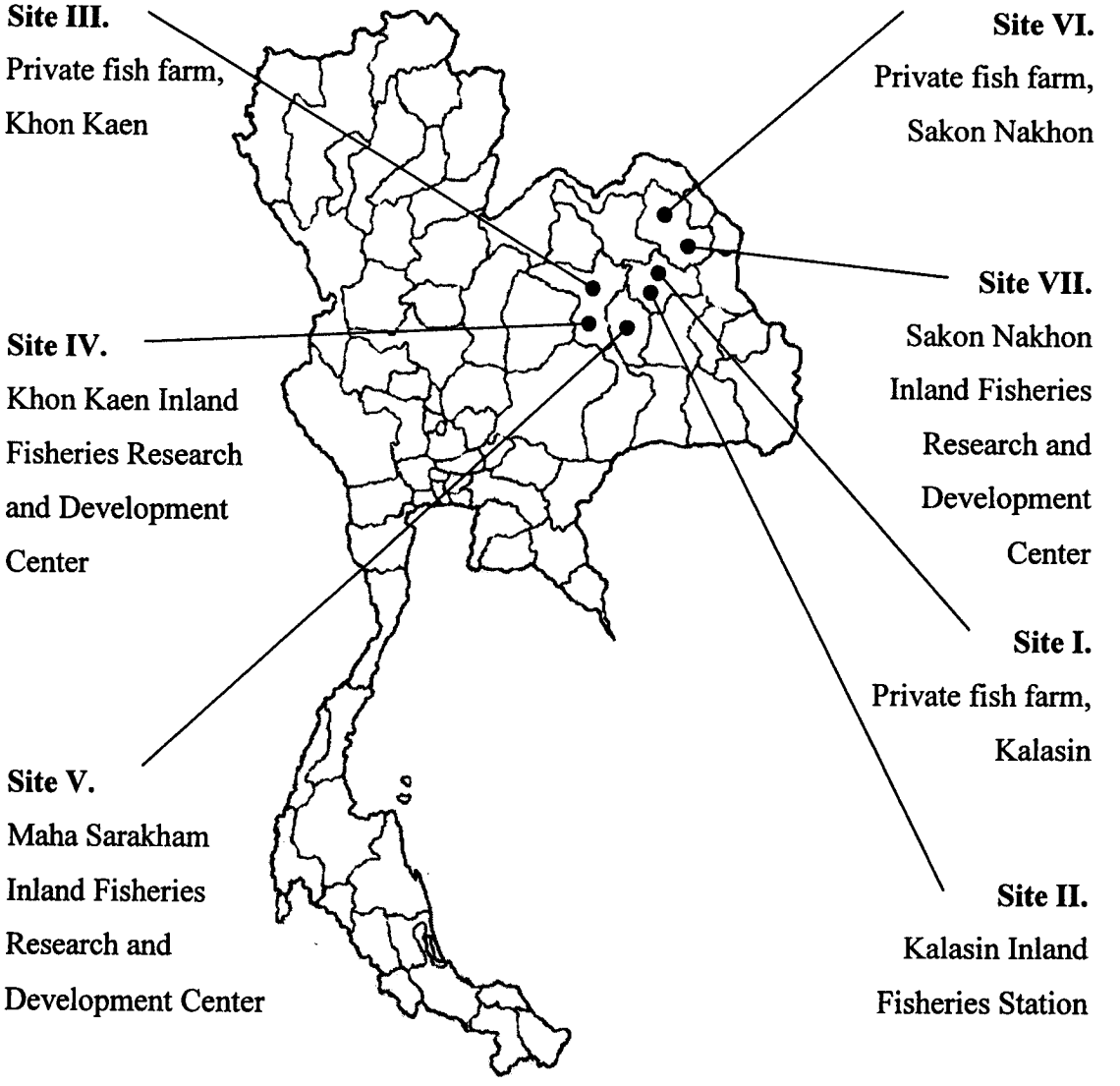


Figure 2.1 Map showing locations of the sampling sites.

Figure 2.2 A fish farm in Khon Kaen showing tilapia eggs in hatching trays. Note the dead eggs (→) and healthy eggs (→).

2.2 Water quality

Water samples were collected from the hatchery stations to examine some physical and chemical parameters such as water temperature, pH, dissolved oxygen (DO), total dissolved solids (TDS), electro-conductivity and salinity (Table 2.1) immediately at all sites on the same sampling dates. Three replicates of each parameter were carried out for this study. Altitude of located sampling site was measured by Altimeter compensated (Barigo, Germany) and water sources for egg incubation from each hatchery were also recorded.

Table 2.1 Analytical parameters and equipments.

Parameters	Equipments
Water temperature (°C)	HACH Session1 pH meter
pH	HACH Session1 pH meter
Dissolved Oxygen (DO) (mg/L)	YSI Model57 Oxygen Meter
Total Dissolved Solids (TDS) (mg/L)	Fisher Scientific Digital Conductivity Resistivity Total Dissolved Solids Temperature Meter09-326-2
Electro-conductivity (μs/cm)	Fisher Scientific Digital Conductivity Resistivity Total Dissolved Solids Temperature Meter 09-326-2
Salinity (%)	Water quality checker U-10

2.3 Egg specimens

Infected eggs were randomly sampled during June-July, October-November 2005 and March-April 2006. They were collected and transported to the Fish Diseases Laboratory, Department of Biology, Faculty of Science, Khon Kaen University. They were examined for fungal hyphae under stereo-microscope (Olympus SZ-ST, Japan).

2.4 Fungal isolation and purification

Five to ten tilapia eggs with fungal infection were washed several times by sterilized tap water (STW) and manipulated as described by Hatai and Egusa (1979). The eggs were inoculated onto 20 mL glucose yeast extract (GY) agar consisting of 1 % glucose, 0.25 % yeast extract and 1.5 % agar (Hatai and Egusa, 1979) (Appendix A). Trace of ampicillin (Sigma, Chemical Co., St. Louis, Missouri, USA) and streptomycin sulfate (Meji Seika, Kaisha, Ltd., Tokyo, Japan) were added onto the medium to inhibit the bacterial growth. The plates were incubated at 25 °C for 24-48 hours for hyphal growth. The edge of agar with growing hyphae was cut and put into 20 mL GY broth (Appendix A) and then incubated at 25 °C for 24-48 hours. After that, the growing hyphae were cut and washed again by STW and incubated in plate of 20 mL STW at 25 °C for 12-20 hours to induce zoospore production. The zoospore suspension of $1x10^3$ zoospores/mL was inoculated onto new GY agar to get a pure single colony (Kitancharoen *et al.*, 1995). The purified fungal isolates were maintained at 25 °C on GY agar, and subcultured to fresh GY agar monthly. To determine the fungal diversity, 10-20 single colonies from a single culture plate were randomly selected to observe the patterns of zoospore discharge.

2.5 Fungal classification and identification

Asexual reproduction of the fungi were prepared as follows: the edge of the single colony was cut (approximately 0.5x0.5 cm) and put into 20 mL GY broth, incubated at 25 °C for 24-48 hours. After that, the edges of young growing mycelia were cut and washed in successive baths of STW and incubated in 20 mL STW at 25 °C for 12-20 hours. The zoosporangial formation and the zoospore discharge pattern were observed under an inverted microscope (Nikon Phase Contrast-2 ELWD 0.3, Japan) to classify the fungal genus.

The sexual stage of fungi was induced with hemp seeds (*Cannabis sativa*). Zoospores were prepared and maintained in the same condition as described above. Then, sterilized hemp seeds were transferred to 20 mL STW containing zoospores. Hemp seed cultures were done at 5, 10, 15, 20 and 25 °C and sexual reproduction (oogonial and antheridial formation) were observed for 1 month. The morphological

structures of reproductive organs of fungi on the hemp seed cultures were identified for the species. In the case of the isolates did not produce reproductive organs during the observation period, they were only classified to genera. The fungi were identified according to Johnson (1956), Scott (1961), Seymour (1970) and Van Der Plaats-Niterink (1981).

3. Results

3.1 Some physical and chemical parameters of incubation water (Appendix B)

Altitude of sampling sites is approximately 140-166 metres above sea level (masl) and different sources of incubation water using in each hatchery station are shown in Table 2.2. The ranges of the different parameters such as temperature, pH, dissolved oxygen, total dissolved solid, electro-conductivity and salinity are presented in Table 2.3.

Table 2.2 Location of sampling sites showing altitude (metres above sea level, masl), sources of incubation water and chemicals used for water treatment.

Sites	Locations	Altitudes (masl)	Water sources	Water treatments
I	Kalasin fish farm Muang District, Kalasin Province	140	Lam Pao Dam	$KMnO_4$, NaCl
II	Kalasin Inland Fisheries Station, Muang District, Kalasin Province	140	Lam Pao Dam	Formalin, $KMnO_4$, NaCl
III	Khon Kaen fish farm, Mancha-Khiri District, Khon Kaen Province	163	Chi River	Formalin, $KMnO_4$, NaCl
IV	Khon Kaen Inland Fisheries Research and Development Center, Muang District, Khon Kaen Province	163	Pong River	Formalin, $KMnO_4$, NaCl
V	Maha Sarakham Inland Fisheries Research and Development Center, Muang District, Maha Sarakham Province	142	Loeng Chan Rapids	Formalin, $KMnO_4$, NaCl
VI	Sakon Nakhon fish farm, Muang District, Sakon Nakhon Province	166	Rain *	NaCl $CaCO_3$
VII	Sakon Nakhon Inland Fisheries Research and Development Center, Muang District, Sakon Nakhon Province	166	Nong Han Lake	Formalin, $KMnO_4$, NaCl, Chlorine

* Nam Un Dam is using during March-April 2006

Table 2.3 Ranges (mean±SD) of the physical and chemical parameters of incubation water from sampling sites during June 2005-April 2006.

Parameters	Sites						
	I	II	III	IV	V	VI	VII
Water temperature (°C)	25.3-32.0 (27.9±3.6)	25.7-32.9 (28.7±3.8)	25.8-27.8 (26.8±1.0)	24.6-27.6 (26.3±1.5)	24.6-26.7 (25.7±1.1)	20.9-28.1 (24.4±3.6)	23.5-27.3 (25.5±1.9)
DO (mg/L)	4.3-4.9 (4.7±3.4)	3.6-5.1 (4.1±0.8)	3.8-4.9 (4.3±0.6)	3.3-4.9 (4.2±0.8)	4.4-5.4 (4.8±0.6)	4.1-5.4 (4.7±0.7)	3.9-5.0 (4.4±0.6)
pH	7.5-7.8 (7.6±0.2)	6.9-7.7 (7.3±0.4)	6.9-7.7 (7.3±0.4)	6.8-7.6 (7.3±0.4)	7.4-8.1 (7.7±0.4)	7.3-7.9 (7.6±0.3)	7.3-8.0 (7.7±0.4)
TDS (mg/L)	215.5-272.0 (236.4±31.0)	186.7-1,963.0 (1108.1±890.1)	1,165.7-1,664.0 (1393.6±251.9)	206.2-385.0 (271.1±99.0)	268.0-1,060.0 (612.3±406.0)	1,666.0-3,870.0 (3022.0±1186.6)	212.7-731.0 (492.6±261.6)
Electro-conductivity (µs/cm)	324.3-373.0 (341.2±27.6)	280.3-2953.3 (1664.5±1339.1)	1,749.0-2,496.7 (2091.9±377.7)	308.3-578.3 (408.0±148.2)	366.3-1,591.0 (906.8±624.9)	2,503.3-5,800.0 (4531.1±1774.5)	320.0-1,097.3 (739.4±392.3)
Salinity (%)	0	0	0-0.01 (0.003±0.006)	0	0	0.01-0.03 (0.02±0.01)	0

3.2 Fungal species and morphology

Sixteen fungal isolates were collected from the dead eggs and were remarked as BKKU 0501-0511 and BKKU 0612-0616. The sampling sites and fungal species are shown in Table 2.4.

Table 2.4 Sampling sites and fungal species obtained from the infected tilapia eggs.

Isolates	Fungal species	Sites	Locations	Dates of sampling
BKKU 0501	*Achlya* sp.	I	Kalasin	June, 2005
BKKU 0502	*Achlya* sp.	II	Kalasin	June, 2005
BKKU 0503	*Achlya* sp.	III	Khon Kaen	June, 2005
BKKU 0504	*A. bisexualis*	IV	Khon Kaen	June, 2005
BKKU 0505	*Aphanomyces* sp.	IV	Khon Kaen	June, 2005
BKKU 0506	*Saprolegnia diclina*	IV	Khon Kaen	June, 2005
BKKU 0507	*Pythium* sp.	IV	Khon Kaen	June, 2005
BKKU 0508	*Aphanomyces* sp.	VII	Sakon Nakhon	July, 2005
BKKU 0509	*A. bisexualis*	I	Kalasin	October, 2005
BKKU 0510	*A. bisexualis*	II	Kalasin	October, 2005
BKKU 0511	*A. bisexualis*	III	Khon Kaen	October, 2005
BKKU 0612	*A. bisexualis*	I	Kalasin	March, 2006
BKKU 0613	*A. bisexualis*	II	Kalasin	March, 2006
BKKU 0614	*A. ambisexualis*	III	Khon Kaen	March, 2006
BKKU 0615	*A. ambisexualis*	VI	Sakon Nakhon	March, 2006
BKKU 0616	*A. bisexualis*	VII	Sakon Nakhon	March, 2006

Taxonomical characteristics of some representative isolates are described below:

***Achlya* sp. BKKU 0501** (Table 2.5)

Hard rough puffy, whitish and moist colony on GY agar and covered entire plate after 5 days incubation at 25 °C. Rigid hyphae penetrated into the agar (Figure 2.3A). Hyphae in GY broth were straight aseptate, stout, sharp tips and a bit branching. The hyphae were 10.5-30.5 (20.2±9.5) μm in width (n=30). In STW culture, typical zoosporangia were straightly filiform and fusiform shape with width 20.0-30.5 (25.5±5.0) x length 145.5-520.0 (357.8±156.7) μm (n=30). Zoospore

formation of fungal asexual stage was occurred about 8-12 hours after mycelia were transferred into STW at 25 °C. Primary zoospores were elongate and discharged from the end of the discharge tube and secondary zoospores were usually persisted at exit pores in the manner of the achlyoid (Figure 2.4). Encysted spores were spherical with 12.5-20.5 (16.5±4.0) μm in diameter (n=30). Oogonial and antheridial formations were not observed at all temperature except gemmae. The gemmae were fusiform and pyriform with single and frequently in chains. From the morphological characteristics and mode of zoospore release as described above, the isolate was identified as belonging to the genus *Achlya* according to Johnson (1956).

The sizes of hyphae and zoosporangia of BKKU 0502 and BKKU 0503 were slightly smaller than BKKU 0501. However, they were all identified as *Achlya* spp. by zoospore discharging pattern.

Table 2.5 Ranges (means±SD) of hyphae and zoosporangial size of three isolates belonging to the genus *Achlya*.

Isolates	Size (μm) (n=30)	
	Hyphae (width)	**Zoosporangia (width x length)**
BKKU 0501	10.5-30.5	20.0-30.5x145.5-520.0
	(20.2±9.5)	(25.5±5.0x357.8±156.7)
BKKU 0502	10.0-30.5	20.0-27.5x140.0-420.0
	(20.0±9.4)	(22.3±2.2x271.7±129.4)
BKKU 0503	10.5-30.0	18.5-29.5x142.5-450.5
	(20.1±9.6)	(24.3±5.1x296.5±154.0)

***A. ambisexualis* BKKU 0614** (Table 2.6)

Hard rough puffy, whitish and moist colony on GY agar and covered entire plate after 5 days incubation at 25 °C. Hyphae penetrated into the agar (Figure 2.3B). The hyphae in GY broth were aseptate and stout with sharp tips. They were 12.5-30.0 (18.9±6.2) μm in width (n=30).

The asexual stage in STW cultures, typical zoosporangia were straightly filiform and fusiform with width 15.0-30.0 (24.5±5.3) x length 190.0-390.0

(287.1±95.1) μm (n=30) and 2-4 rows of zoospores per zoosporangia. Zoospore formation was occurred about 8-12 hours after mycelia were transferred into STW at 25 °C. Zoospores were discharged in achlyoid. Encysted spores were 10.0-12.5 (11.2±1.1) μm in diameter (n=30).

The sexual stages were not observed at 5 and 10 °C for 4 weeks, except only gemmae were observed at 10 °C for 7 days. Oogonia were formed on hemp seed cultures after incubation at 15, 20 and 25 °C for 3-4 days and then formed only at the lateral parts of principal vegetative thalii. The oogonia were mainly pyriform and a scarcely spherical with width 50.0-85.0 (64.6±9.6) x length 57.5-100.0 (70.4±10.9) μm (n=30) and oogonial walls were conspicuously pitted under the points of attachment of antheridial cells. Oogonial stalks were straight. The fungus showed a single branch or the sparingly diclinous of antheridial branches. The antheridial cells appresssed the oogonia by lateral and short projection. Oospores were mature for 5, 4 and 3 days after oogonia formation at 15, 20 and 25 °C, respectively. Mature oospores were spherical, eccentric with 20.0-27.5 (24.2±3.2) μm in diameter (n=30) (Figure 2.5). The mature oospores were not abortively filling the oogonium. There were 3.0-11.0 (6.9±3.2) oospores per oogonium (n=30). Gemmae were fusiform, clavate, and spherical with single and frequently in chains (Figure 2.6). From the morphological characteristics and mode of zoospore release as described above, the isolate was identified as *A. ambisexualis* according to Johnson (1956).

The sizes of zoosporangia, oogonia and oospores of BKKU 0615 were slightly larger than BKKU 0614 and it was identified as well as *A. ambisexulais* (Johnson, 1956).

Table 2.6 Ranges (means±SD) of size of oogonia, oospores and oospore numbers per oogonium of two isolates of *A. ambisexualis*.

Isolates	Size (μm) (n=30)		Oospores/Oogonium
	Oogonia (width x length)	Oospores	
BKKU 0614	50.0-85.0x57.5-100.0	20.0-27.5	3.0-11.0
	(64.6±9.6x70.4±10.9)	(24.2±3.2)	(6.9±3.2)
BKKU 0615	55.0-112.5x57.5-115.0	25.0-30.0	1.0-15.0
	(79.2±21.2x83.2±24.7)	(26.1±0.8)	(7.6±6.2)

***A. bisexualis* BKKU 0504** (Table 2.7)

Hard rough puffy, whitish and moist colony on GY agar and covered entire plate after 5 days at 25 °C. Hyphae penetrated into the agar (Figure 2.3C). The hyphae in GY broth were aseptate, stout and sharp tips. The hyphae were 13.0-27.5 (20.2±7.2) μm in width (n=30).

The asexual stage in STW cultures, typical zoosporangia were straightly filiform and occasionally fusiform shape with width 30.0-60.0 (47.5±10.5) x length 170.0-520.0 (340.5±165.5) μm (n=30) and 2-3 rows of zoospores per zoosporangium. Zoospore formation was occurred about 8-12 hours after mycelia were transferred into STW at 25 °C. Zoospores were discharged in achlyoid. Encysted spores were globose and 12.5-17.5 (15.5±1.5) μm in diameter (n=30).

The sexual stages were not observed at 5 and 10 °C for 4 weeks, except only gemmae were observed at 10 °C for 7 days. Oogonia were formed on the hemp seed cultures after incubation at 15, 20 and 25 °C for 3-4 days and then formed at the terminal or lateral parts of principal vegetative hyphae. The oogonia were mainly spherical or pyriform with width 45.0-65.0 (55.4±9.5) x length 55.0-97.5 (75.3±18.5) μm (n=30) and oogonial walls were conspicuously pitted under the points of attachment of antheridial cells. Oogonial stalks were stout and straight. The fungus showed a single branch or the sparingly diclinous of antheridial branches. The antheridial cells appresssed the oogonia by lateral, apical and short projection. Oospores were mature 3 days after oogonial formation at 15, 20 and 25 °C. The mature oospores were spherical, eccentric with 22.5-27.5 (24.3±1.5) μm in diameter

(n=30) and were not abortively filling the oogonium (Figure 2.7). There were 2-8 (4.5±2.3) oospores per oogonium (n=30). Gemmae were very abundant, filiform, fusiform, and pyriform with single and frequently in chains (Figure 2.8). From the morphological characteristics and mode of zoospore release as described above, the isolate was identified as *A. bisexualis* according to Johnson (1956).

The size of zoosporangia, oogonia and oospores of BKKU 0509, BKKU 0510, BKKU 0511, BKKU 0612, BKKU 0613 and BKKU 0616 were smaller than BKKU 0504 and all isolates were identified as *A. bisexualis* according to Johnson (1956).

Table 2.7 Ranges (means±SD) of size of oogonia, oospores and oospore numbers per oogonium of seven isolates of *A. bisexualis*.

Isolates	Size (μm) (n=30)		Oospores/Oogonium
	Oogonia (width x length)	Oospores	
BKKU 0504	45.0-65.0x55.0-97.5	22.5-27.5	2.0-8.0
	(55.4±9.5x75.3±18.5)	(24.3±1.5)	(4.5±2.3)
BKKU 0509	35.0-50.0x42.5-80.0	15.0-17.5	4.0-12.0
	(43.3±6.5x64.3±15.5)	(16.0±0.6)	(6.8±2.5)
BKKU 0510	30.0-40.0x32.5-50.0	12.5-15.0	3.0-10.0
	(37.0±2.5x41.8±6.3)	(14.8±0.1)	(5.1±2.0)
BKKU 0511	30.0-47.5x32.5-57.5	12.5-15.0	3.0-15.0
	(37.3±7.2x45.8±10.2)	(14.0±0.8)	(7.1±3.9)
BKKU 0612	42.5-75.0x57.5-95.0	12.5-27.5	1.0-9.0
	(50.1±6.8x70.5±12.5)	(21.8±4.5)	(4.2±3.0)
BKKU 0613	27.5-47.5x27.5-72.5	12.5-15.0	2.0-8.0
	(32.3±4.5x53.3±18.5)	(13.8±0.7)	(4.8±2.5)
BKKU 0616	40.0-70.0x55.0-90.0	20.0-27.5	2.0-6.0
	(55.3±12.5x74.7±14.3)	(23.5±2.2)	(4.4±1.2)

***Aphanomyces* sp. BKKU 0505** (Table 2.8)

Flat, fine, dense and whitish colony on GY agar and reached full plate after 4 days at 25 °C. Fungal colony could only grow on surface of GY agar (Figure 2.3D). The hyphae in GY broth were aseptate, slender, round-tip hyphae with 2.0-7.5

(5.4±1.5) μm in width (n=30) and were not branching. In STW cultures, zoosporangia were not wider than the hyphae and were predominantly filiform with single row of zoospores. Zoospore formation was occurred about 8-12 hours after mycelia were transferred into STW at 25 °C. Zoospores were discharged in achlyoid and sometimes the zoospores were emerged laterally from the hyphae and encysted immediately (Figure 2.9). Encysted spores were spherical with 2.0-5.5 (3.8±1.4) μm in diameter (n=30). Oogonial and antheridial formations were not observed at all temperature. From these characteristics, the isolate was identified to the genus *Aphanomyces* as described by Scott (1961).

The sizes of hyphae and encysted zoospores of BKKU 0508 were smaller than BKKU 0505 and they were all identified to the genus *Aphanomyces.*

Table 2.8 Ranges (means±SD) of hyphae and encysted zoospores of two isolates of the genus *Aphanomyces*.

Isolates	Size (μm) (n=30)	
	Hyphae (width)	**Encysted zoospores**
BKKU 0505	2.0-7.5	2.0-5.5
	(5.4±1.5)	(3.8±1.4)
BKKU 0508	2.0-7.0	2.0-5.0
	(5.1±1.7)	(3.4±1.2)

***Saprolegnia diclina* BKKU 0506**

Soft puffy, whitish and moist colony on GY agar and reached full plate after 2 days incubation at 25 °C. Fungal colony could only grow on surface of GY agar (Figure 2.3E). Hyphae in GY broth exhibited aseptate, slender, round-tip with 5.0-17.5 (10.25±5.24) μm in width (n=30).

The asexual stage in STW cultures, zoosporangia were predominantly clavate or filiform and they were frequently irregular, straight or bent with width 95.0-225.0 (154.6±59.3) x length 125.0-225.0 (177.0±46.2) μm (n=30) and 4-6 rows of zoospores per zoosporangium. Zoospore formation was occurred about 8-16 hours after mycelia were transferred into STW at 25 °C. Primary zoospores were elongate

and irregular shape and discharged fashion through the exit pore and swimming away from tip of zoosporangium in the manner of the saprolegnoid (Figures 2.10). Encysted spores were 7.5-12.0 (9.3±1.7) μm in diameter (n=30).

The sexual stages were not observed at 5 °C for 4 weeks. Oogonia were formed on the hemp seed cultures after incubation for 8 days at 10 °C and 3-6 days at 15, 20 and 25 °C. Then, the oogonia were formed at the terminal or lateral parts of principal vegetative thalii. The oogonia were spherical and oval with width 35.0-89.5 (60.3±24.2) x length 39.0-95.0 (69.4±24.6) μm (n=30) and oogonial walls were pitted under the points of antheridia cell attachment. The isolate revealed a dominance of diclinous antheridial branches which abundantly surrounded the oogonium (Figure 2.11). The antheridial cells were tubular, clavate or irregular and laterally or apically appressed on the oogonial wall. Oospores were mature for 10 days at 10 °C, 7 days at 15 °C and 3 days at 20 °C and 25 °C after the oogonia formation. The structure of mature oospore was centric with 15.0-30.0 (22.5±7.5) μm in diameter (n=30) and there was 1-14 (8.6±5.4) oospores per oogonium (n=30). Gemmae were spherical which arranged in a single and chains (Figure 2.12). From these characteristics, the isolate was identified as *Saprolegnia diclina* according to Seymour (1970).

***Pythium* sp. BKKU 0507**

Flat, fine radial and chrysanthemum, dense, and whitish colony on GY agar. The colony was covered entire plate after 3 days at 25 °C and was submerged the agar (Figure 2.3F). The hyphae in GY broth were aseptate, slender and round-tip with 3.5-7.0 (5.1±1.4) μm in width (n=30) and branched. Zoosporangia were terminally filamentous and were not different from the hyphae. Zoospores moved through the sporangia and formed vesicle at the sporangial tip. Then, the zoospores swam away from the vesicle in the manner of the pythyoid (Figure 2.13). Oogonial and antheridial formations were not observed at all temperature. From these characteristics, the isolate was identified as *Pythium* sp. as described by Van Der Plaats-Niterink (1981).

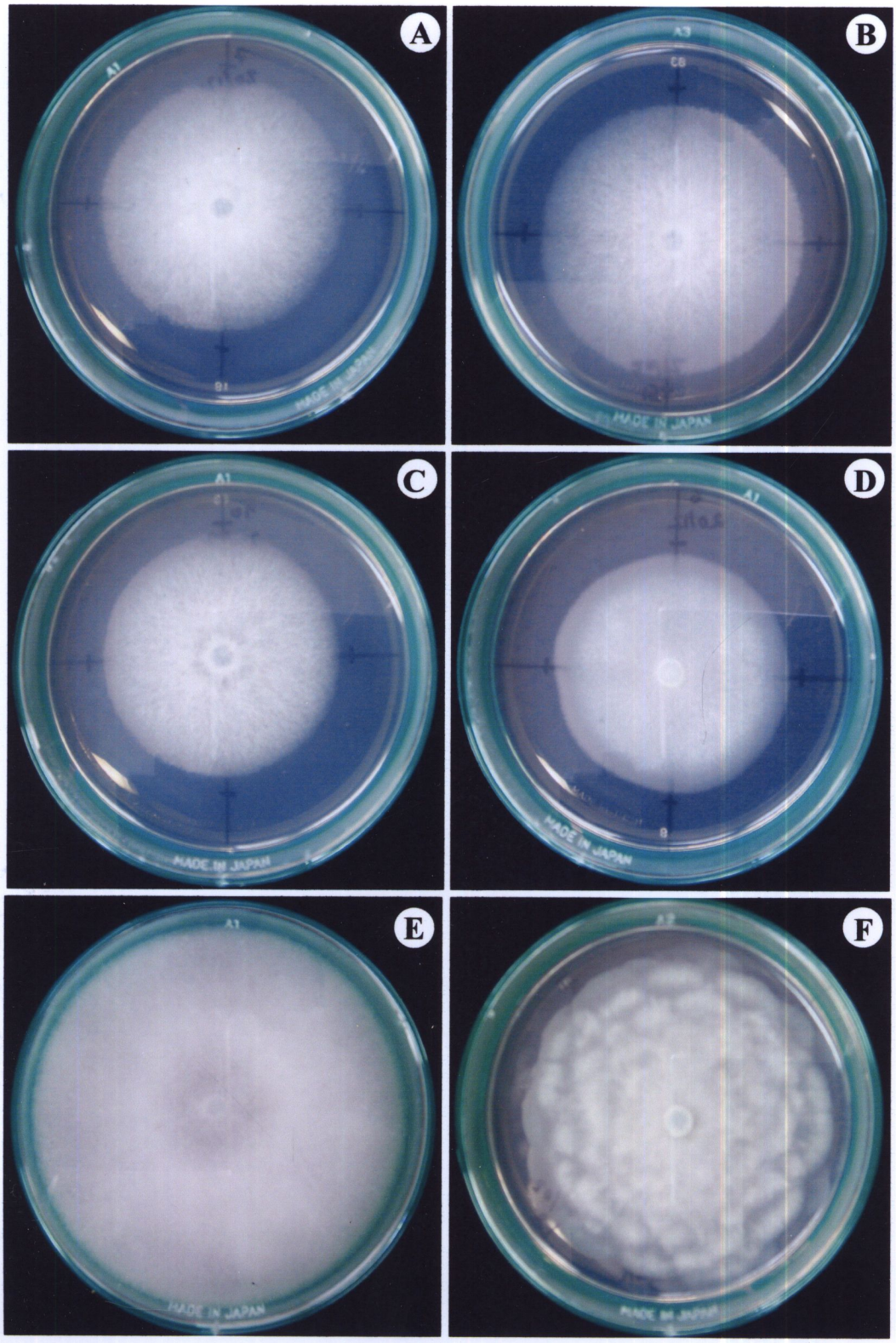


Figure 2.3 Fungal colonies of various genera found in tilapia eggs after incubation at 25 °C for 3 days. A. *Achlya* spp. B. *A. ambisexualis* C. *A. bisexualis* D. *Aphanomyces* spp. E. *S. diclina* F. *Pythium* sp.

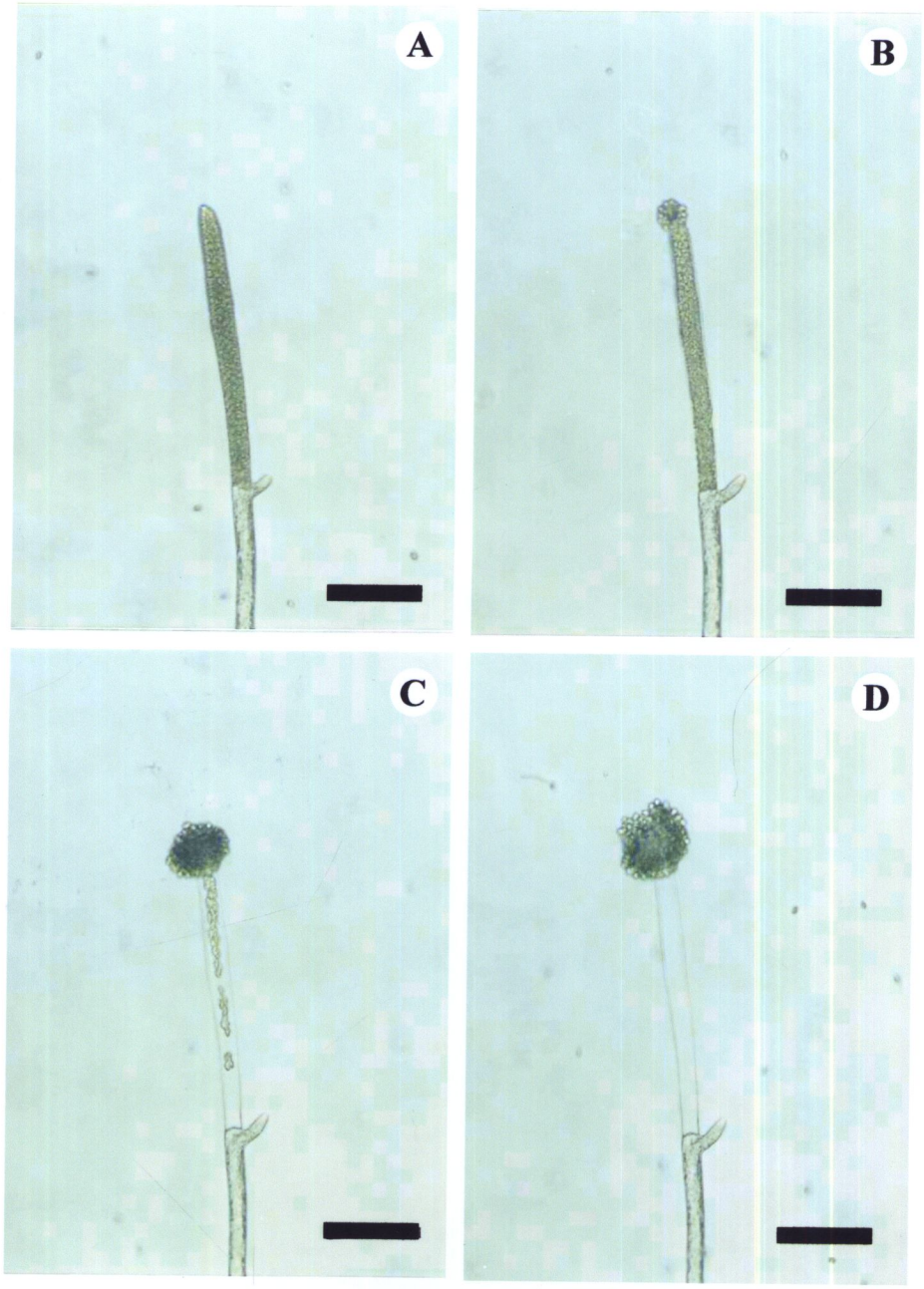


Figure 2.4 Zoospore production of *Achlya* sp. BKKU 0501 in the manner of the achlyoid (bar=20 μm) A. Zoosporangium with 3-4 rows of zoospores. B-C. The zoosporangium started to release primary zoospores and released more zoospores. D. The primary zoospores encysted at tip of zoosporangium as a cluster.

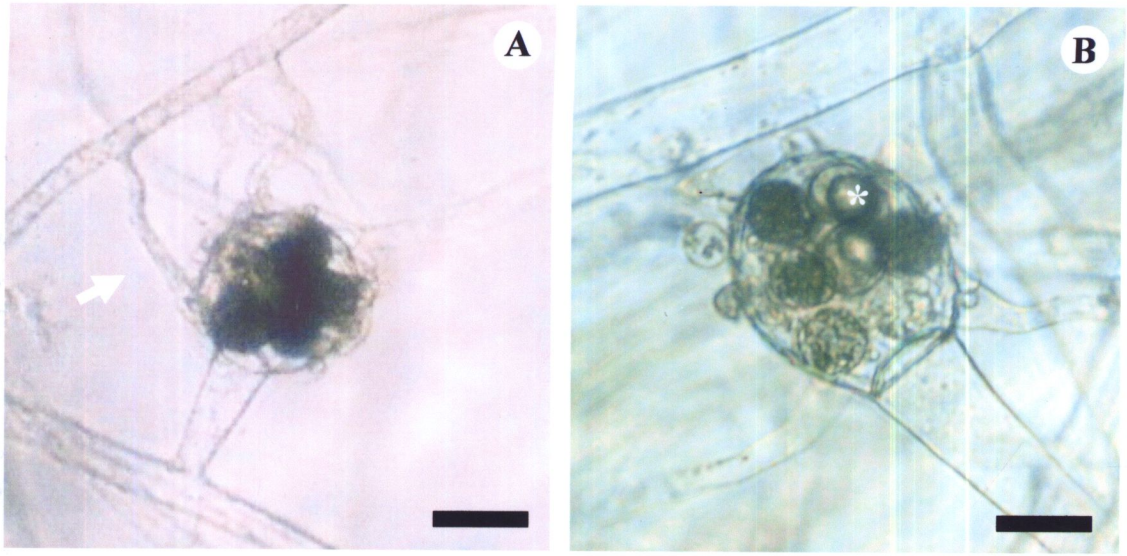


Figure 2.5 Sexual stage of *A. ambisexualis* BKKU 0614 (bar=20 μm) A. Oogonium with young oospores and diclinous antheridial branch (➡). B. Matured oogonium with eccentric oospores (*).

Figure 2.6 Asexual stage of *A. ambisexualis* BKKU 0614 showing fusiform gemmae (bar=20 μm).

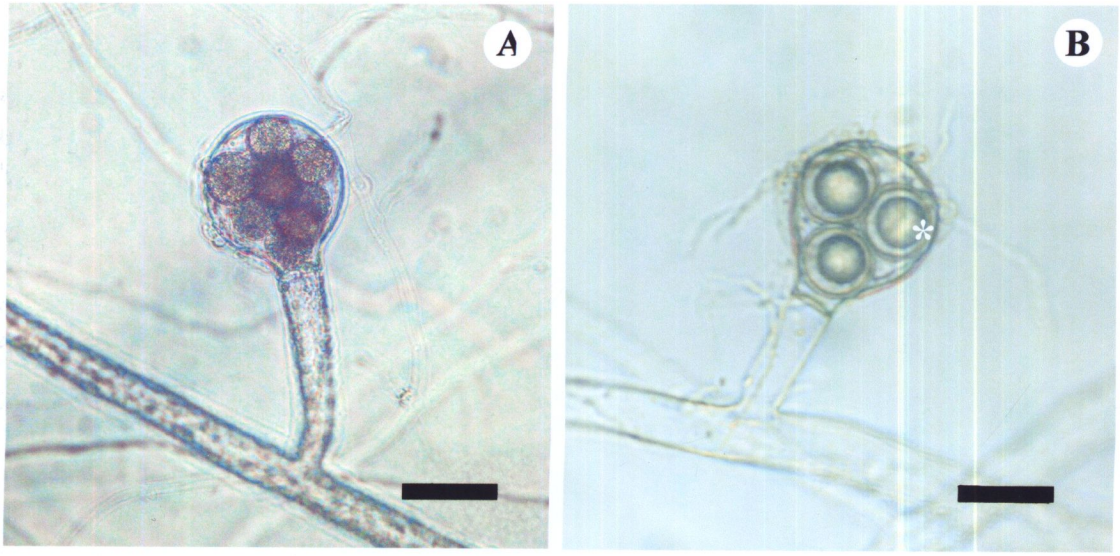


Figure 2.7 Sexual stage of *A. bisexualis* BKKU 0504 (bar=30 μm) A. Oogonium with young oospores. B. Matured oogonium with eccentric oospores (*).

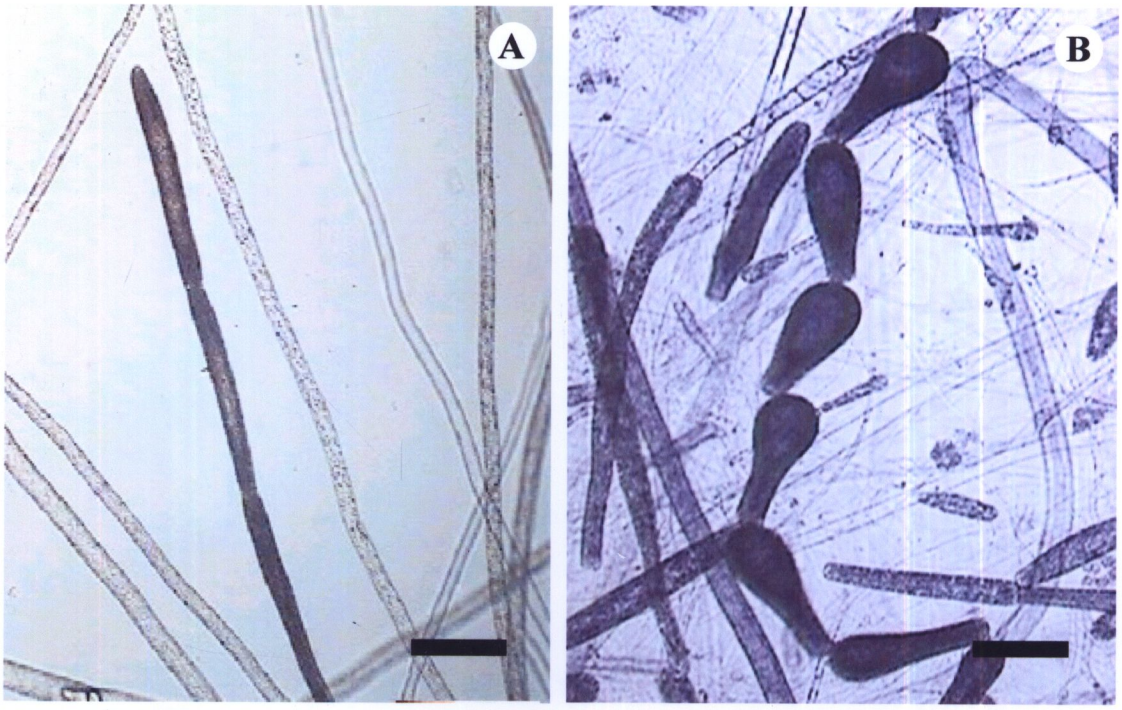


Figure 2.8 Asexual reproduction of *A. bisexualis* BKKU 0504 (bar=20 μm) A. Filiform gemmae in chain. B. Pyriform gemmae in chain.

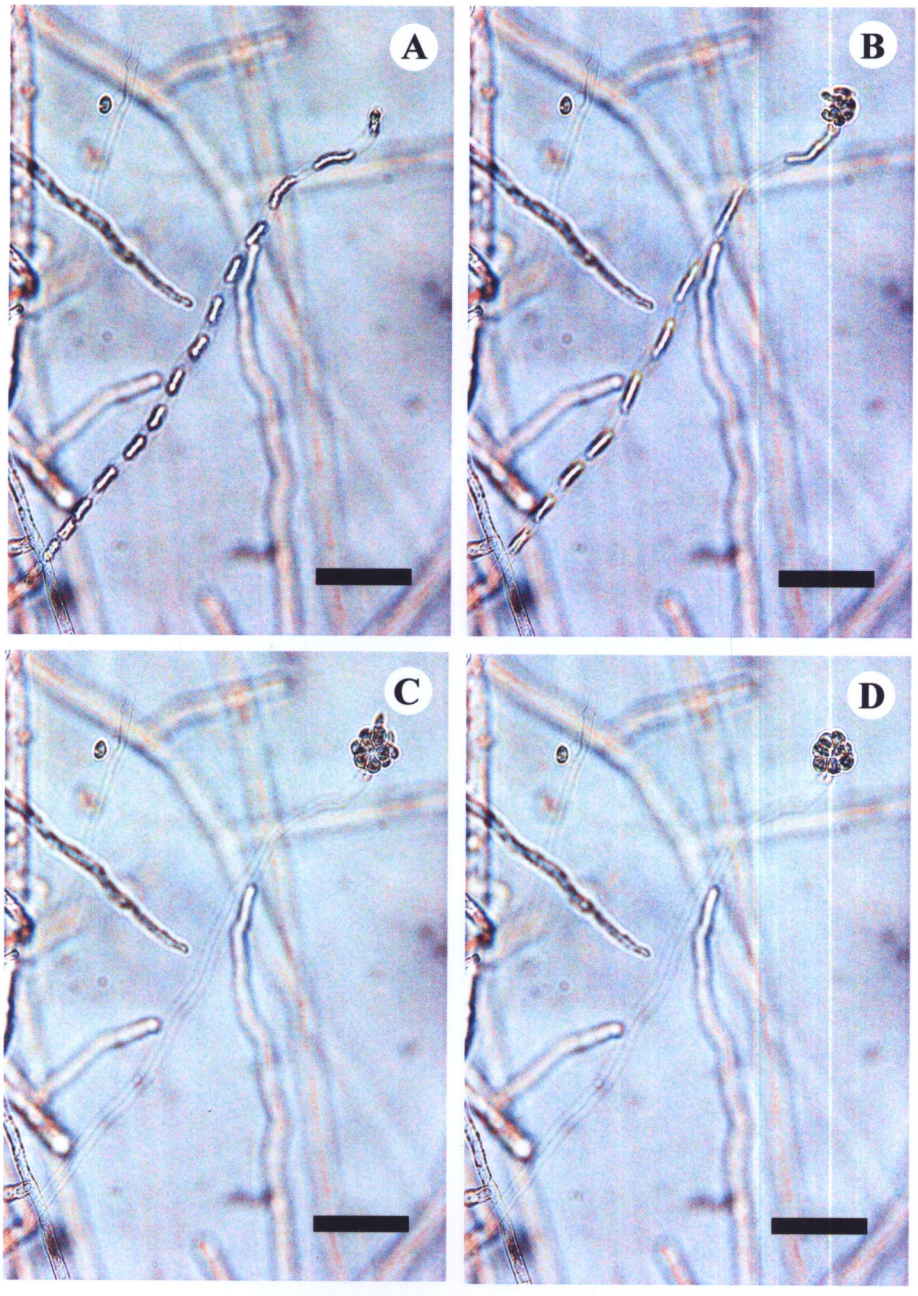


Figure 2.9 Zoospore production of *Aphanomyces* sp. BKKU 0505 in the manner of the achlyoid (bar=20 μm) A. A single row of zoospores in the zoosporangium. B-D. Primary zoospores discharged from the zoosporangium and encysted at the top of the zoosporangium.

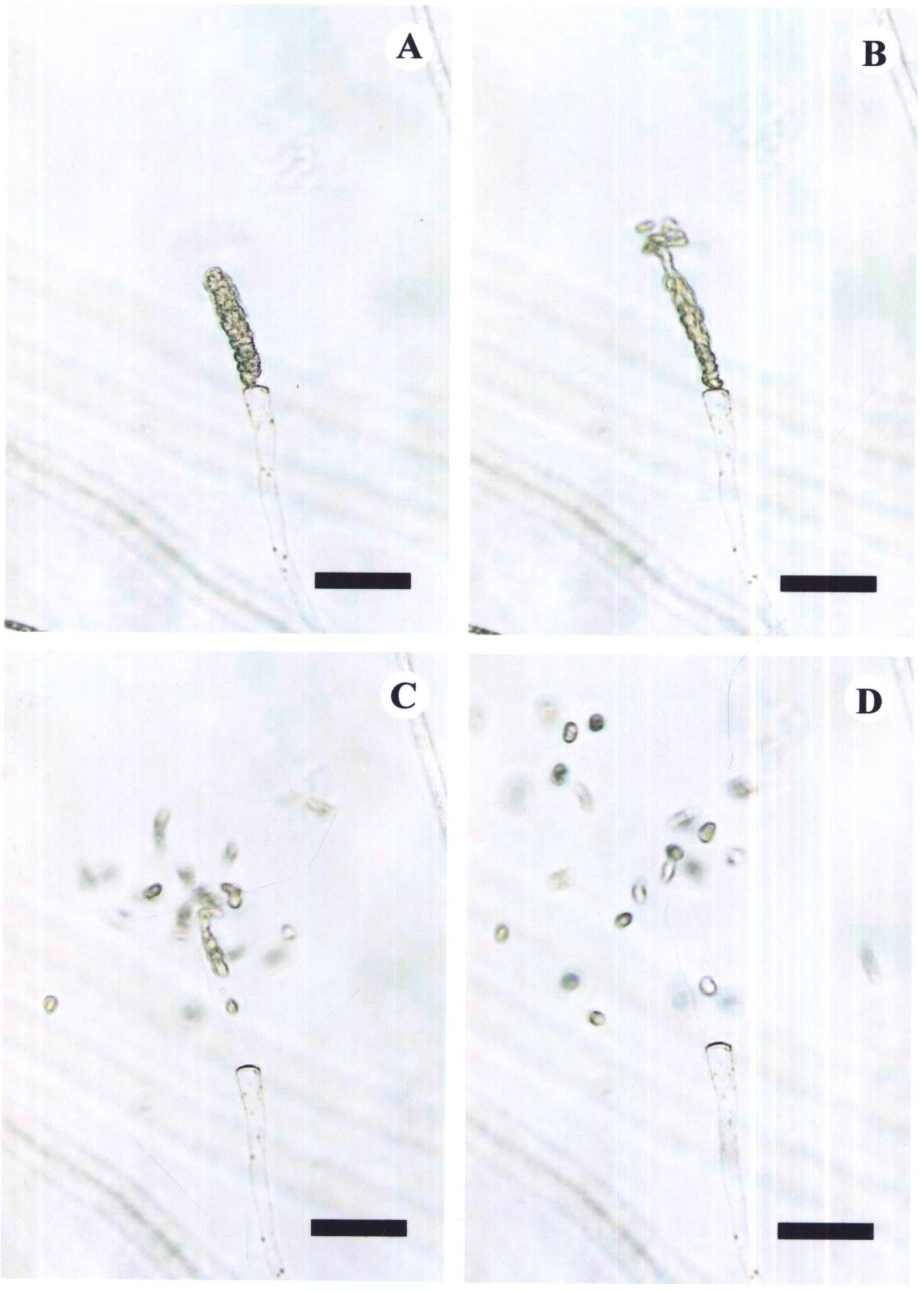


Figure 2.10 Zoospore production of *S. diclina* BKKU 0506 in the manner of saprolegnoid (bar=20 μm) A. Matured zoosporangium with primary zoospores. B. The sporangium released primary zoospores. C-D. The primary zoospores swam away from zoosporangium in the water.

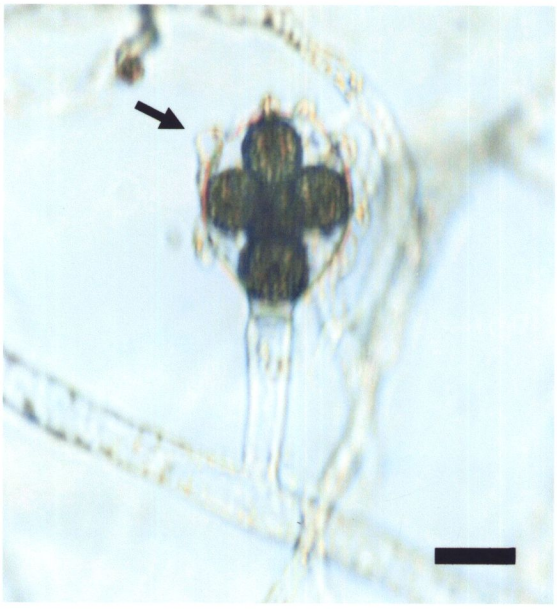


Figure 2.11 Sexual stage of *S. diclina* BKKU 0506 showing oogonium with young oospores and diclinous antheridial branch (➡) (bar=5 μm).

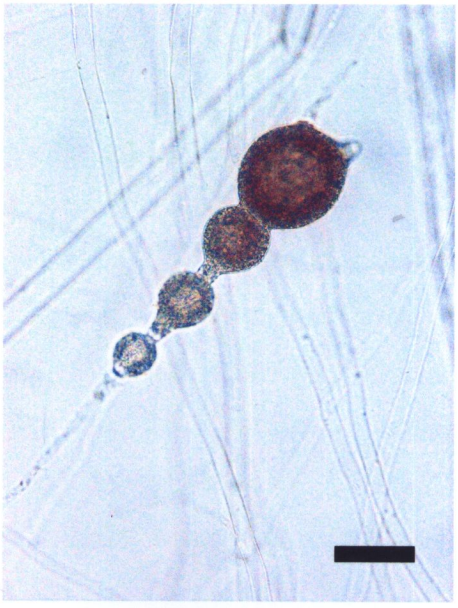


Figure 2.12 Asexual reproduction of *S. diclina* BKKU 0506 showing spherical gemmae in chain (bar=20 μm).

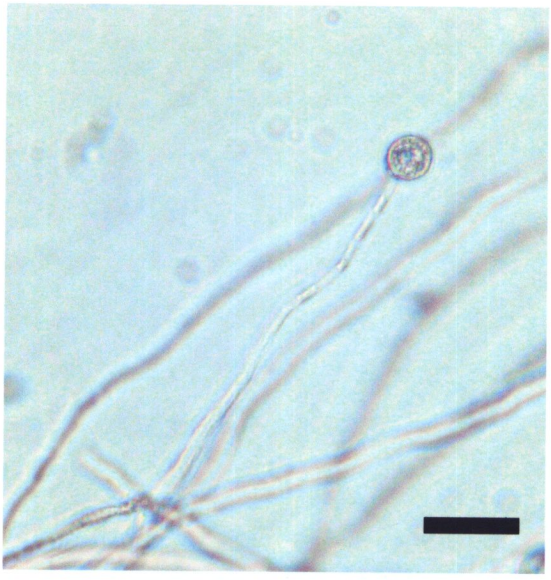


Figure 2.13 Zoospore production of *Pythium* sp. BKKU 0507 in the manner of the pythyiod showing a vesicle with zoospores (bar=20 μm).

3.2 Occurrence of fungal isolates

A percentage of total fungal isolates from all sampling sites in each sampling period is presented in Figure 2.14. The occurrence of fungal isolates such as 37 % (3/8) *Achlya* spp., 24 % (2/8) *Aphanomyces* spp., 13 % (1/8) *A. bisexualis*, 13 % (1/8) *S. diclina* and 13 % (1/8) *Pythium* sp. were found during June-July 2005. Only 100 % (3/3) *A. bisexualis* was found during October-November 2005. Whereas 60 % (3/5) *A. bisexualis* and 40 % (2/5) *A. ambisexualis* were appeared in March-April 2006.

The occurrences of aquatic fungi from each sampling site during the study period from June 2005 to April 2006 are given in Table 2.9. *Achlya* sp. BKKU 0501 and *A. bisexualis* BKKU 0509 and BKKU 0612 were isolated from site I which is a private farm and site II, Kalasin Inland Fisheries Station, both sites located with the same altitude 140 masl and received water from Lam Pao Dam. *Achlya* sp. BKKU 0503, *A. bisexualis* BKKU 0511 and *A. ambisexualis* BKKU 0614 were isolated from site III which was the private farm located in Khon Kaen province with 163 masl and received water from the Chi River. *A. bisexualis* BKKU 0504, *Aphanomyces* sp. BKKU 0505, *S. diclina* BKKU 0506 and *Pythium* sp. BKKU 0507 were only found from site IV in June 2005. This site is Khon Kaen Inland Fisheries Research and

Development Center had same altitude as site III but it received water from the Pong River. Site V, no fungi were found throughout the sampling period. This site was Maha Sarakham Inland Fisheries Research and Development Center and located in Maha Sarakham province with 142 masl and water used for incubation eggs from Loeng Chan Rapids. *A. ambisexualis* BKKU 0615 was isolated from site VI in March 2006 and there were no fungi occurred in June-July and October-November 2005, Site VI is the private farm and located at Sakon Nakhon province with 166 masl.

Aphanomyces sp. BKKU 0508 occurred in July 2005 and *A. bisexualis* isolate BKKU 0616 occurred in March 2006 at site VII. This site is Sakon Nakhon Inland Fisheries Research and Development Center, situates in Sakon Nakhon province with 166 masl and water from Nong Han Lake was used for incubation eggs.

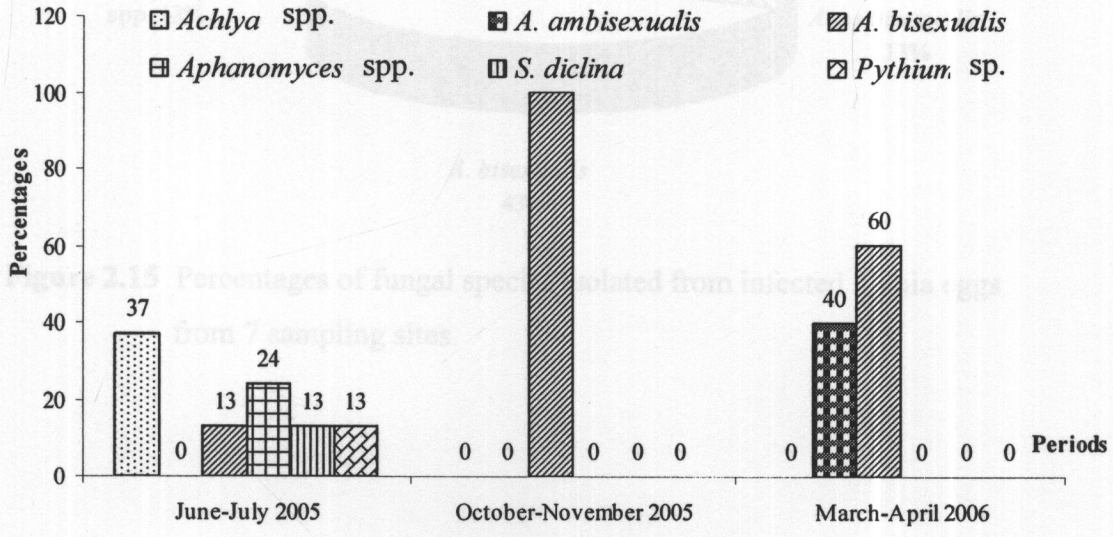


Figure 2.14 Number of fungi isolated from tilapia eggs with fungal infection during June-July 2005, October-November 2005 and March-April 2006.

3.3 Species diversity

From the characteristics of asexual stage, all isolates were classified to families Saproegniales and Pythiaceae. Family Saproegniaceae was consisted of 3 genera i.e. *Achlya* [3 isolates (19 %) *Achlya* spp., 2 isolates (13 %) *A. ambisexualis* and 7 isolates (43 %) *A. bisexualis*], *Aphanomyces* [2 isolates (13 %) *Aphanomyces* spp.], and *Saprolegnia* [1 isolate (6 %) *S. diclina*] and family Pythiaceae was composed of 1 genus: *Pythium* [(1 isolate (6 %) *Pythium* sp.)] as shown in Figure 2.15.

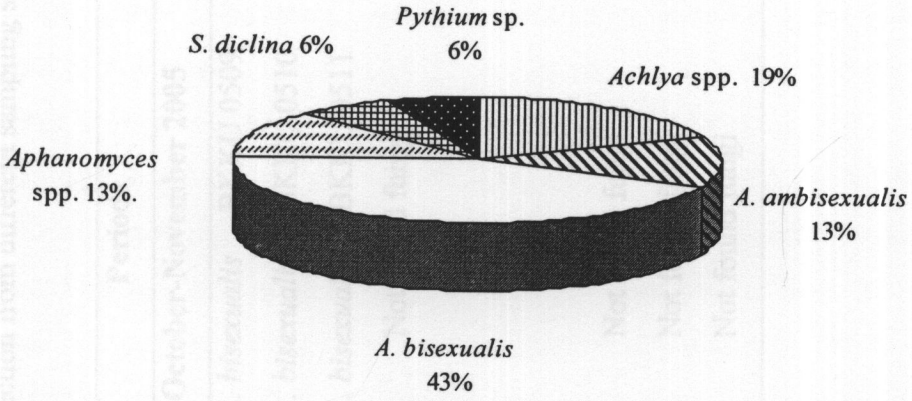


Figure 2.15 Percentages of fungal species isolated from infected tilapia eggs from 7 sampling sites.

Table 2.9 Fungal isolates from tilapia eggs with fungal infection from different sampling sites during June 2005-April 2006.

Sites	Periods		
	June-July 2005	**October-November 2005**	**March-April 2006**
I	*Achlya* sp. BKKU 0501	*A. bisexualis* BKKU 0509	*A. bisexualis* BKKU 0612
II	*Achlya* sp. BKKU 0502	*A. bisexualis* BKKU 0510	*A. bisexualis* BKKU 0613
III	*Achlya* sp. BKKU 0503	*A. bisexualis* BKKU 0511	*A. ambisexualis* BKKU 0614
IV	*A. bisexualis* BKKU 0504	Not found fungi	Not found fungi
	Aphanomyces sp. BKKU 0505		
	S. diclina BKKU 0506		
	Pythium sp. BKKU 0507		
V	Not found fungi	Not found fungi	Not found fungi
VI	Not found fungi	Not found fungi	*A. ambisexualis* BKKU 0615
VII	*Aphanomyces* sp. BKKU 0508	Not found fungi	*A. bisexualis* BKKU 0616

4. Discussion

The present study, *Achlya, Aphanomyces, Saprolegnia* and *Pythium* were isolated from tilapia eggs with fungal infection. *Achlya* was the most dominant genus in samples isolated from 6 out of 7 sampling sites during the collecting period. It was similar to the report of Yuasa *et al.* (2000) that they isolated *Achlya, Aphanomyces* and *Saprolegnia* from the fungal infected tilapia eggs, except *Pythium,* and they could very often isolate the fungi of genus *Achlya* of various fish species in Northeast of Thailand. It may indicate that *Achlya* was the most common genus throughout the year which was similar to Chaudhuri *et al.* (1947) (*cited by* El-Hissy and Khallil, 1991). According to Nejadsattari (2000), *Achlya* is a large genus and contains many species in aquatic ecosystems. In the present study, it showed that genus *Achlya* consisted of *Achlya* spp. (3 out of 16, 19 %), *A. ambisexualis* (2 out of 16, 13 %) and *A. bisexualis* (7 out of 16, 43 %), respectively.

It was found that *Achlya* was obtained from sites I, II, III and VI, whereas all 4 genera were isolated from site IV only. Some physical and chemical analysis of the water used for egg incubation at site IV revealed receiving water from the Pong River exhibited low dissolved oxygen, pH, electro-conductivity and null salinity comparing with different sampling sites. Misra (1982) states that various quantity of dissolved oxygen, pH and temperature may affect on the variation in number of fungi. In this study, no fungi were found from site V throughout the sampling periods. It may be due to a low density of incubated eggs in culture system (personal communication). This is agreement with Rach *et al.* (1997), fish eggs incubated in high density culture system provide ideal condition outbreaks. According to Piper *et al.* (1982) (*cited by* Rach *et al.*, 1997), if a fungal infection starts, it can spread rapidly from infected to healthy eggs. From this study, *Achlya* was also isolated from site VI only in March 2006 and there were no fungi occurred in June-July and October-November 2005. It may be due to rainy water with high concentration of total dissolved solids, electro-conductivity and salinity (Table 2.9) was used for egg incubation at this time. After that water from Nam Un Dam was used instead of rainy water in March-April 2006. It was found that *Aphanomyces* and *Achlya* were obtained from site VII as well.

From this study, the occurrence of fungi may be depended on water sources for egg incubation such as rain, dam, river, reservoir and lake (Table 2.2). This

finding is similar to the report of Yuasa *et al.* (2000) that the differences in water sources used for incubation eggs may be affected on the distribution of fungi. Rach *et al.* (1997) concluded that the occurrence and severity of a fungal outbreak is dependent on the water source, temperature, organic load and incubation period. Gupta and Mehrota (1989) also stated that a major environmental factor controlled the occurrence of aquatic fungi is water temperature. From the present investigation, the occurrence of aquatic fungi was neither associated with the water temperature nor the period of sampling. Due to the ranges of water temperature were not different between sampling sites and the collecting periods (personal recorded). It was also found that altitude of sampling site location had no effect on distribution of fungi.

Therefore, it may conclude that the occurrence of fungi was not associated with the host eggs, location of sampling sites and periods of collection but it may be depended on quality of the water from different sources into egg incubation tank and water itself which may promote mycoflora species diversity.

CHAPTER III
SOME BIOLOGICAL CHARACTERISTICS OF FUNGI ISOLATED FROM TILAPIA EGGS WITH FUNGAL INFECTION

1. Introduction

Although fungal diseases of fish and fish eggs have been known of for many years (Srivastava, 1980; Post, 1983; Kitancharoen *et al.*, 1997; Nejadsattari, 2000), fungal infection of fish and fish eggs are still problematic for culturists. *Achlya, Aphanomyces* and *Sprolegnia* are three genera of family Saprolegniaceae which cause diseases in fish and fish eggs (Czeczuga and Woronowicz, 1993; Wada *et al.*, 1993; Kitancharoen and Hatai, 1997; Paxton and Willoughby, 2000; Hussein *et al.*, 2001). They were widely spread throughout in natural and fish pond water (Schmitt and Beneke, 1962; Kitancharoen *et al.*, 1995; El-Hissy *et al.*, 1992; Czeczuga and Muszynska, 1997; Steciow, 2001). From the present study, fungi in family Saprolegniaceae are major fungal flora appeared to be responsible for the infection. Especially genus *Achlya*, a second genus followed from genus *Saprolegnia*, is well known as one of important genera which causing fungal disease in many fish and their eggs in Thailand (Willoughby and Lilley, 1992). Identification of these fungi is based on morphological structures of hyphae, mode of typical zoospore released and reproductive organs of fungi (Post, 1983). But the identification from the morphological characteristics of fungi is sometimes not obscured because some fungi had a similarity of morphology and were difficult to unidentifiable.

Therefore, study of some biological characteristics of fungi such as effect of different temperatures, various pHs and various salinity of sodium chloride (NaCl) on growth of hyphae and zoospore germination were investigated in order to classify fungal flora (Olàh and Farkas, 1978; Kitancharoen *et al.*, 1996). In addition, the biological characteristic among the fungal flora may be related with the different degrees of pathogenicity and host specificity (Willoughby and Copland, 1984; Kitancharoen *et al.*, 1996).

The purposes of this chapter were to study some biological characteristics such as temperature, pH and salinity of NaCl on vegetative growth and zoospore germination of fungi isolates from tilapia eggs.

2. Materials and Methods

2.1 Fungi and culture condition

Sixteen purified fungal isolates used in this study are the same as fungi isolated from tilapia eggs in Table 2.4 (Chapter II). The fungi were routinely maintained on GY agar at 25 °C and subcultured to fresh GY agar every month.

2.2 Effect of various temperatures on vegetative growth

GY agar with pure fungal colony of 16 isolates studied was incubated at 25 °C for 24-48 hours were used in this experiment. The circular blocks of actively growing edge of the culture colony were cut with a No. 2 cork borer (5.5 mm in diameter) and centrally placed onto Petri dishes (100x22 mm) which containing of 20 mL GY agar. Plates were incubated at different temperatures 5, 10, 15, 20, 25, 30, 35 and 40 °C. Colony diameters were measured daily with a vernier caliper for 7 days, and the result showed as mean of two perpendicular radii. In a case of negative test during experiment, the tested agar blocks were incubated again at 25 °C to reconfirm the survival of fungi. Determination of colony radius (mm) was calculated from radius of fungal growth (mm) subtracted with radius of centrally circular agar block (mm).

2.3 Effect of various temperatures on zoospore production

Preparation of circular blocks of 16 fungal isolates was the same procedure as previous mention and incubated in 20 mL of GY broth at 25 °C for 48 hours. The hyphae were cut and washed several times with STW. Approximately the same amount of hyphae was put into small Petri dishes (50x15 mm) containing of 10 mL STW and incubated at 10, 15, 20, 25 and 30 °C. The numbers of motile zoospores were counted using a Neubauer counting chamber (Erma®) every 24 hours for 1 week and at day 14, 21 and 28.

2.4 Effect of various pHs on vegetative growth

GY broth was adjusted to various pHs 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, 10.0, 11.0 and 12.0 by adding 1N HCL or 1N NaOH. After that the GY broth was filtered through 0.2 μm millipore filter paper (Sartorius, Hannover, Germany) and poured into sterilized test tubes. The GY agar blocks with young mycelia were prepared the same procedure as described before and cut off with a No.2 cork borer. After that the mycelia were transferred into test tubes containing of 10.0 mL GY broth with various pH and incubated at 25 °C. The growth of fungal colony was observed by naked eye and measured daily by the vernier caliper for 5 days. Determination of fungal colony was calculated from length of fungal colony (mm) subtract with length of circular agar block (mm).

2.5 Effect of various pHs on zoospore germination

The zoospore suspension of each isolate was obtained as follows: the edge of the fungal colony on GY agar was cut and put into 20 mL of GY broth which was incubated at 25 °C for 48 hours. The mycelia were washed several times with STW, then placed in STW and kept at 25 °C for 48 hours to obtain high numbers of zoospores. The numbers of zoospores of each isolate were adjusted to $1x10^3$ zoospores/mL. A 100 μL portion of zoospore suspension was transferred into the Petri dishes containing of 10 mL of GY broth with various pHs as the Experiment 2.4. The plates were incubated at 25 °C for 24 hours to observe zoospore germination and hyphal growth. Abnormal growth of fungal hyphae was observed at day 7.

2.6 Effect of salinity of NaCl on vegetative growth

The agar blocks with the actively growing hyphae on GY agar were prepared with the same method as previously described and put into the center of 20 mL of the GY agar plates containing different concentrations of NaCl 0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5 and 4.0 percent (%), then incubated at 25 °C. The radial growth of colony from the blocks was measured daily with the vernier caliper for 7 days and was calculated as the same as in Experiment 2.2.

Three replicates of each experiment were carried out.

3. Results

3.1 Effect of various temperatures on vegetative growth

All isolates of fungi were able to grow at the temperature range of 10-35 °C (Figures 3.1 and 3.2). Slightly growths were observed at 5 °C, but they were still able to survive after they were reincubated at 25 °C. However, neither isolates were able to grow at 40 °C. Among 4 fungal genera, *Achlya* spp. BKKU 0501, BKKU 0502 and BKKU 0503 showed fast growing between 20 and 30 °C with maximal growth at 35 °C. *A. ambisexualis* BKKU 0614 and BKKU 0615 showed rapid growth at 20-35 °C with maximum at 25-35 °C. *A. bisexualis* BKKU 0504, 0505 and 0511 showed fast growth at 20-35 °C with maximal growth at 35 °C. Whereas *A. bisexualis* BKKU 0510, 0612, 0613 and 0616 grew rapidly at 10-15 °C and more slowly at 20 °C but they had fast growing again at 25-35 °C with maximal growth at 35 °C. *Aphanomyces* spp. BKKU 0505 and BKKU 0508 showed maximal growth at 30-35 °C and 25-35 °C, respectively. *S. diclina* BKKU 0506 showed maximal growth between 20 and 35 °C as well as *Pythium* sp. BKKU 0507, while *S. diclina* BKKU 0506 covered entire plate at 2 days after inoculation at 20 °C comparing to all other isolates.

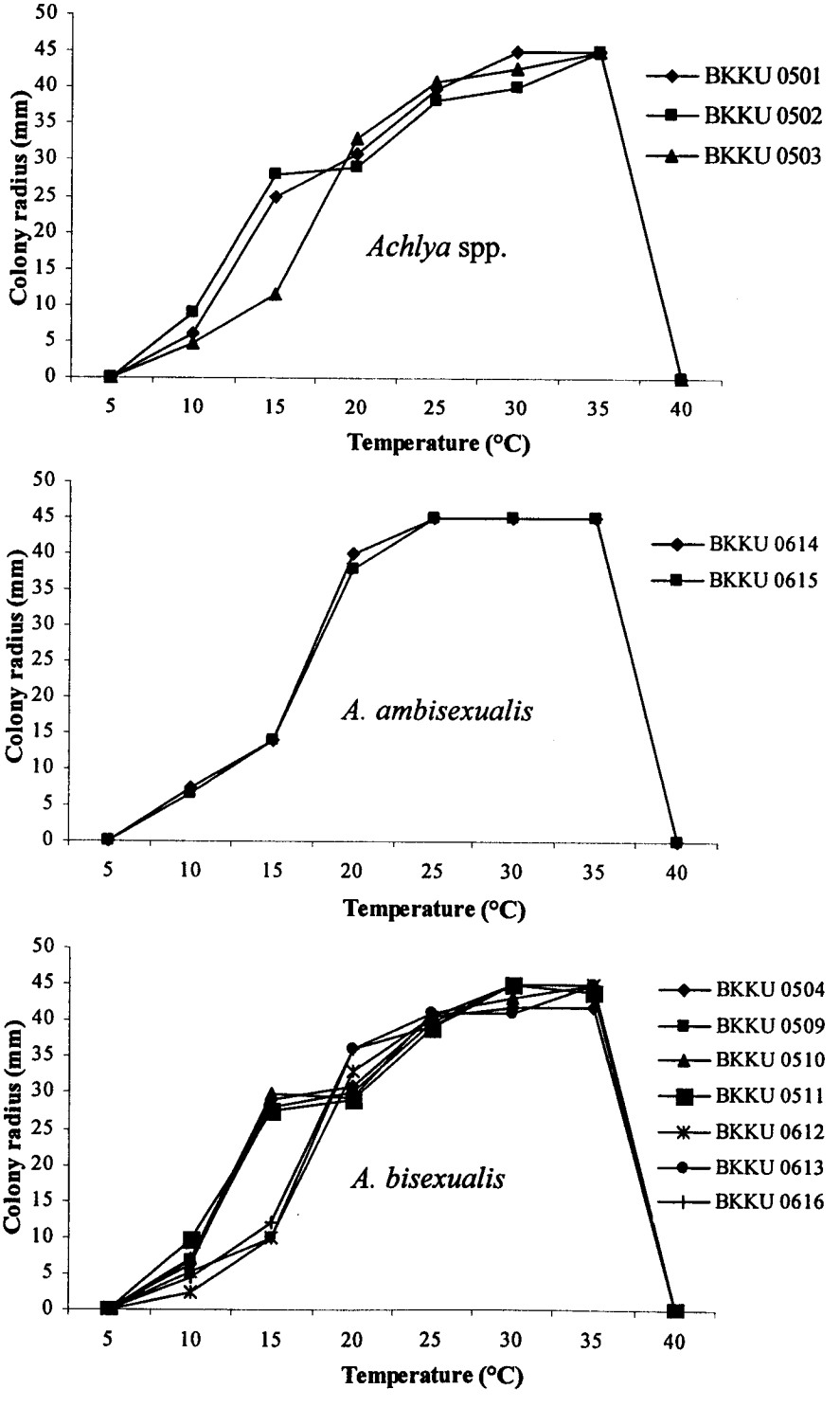


Figure 3.1 Effect of various temperatures on vegetative growth of *Achlya* spp., *A. ambisexualis* and *A. bisexualis* at 4 days after inoculation.

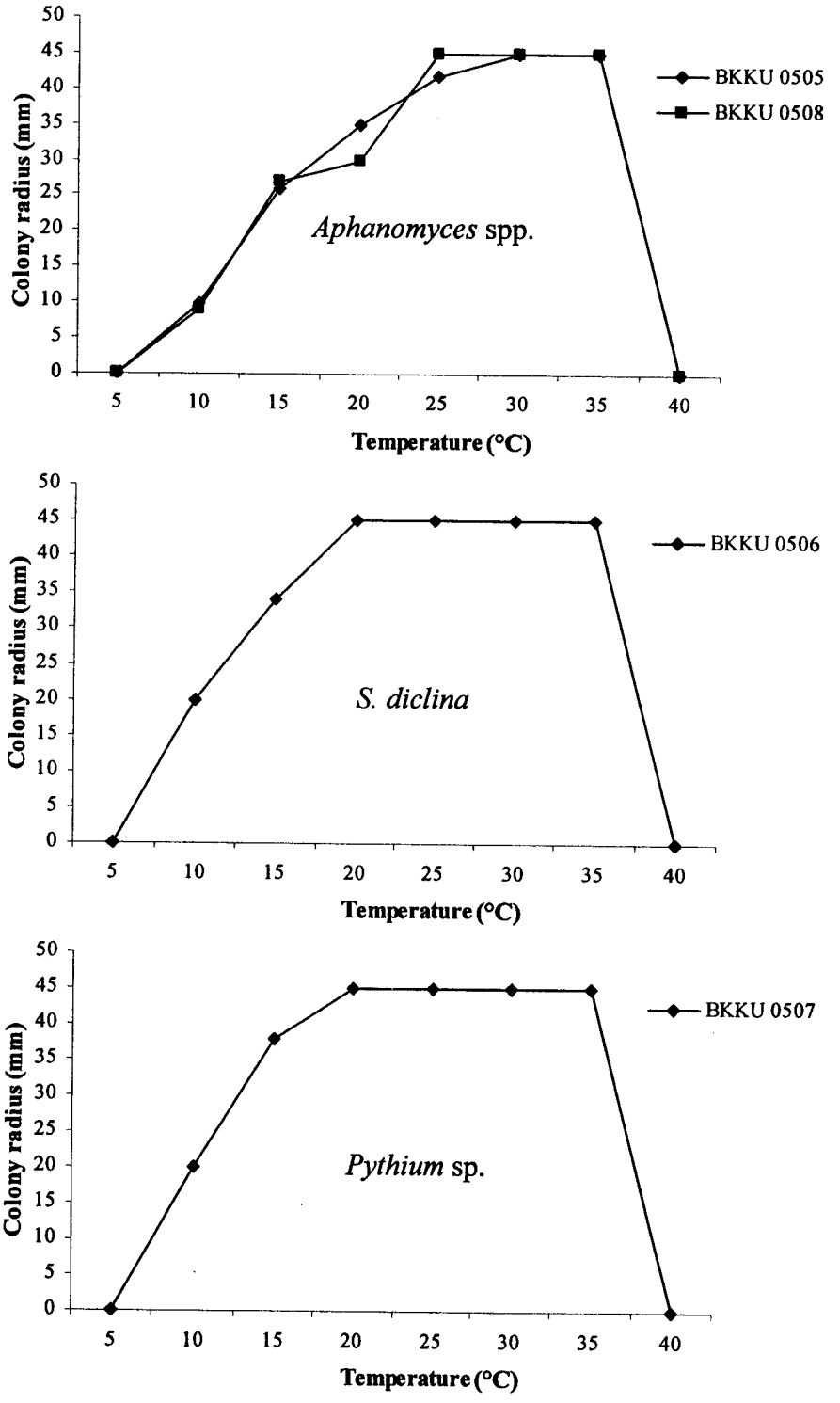


Figure 3.2 Effect of various temperatures on vegetative growth of *Aphanomyces* spp., *S. diclina* and *Pythium* sp. at 4 days after inoculation.

3.2 Effect of various temperatures on zoospore production

Neither all isolates produced zoospores at 10 °C within 1 month observation. As shown in Table 3.1, at 15 °C *Achlya* spp. BKKU 0501 and BKKU 0502, *A. ambisexualis* BKKU 0614 and BKKU 0615, *A. bisexualis* BKKU 0612 and BKKU 0616 were able to produce a few numbers of zoospores from day 1 to day 3, except for *Achlya* sp. BKKU 0503, *A. bisexualis* BKKU 0504, BKKU 0509, BKKU 0510 and BKKU 0613 started to produce zoospores on day 2 after incubation. Neithier *A. bisexualis* BKKU 0511 nor *Aphanomyces* spp. BKKU 0505 and BKKU 0508 produced zoospores. While *S. diclina* BKKU 0506 was able to produce zoospores in amount of $5.0x10^2$-$9.9x10^2$ zoospores/mL on day 1.

The results of zoospore production at 20 °C are presented in Table 3.2. Most isolates of fungi started to produce zoospores on day 1 after incubation and prolonged their production to days 3-5 for genus *Achlya*. In the case of *A. bisexualis* BKKU 0504 and BKKU 0616 were able to produce zoospores again on days 14 and 21 after incubation. *Aphanomyces* sp. BKKU 0505 could produce $1.0x10^3$-$2.0x10^3$ zoospores/mL on day 1 with a small amount to count on days 2-3, while *Aphanomyces* sp. BKKU 0508 was able to produce $2.0x10^3$-$2.9x10^3$ zoospores/mL on day 1, and decreased to $1.0x10^3$-$1.9x10^3$ zoospores/mL on day 2 and very low amount of zoospore on day 3. Whereas, *S. diclina* BKKU 0506 could produce maximum numbers $4.0x10^3$-$4.9x10^3$ zoospores/mL on day 1 and its production was gradually decreased after the prolonged period of incubation to day 7.

Table 3.3 shows that at 25 °C *A. bisexualis* BKKU 0511 could produce $4.0x10^3$-$4.9x10^3$ zoospores/mL on day 1 and gradually decreased to too few to count on day 4 and produced zoospores again on day 14 after incubation. Most isolates of *Achlya*, 10 out of 17 isolates could produce zoospores from day 1 up to day 14 and BKKU 0502, BKKU 0612 and BKKU 0616 could be prolonged their production to day 21 after incubation. *Aphanomyces* sp. BKKU 0505 and BKKU 0508 produced $3.0x10^3$-$3.9x10^3$ zoospores/mL on day 1 and their production was decreased on days 3 and 4, respectively. Whereas, *S. diclina* BKKU 0506 was only one isolate that produced maximum number $5.0x10^3$-$5.9x10^3$ zoospores/mL on day 1 and its production was declined along the following incubation days until day 7.

It was found that the 30 °C delayed the production of zoospores and caused lower numbers of zoospore production as presented in Table 3.4. *Achlya* spp. BKKU 0502 and BKKU 0503 could prolong their zoospore production until day 5 after incubation comparing with other isolates of genus *Achlya*, while *A. bisexualis* BKKU 0511 and *Aphanomyces* sp. BKKU 0505 produced a few zoospores to count in day 1 incubation. Whereas *S. diclina* BKKU 0506 could produce $1.0x10^3$-$1.9x10^3$ zoospores/mL on day 1, and zoospore number could be counted at days 2 and 3 after incubation.

In this study, it was found that *Pythium* sp. BKKU 0507 did not produce zoospores at any temperature tested.

Table 3.1 Zoospore production of the fungi isolated from tilapia eggs at 15 °C.

Isolates	Observation period (days)*									
	1	2	3	4	5	6	7	14	21	28
***Achlya* spp.**										
BKKU 0501	U		U							
BKKU 0502	U		U							
BKKU 0503				U						
A. ambisexualis										
BKKU 0614			U							
BKKU 0615	U			U						
A. bisexualis										
BKKU 0504			U							
BKKU 0509				U						
BKKU 0510		U								
BKKU 0511										
BKKU 0612		U	U							
BKKU 0613		U	U							
BKKU 0616			U							
***Aphanomyces* spp.**										
BKKU 0505										
BKKU 0508										
S. diclina										
BKKU 0506		U								
***Pythium* sp.**										
BKKU 0507										

Table 3.2 Zoospore production of the fungi isolated from tilapia eggs at 20 °C.

Isolates	Observation period (days)*									
	1	2	3	4	5	6	7	14	21	28
***Achlya* spp.**										
BKKU 0501			U							
BKKU 0502				U						
BKKU 0503					U					
A. ambisexualis										
BKKU 0614					U					
BKKU 0615					U					
A. bisexualis										
BKKU 0504				U				U	U	
BKKU 0509				U	U					
BKKU 0510			U							
BKKU 0511			U							
BKKU 0612					U	U				
BKKU 0613			U	U						
BKKU 0616						U		U	U	
***Aphanomyces* spp.**										
BKKU 0505		U	U							
BKKU 0508			U							
S. diclina										
BKKU 0506							U			
***Pythium* sp.**										
BKKU 0507										

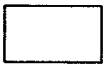
Table 3.3 Zoospore production of the fungi isolated from tilapia eggs at 25 °C.

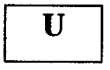
Isolates	Observation period (days)*									
	1	2	3	4	5	6	7	14	21	28
***Achlya* spp.**										
BKKU 0501						U	U	U		
BKKU 0502								U	U	
BKKU 0503						U	U			
A. ambisexualis										
BKKU 0614						U	U	U		
BKKU 0615						U	U	U		
A. bisexualis										
BKKU 0504								U		
BKKU 0509							U	U		
BKKU 0510						U		U		
BKKU 0511				U				U		
BKKU 0612								U	U	
BKKU 0613							U			
BKKU 0616								U	U	
***Aphanomyces* spp.**										
BKKU 0505			U							
BKKU 0508				U						
S. diclina										
BKKU 0506							U			
***Pythium* sp.**										
BKKU 0507										

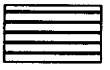
Table 3.4 Zoospore production of the fungi isolated from tilapia eggs at 30 °C.

Isolates	Observation period (days)*									
	1	2	3	4	5	6	7	14	21	28
***Achlya* spp.**										
BKKU 0501			U							
BKKU 0502				U	U					
BKKU 0503	U				U					
A. ambisexualis										
BKKU 0614			U							
BKKU 0615			U							
A. bisexualis										
BKKU 0504										
BKKU 0509			U							
BKKU 0510		U								
BKKU 0511	U	U								
BKKU 0612			U							
BKKU 0613			U							
BKKU 0616			U							
***Aphanomyces* spp.**										
BKKU 0505	U									
BKKU 0508		U								
S. diclina										
BKKU 0506		U	U							
***Pythium* sp.**										
BKKU 0507										

Remarks: No. of zoospores per mL.


None


U Too few to count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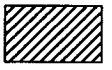
$1.0x10^3$ - $1.9x10^3$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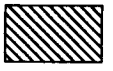
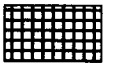
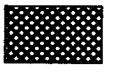

$3.0x10^3$ - $3.9x10^3$


$4.0x10^3$ - $4.9x10^3$


$5.0x10^3$ - $5.9x10^3$

3.3 Effect of various pHs on vegetative growth

Almost isolates of *Achlya* grew in GY broth with a wide range of pH 4.0-11.0, except *Achlya* spp. BKKU 0501 and BKKU 0503 could only grow at pH 4.0-10.0. All isolates of *Achlya* grew with optimal pH 6.0-8.0. *Aphanomyces* spp. BKKU 0505 and BKKU 0508 and *S. diclina* BKKU 0506 could grow in a wide range of pH 5.0-11.0 with optimal pH 6.0-9.0. Whereas *Pythium* sp. BKKU 0507 grew in GY broth at pH range of 5.0-10.0 with optimal pH 6.0-7.0 (Table 3.5).

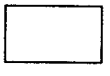
3.4 Effect of various pHs on zoospore germination

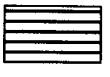
As presented in Table 3.6, zoospores of almost fungal isolates could germinate in GY broth with a wide range of pH 4.0-11.0 during 24 hours incubation. Except *Pythium* did not produce zoospores during this study. In addition, lower and higher pHs of GY broth could cause abnormality of hyphae germinated from zoospores. *A. bisexualis* BKKU 0612 in GY broth pH 4.0 showed vacuoles inside growing hyphae from zoospore germination (Figure 3.3A) and *A. bisexualis* BKKU 0509 in GY broth pH 11.0 showed abnormal branching of hyphae (Figure 3.3B) at 25 °C for 7 days after incubation.

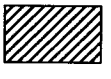
Table 3.5 Effect of various pHs on growth of fungi isolates from fungal infected tilapia eggs at 25 °C for 3 days after incubation.

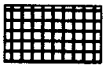
Isolates	pHs									
	3.0	4.0	5.0	6.0	7.0	8.0	9.0	10.0	11.0	12.0
***Achlya* spp.**										
BKKU 0501										
BKKU 0502										
BKKU 0503										
A. ambisexualis										
BKKU 0614										
BKKU 0615										
A. bisexualis										
BKKU 0504										
BKKU 0509										
BKKU 0510										
BKKU 0511										
BKKU 0612										
BKKU 0613										
BKKU 0616										
***Aphanomyces* spp.**										
BKKU 0505										
BKKU 0508										
S. diclina										
BKKU 0506										
***Pythium* sp.**										
BKKU 0507										

Remarks: hyphal length (mm)


no growth


hyphal length less than 5.0 mm


hyphal length between 5.0-9.9 mm


hyphal length between 10.0-14.9 mm


hyphal length between 15.0-19.9 mm


hyphal length more than 19.9 mm

Table 3.6 Effect of various pHs on zoospore germination of fungi isolates from fungal infected tilapia eggs at 25 °C for 24 hours after incubation.

Isolates	pHs									
	3.0	4.0	5.0	6.0	7.0	8.0	9.0	10.0	11.0	12.0
***Achlya* spp.**										
BKKU 0501	-[a]	+	+	+	+	+	+	+	+	-
BKKU 0502	-	+	+	+	+	+	+	+	+	-
BKKU 0503	-	+	+	+	+	+	+	+	+	-
A. ambisexualis										
BKKU 0614	-	+	+	+	+	+	+	+	+	-
BKKU 0615	-	+	+	+	+	+	+	+	+	-
A. bisexualis										
BKKU 0504	-	+	+	+	+	+	+	+	+	-
BKKU 0509	-	+	+	+	+	+	+	+	+	-
BKKU 0510	-	+	+	+	+	+	+	+	+	-
BKKU 0511	-	+	+	+	+	+	+	+	+	-
BKKU 0612	-	+	+	+	+	+	+	+	+	-
BKKU 0613	-	+	+	+	+	+	+	+	+	-
BKKU 0616	-	+	+	+	+	+	+	+	+	-
***Aphanomyces* spp.**										
BKKU 0505	-	+	+	+	+	+	+	+	+	-
BKKU 0508	-	+	+	+	+	+	+	+	+	-
S. diclina										
BKKU 0506	-	+	+	+	+	+	+	+	+	-
***Pythium* sp.**										
BKKU 0507	*									

[a] zoospore germination: - no germination and + germination

* did not produce zoospores

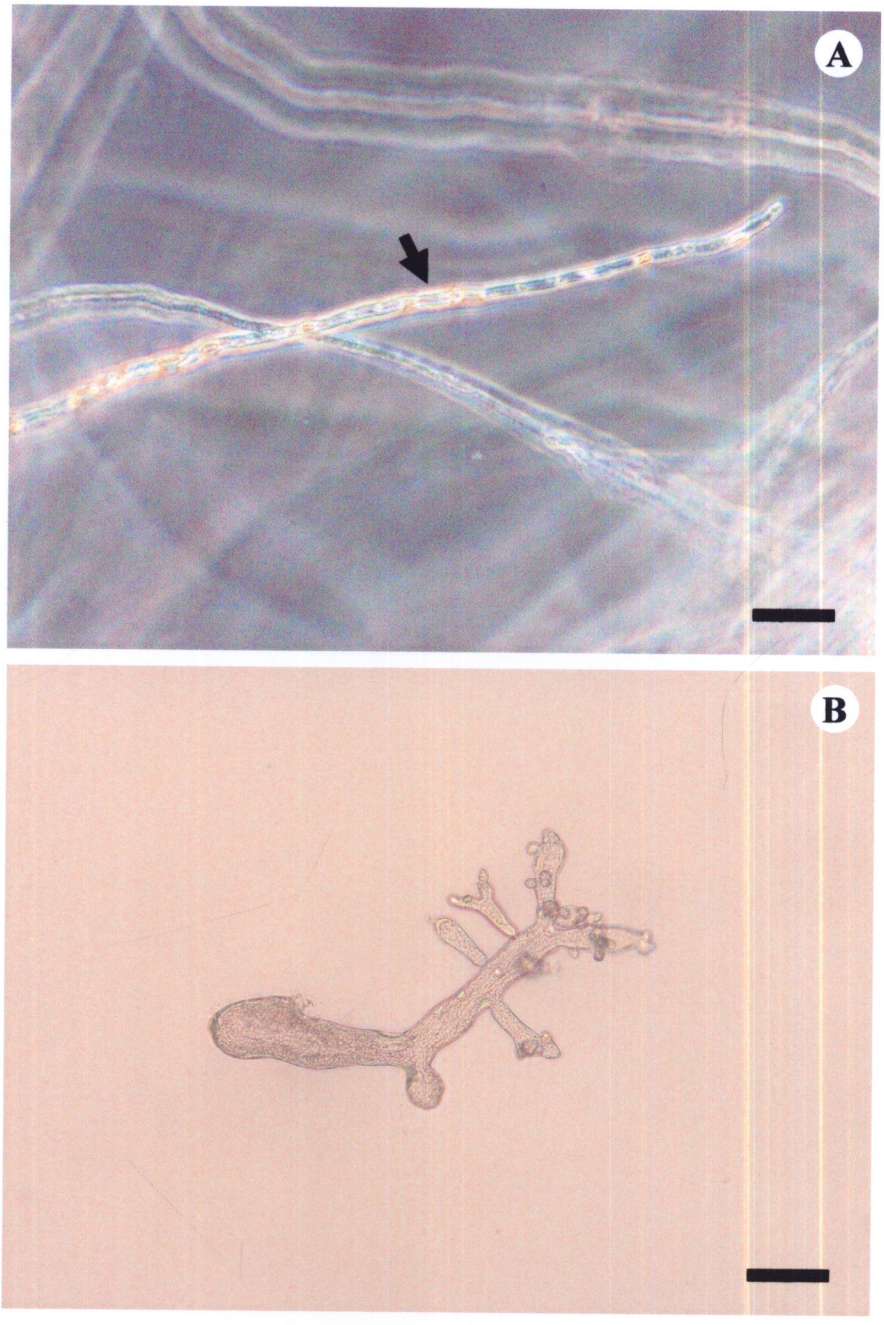


Figure 3.3 The isolates BKKU 0612 and BKKU 0509 of *A. bisexualis* showing abnormal structures (bar=60 μm) A. Hyphae showing vacuolation (➡) at pH 4.0. B. Hyphae showing abnormal branching at pH 11.0.

3.5 Effect of various salinity of NaCl on vegetative growth

All isolates of genera *Achlya* and *Aphanomyces* had maximal growth in GY agar without NaCl (0 %). All isolates of *Achlya* were tolerated up to 1.5 % NaCl as well as *Aphanomyces* sp. BKKU 0505. Whereas *Aphanomyces* sp. BKKU 0508 grew up to 2.0 % NaCl. *S. diclina* BKKU 0506 grew well in 0-1.5 % NaCl and was tolerated up to 3.0 % NaCl. *Pythium* sp. BKKU 0507 grew well in 0-0.5 % NaCl and tolerated up to 2.0 % NaCl (Figures 3.4-3.5).

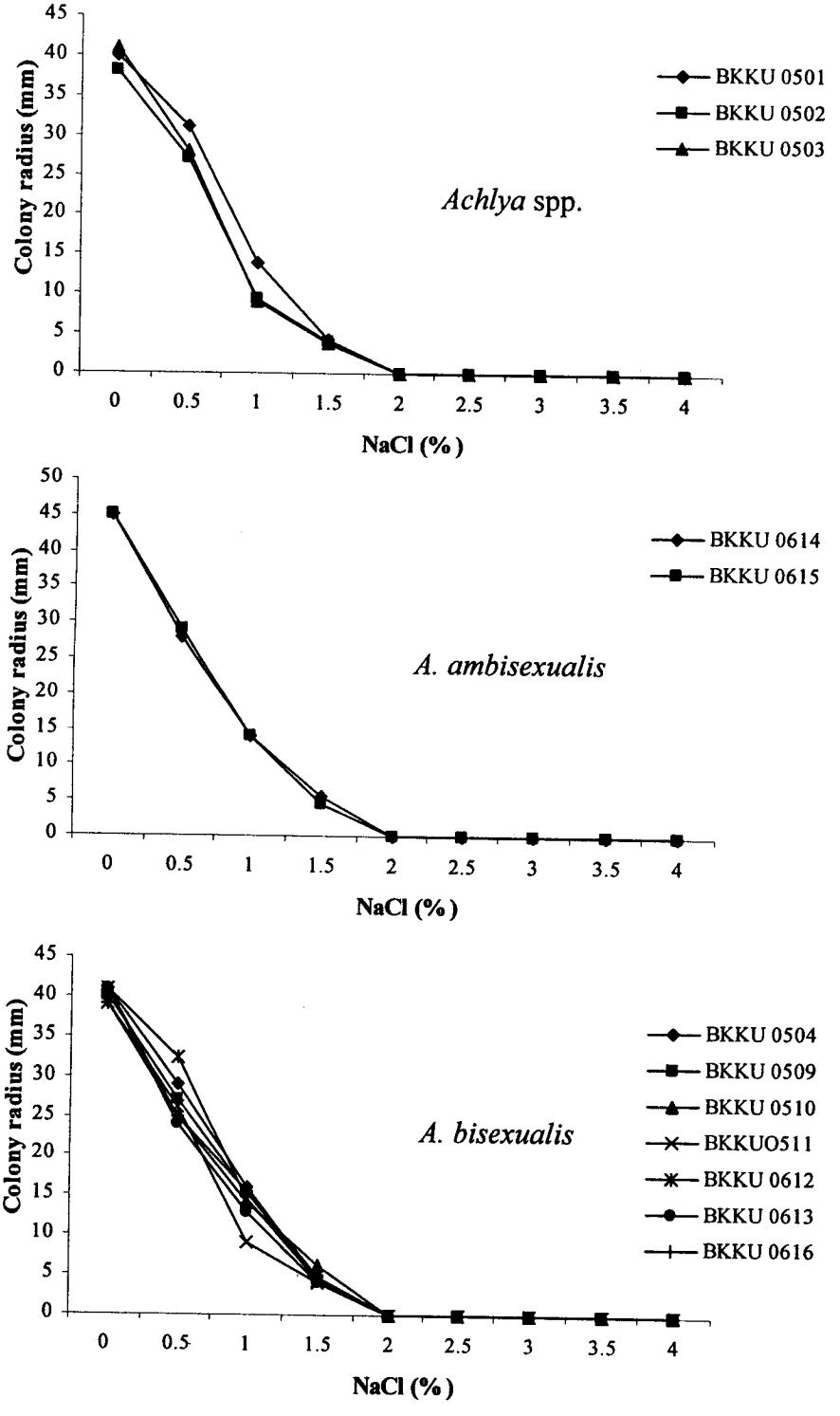


Figure 3.4 Effect of various salinity of NaCl on vegetative growth of *Achlya* spp., *A. ambisexualis* and *A. bisexualis* at 4 days after inoculation.

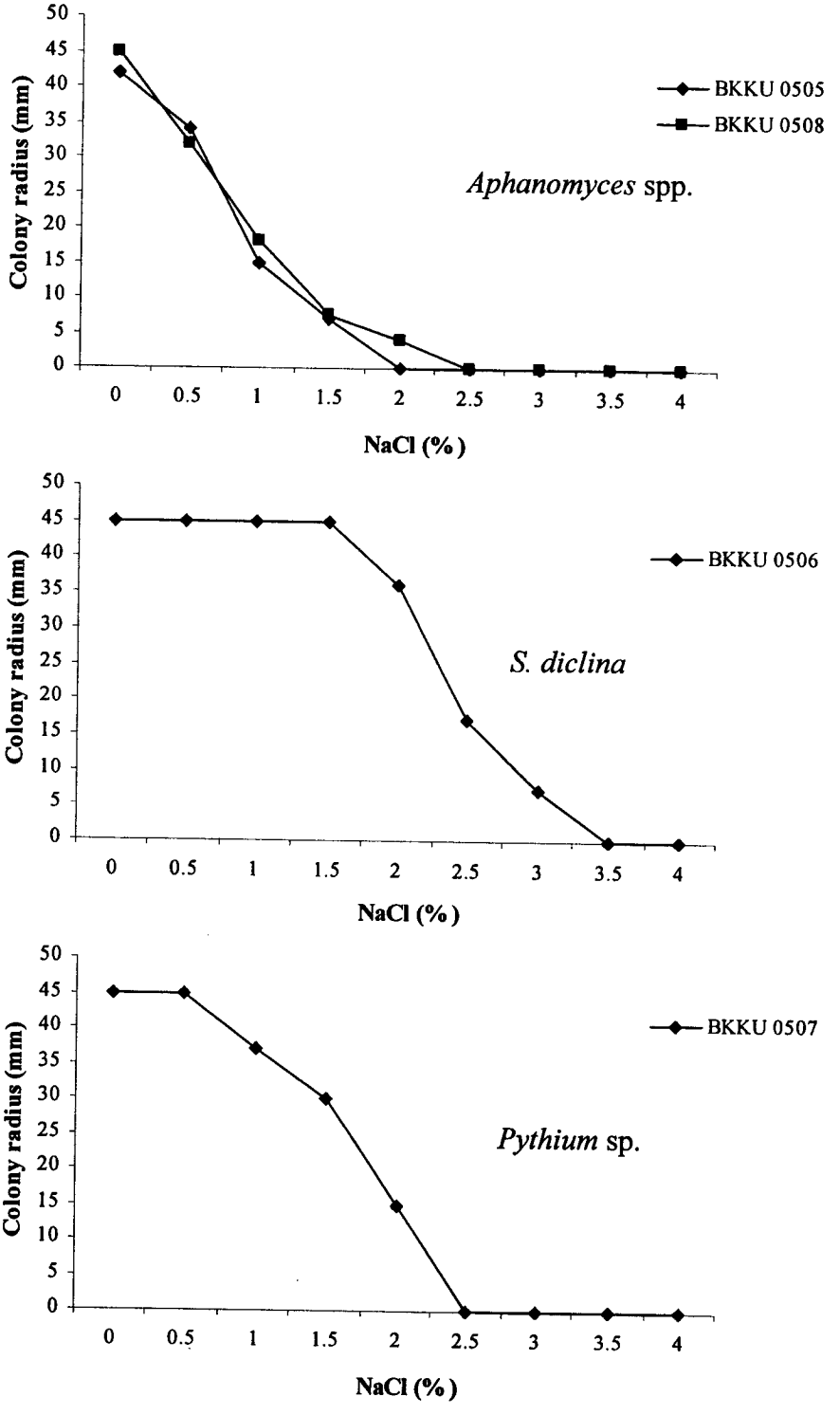


Figure 3.5 Effect of various salinity of NaCl on vegetative growth of *Aphanomyces* spp., *S. diclina* and *Pythium* sp. at 4 days after inoculation.

4. Discussion

As shown in Figures 3.1 and 3.2, the ranges of water temperature for vegetative growth of the fungal isolates were between 10 °C to 35 °C. Optimal temperature for mycelial growth of *Achlya* and *Aphanomyces* ranged between 25 °C to 35 °C, whereas *Saprolegnia* and *Pythium* were 20-35 °C. The results obtained in the present study are higher than those of Yuasa *et al.* (2000) reported that all these genera grew at a range of 5-30 °C with optimal temperature at 25 °C. Similar data have been reported by many authors as follows: Olàh and Farkas (1978) reported that the vegetative growth of *Achlya* and *Saprolegnia* isolated from water and diseased carp could grow at 10-35 and 5-30 °C with optimum temperature at 25-35 and 25 °C, respectively. Munchan (2003) showed that *Achlya* spp. and *S. diclina* isolates from ornamental fish could grow at 5-35 °C with the optimum temperature at 30-35 °C and 20-35 °C, respectively, as well as Chukanhom and Hatai (2004) reported that *S. diclina* grew at a range of 5-35 °C with the optimal temperature at 25-30 °C. Rakmanee (2004) also reported that both *Achlya* spp. and *A. ambisexualis* isolated from African catfish and common carp eggs could grow at a range of temperature 15-30 °C with the optimal temperature at 25-30 °C, while both *Aphanomyces* sp. and *S. diclina* grew at 10-30 °C with the optimal temperature at 30 °C and 25 °C, respectively. The present study is convinced that all fungi isolates did not show any relation to a particular collection period but they could occur whole year round (as mentioned in Chapter II). This may be due to the climatic condition of Thailand was characterized by air temperature range of 21.5 °C to 34.0 °C (personal recorded during June 2005 to April 2006). In addition, water temperatures using for eggs incubation from the hatcheries are approximately 25-32 °C and it has never been varied up to 35 °C (see Table 2.9, Chapter II). From the optimal temperature *in vitro* for hyphal growth of fungi were 20-35 °C which was coincided with incubation water temperature from each hatchery. This finding supports that fungal isolates were able to grow in incubation water. Therefore, all these fungal isolate adapt to survive in higher temperature. It may conclude that these isolates are mesophilic fungi (Griffin, 1994).

The present study revealed that the zoospore production was temperature dependent. The production changed with the variation of temperatures as shown in Tables 3.1-3.4. The zoospore productions differed between *Achlya*, *Apahnomyces* and *Saprolegnia*. No zoospores of *Pythium* were produced at any temperature tested. From Table 3.3, it shows that the zoospore production of these 3 genera was maximum at 25 °C which was same as Rakmanee (2004). Yuasa *et al.* (2000) have indicated that *Saprolegnia* produced maximal zoospores at lower temperature 10 °C as well as *Achlya*. While Yuasa *et al.* (2000) reported that maximum zoospore production of *Aphanomyces* and *Pythium* occurred at 20 °C. This finding revealed that the optimal temperature for the zoospore production of 3 genera was 25 °C, it may be coincided with the water temperature from natural or fish ponds that using for egg incubation (see Table 2.9, Chapter II). All fungal isolates produced many zoospores throughout the year because the water temperature using in hatcheries was relatively constant in range of 25-30 °C for the whole year round and it was never higher than 35 °C (personal record for 1 year). It may be concluded that zoospore production and zoospore attachment on tilapia eggs depend not only on the eggs themselves but also on the temperature of water using for egg incubation.

It is known that water moulds can grow well in very dilute nutrients (Willoughby, 1985) and prefer pH from neutral to weak alkaline conditions (Wood *et al.*, 1986). From the present study, it was found that maximal growths of 4 fungal genera were pH dependent. This result is similar to the report of Rakmanee (2004) that *Achlya*, *Apahnomyces* and *Saprolegnia* grew maximum at pH range 6.0-8.0, 8.0-10.0 and 7.0-9.0, respectively. Yuasa *et al.* (2000) reported that the optimal pH for vegetative growth of *Achlya* and *Saprolegnia* was the same as 7.0-8.0 but *Apahnomyces* and *Pythium* grew in a wider range pH of 6.0-8.0. Munchan (2003) also reported the optimal pH for mycelial growth of *Achlya* and *Saprolegnia* were 5.0-8.0 and 5.0-10.0, respectively. Whereas Olàh and Farkas (1978) reported that the optimal pH for mycelial growth of *Achlya* and *Saprolegnia* was lower as 3.8-6.9 and 3.8-9.0, respectively. From the Table 3.6, the zoospore germination of *Achlya*, *Apahnomyces* and *Saprolegnia* occurred at the same range of pH 4.0-11.0. *Pythium* did not produce zoospores at any pHs tested. Yuasa *et al.* (2000) found that

zoospores of *Achlya*, *Aphanomyces* and *Pythium* could germinate at pH range of 5.0-11.0, except *Saprolegnia* at pH range of 3.8-11.0. Whereas Rakmanee (2004) reported those zoospores of *Achlya* and *Saprolegnia* could germinate at pH 4.0-11.0 and *Aphanomyces* was able to germinate at pH 5.0-11.0. As shown in Table 2.9 (Chapter II), water pH from various sources using for tilapia egg incubation is approximately 6.8-8.1, this result supported the above study on the optimal pH for the mycelial growth of 4 fungal genera was 6.0-9.0.

In addition, it was found that *A. bisexualis* BKKU 0612 at pH 4.0 showed vacuolation in growing portion of hyphae (Figure 3.3A). While *A. bisexualis* BKKU 0509 produced abnormal branching from germinating hyphae at pH 11.0 (Figure 3.3B). Therefore, lower and higher pHs had an effect on the development of hyphae.

The comparative study at various optimal pH conditions, all isolates have been classified into 3 groups. These are (1) weak acid-weak basic groups were made up of *Achlya* with maximal growth at 6.0-8.0 and *Aphanomyces* 6.0-9.0, respective, (2) neutral-weak basic group contains of *Saprolegnia* with maximal growth at pHs 7.0-9.0, and (3) weak acid-neutral group, *Pythium* with maximal growth at pH 6.0-7.0. This result agrees with those obtained by Roberts (1963) (*cited in* Dix and Webster, 1995) and Kitancharoen *et al.* (1996) who reported that the capability of fungal growth at various pH conditions were used for fungal classification and identification. From the above studies on the vegetative growth and zoospore germination reveals that they were affected by pH. This result agrees with those obtained by Olàh and Farkas (1978) who reported that pH of water has an important role for fungal growth and distribution. Hence, it can be concluded that water pH plays as a major environmental factor for fungal growth and classification.

In the present study, 4 fungal genera could grow and tolerate up to various concentrations of NaCl *in vitro* condition. This result was similar to the reports of Munchan (2003), Rakmanee *et al.* (2004) and Chukanhom and Hatai (2004) that *Achlya* tolerated in GY agar containing 1.0-1.5 % NaCl. Rakmanee (2004) reported that *Aphanomyces* spp. was able to tolerate in NaCl up to 2.0 %. While *Saprolegnia* grew in 3.0-3.5 % NaCl which was same as those obtained by Munchan (2003), Rakmanee *et al.* (2004) and Chukanhom and Hatai (2004). From the survey, *Achlya*, *Aphanomyces*, *Saprolegnia* and *Pythium* only occurred in salinity 0-0.01 %. Neither

of fungi appeared in salinity 0.02 % nor 0.03 % (Table 2.9, Chapter II). This result was correlated with salinity of incubation water which was range of 0-0.03 % (Table 2.9, Chapter II). Therefore, it may conclude that 4 fungal genera were able to survive in salinity condition which is similar to the report of Chukanhom and Hatai (2004) that fungi could survive in high salinity freshwater and also in brackish water.

Hence, biological characteristics of fungal isolates in the present study can be used for genera classification and may play a major role in fungal distribution.

CHAPTER IV
HISTOPATHOLOGY OF TILAPIA EGGS WITH FUNGAL INFECTION

1. Introduction

Tilapias are warm-water species, stop growing at temperature below 16 °C and do not survive below 10 °C. Most tilapias need water temperature of at least 20 °C to breed new fingerlings. As well as photoperiodicity and light intensity are ones of the factors in breeding (Jalabert and Zohar, 1982). Tilapias all exhibit a high degree of parental care and in this function they are mouth-brooders to protection for eggs and fry against predators. When they are mature, they can spawn year-round if the water temperature stays above 24 °C. They lay their eggs about 2,000 eggs per batch and the eggs are large with 3-5 mm which contain sufficient yolk to sustain the newly hatched for approximately 7-8 days after hatching at 27 °C (Suresh, 2003). Tilapia eggs are usually not embedded or non-adhesived to substrate because they do not produce sticky material which is mucopolysaccharides (Jalabert and Zohar, 1982). The eggs were quite dense and sink to the bottom when they were released (Chapman, 2000).

According to Ogbonna and Alabi (1991), hatching and rearing tanks or lagoons with their dense fish populations provide good conditions for the epidemic spread of pathogen and leading to the death of other viable fish. Fungal infection of eggs masses can have economically important for eggs quality and seed production of new offspring (Luzur, 2002). Microscopic examination of hyphal growth was carried out on fish individual in many species but there is little known on microscopic structure of fungal infected tilapia eggs. Therefore, this chapter reports on the microscopic structures of healthy and fungal infected tilapia eggs.

2. Materials and Methods

2.1 Source of specimens

Healthy eggs of tilapia samples were obtained from Khon Kaen Inland Fisheries Research and Development Center hatchery, Khon Kaen Province. The fungal infected eggs were randomly collected from 7 sampling sites (see Table 2.2, Chapter II). All eggs were brought back to the Fish Diseases Laboratory, Department of Biology, Faculty of Science, Khon Kaen University.

2.2 Preparation procedures for light microscopy

The eggs with and without fungal infection were fixed in 10 % phosphate buffer formalin (10 % PBF) for 24 hours and were processed for routine paraffin method. All samples were dehydrated with a graded series of ethanol by Automatic Tissue Processor (Leica TP 1020) and embedded in paraffin wax by Paraffin Embedding Center (Leica EG 1160). Sections were cut at 5 μm and serial sections were stained with Haematoxylin and Eosin (H&E) (Humason, 1972). The histological sections were examined microscopically at magnifications of x40, x100 and x400 by Nomarski microscope (Olympus model BX 51TF, Japan).

3. Results

3.1 External morphology

As shown in Figure 4.1A, healthy tilapia eggs were large, ovoid shaped with light or dark yellow-brown yolk and the eggs were covered with transparent visibility gelatinous-like membrane. The fungal infected tilapia eggs were covered with numerous, white or grey cotton-like mycelia which were seen by naked-eye (Figure 4.1B).

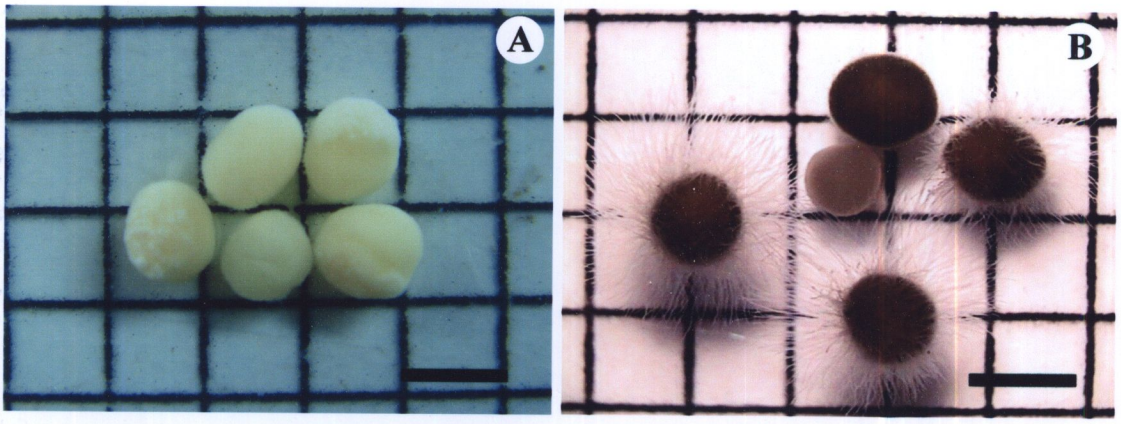


Figure 4.1 Tilapia eggs (bar=4 mm) A. Healthy eggs without fungal infection. B. Eggs with fungal infection.

3.2 Microscopic examination of tilapia eggs

Histological examination of mature tilapia eggs is similar to those described in channel catfish (*Ictalurus punctatus*) (Grizzle and Rogers, 1976), striped bass (Groman, 1982) and walking catfish (*Clarius batrachus*) (Chinabut *et al.*, 1991). The mature oocyte was composed of thick layer of egg envelope and eosinophilic yolk granules accumulated in cytoplasm (Figure 4.2).

All of the fungal infected eggs collected from 7 sampling sites were microscopic examined. There were numerous mats of hyphae surrounded the eggs and attached the outer surface of egg envelope (Figure 4.3). The highly invasive of fungal hyphae through yolk granules were observed and accumulated in cytoplasm (Figure 4.4). Some portion of egg envelope was disintegrated (Figure 4.5). Invasive pores of hyphae were also seen within some areas of egg envelope (Figure 4.6).

In some eggs, degenerative change in the yolk granules caused vacuolation in the cytoplasm (Figure 4.7) which hyphae accumulated (Figure 4.8). Zoospore germination was also observed outside the egg envelope (Figure 4.9). In addition, enormous numbers of growing bacteria were also seen beneath the egg envelope and among necrotic yolk granules (Figure 4.10).

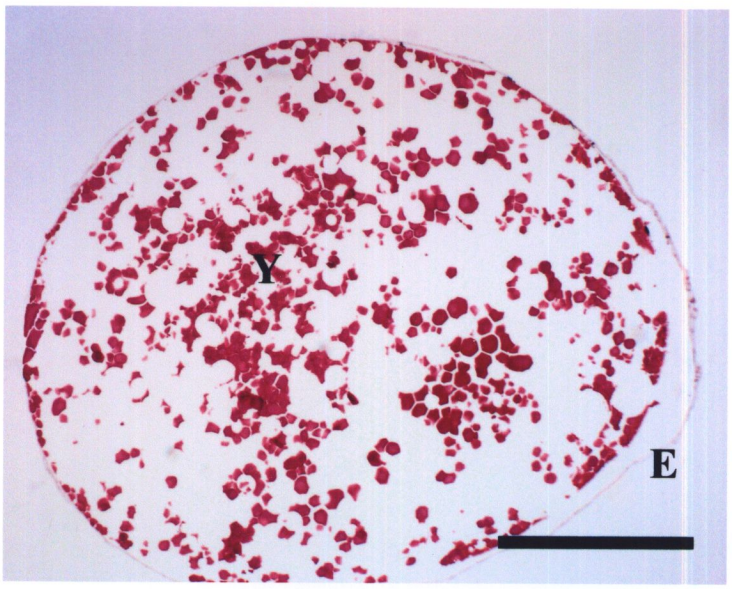


Figure 4.2 Cross-section of healthy matured egg showing eosinophilic yolk granules (Y) and egg envelope (E) (H&E, bar=400 μm).

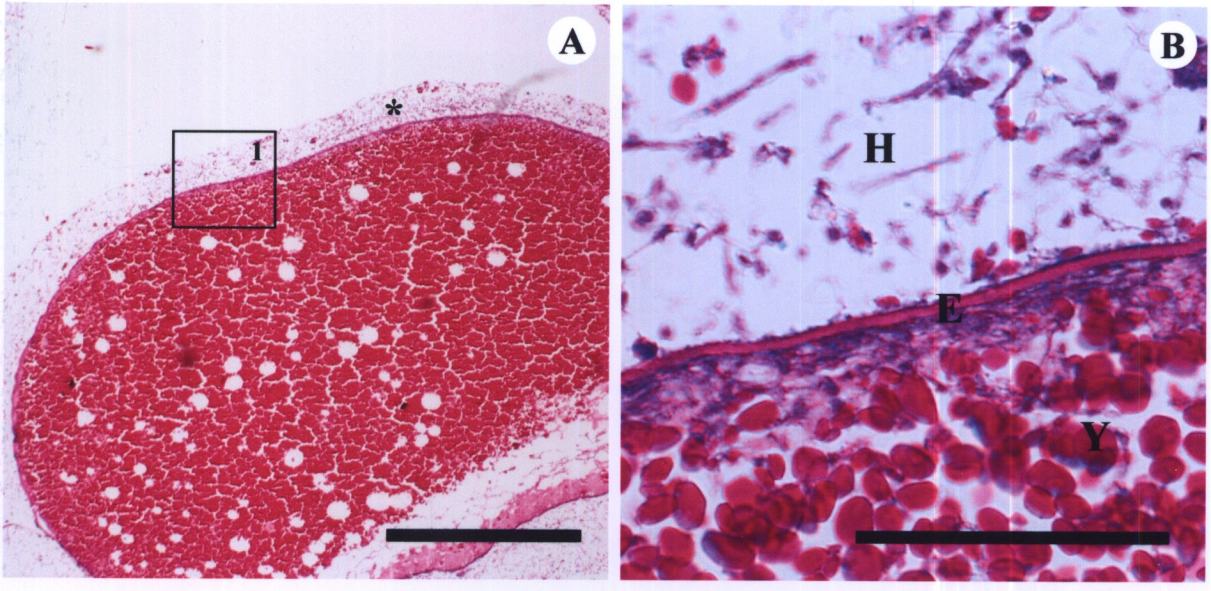


Figure 4.3 Cross-section of fungal infected egg (H&E) A. Low magnification of egg showing numerous fungal mats (*) (bar=400 μm). B. High magnification of fungal mats area 1 seen in Figure 4.3A showing hyphae (H) on outer layer of egg envelope (E) (Y=Yolk granule) (bar=50 μm).

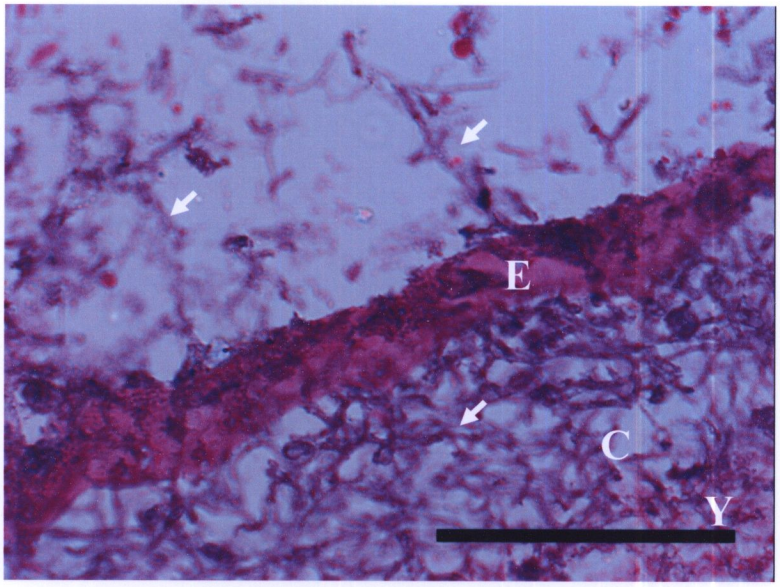


Figure 4.4 Cross-section of fungal infected egg showing numerous fungal hyphae (➡) attached outer layer of egg envelope (E) and accumulated in cytoplasm (C) (Y=Yolk granule) (H&E, bar=50 μm).

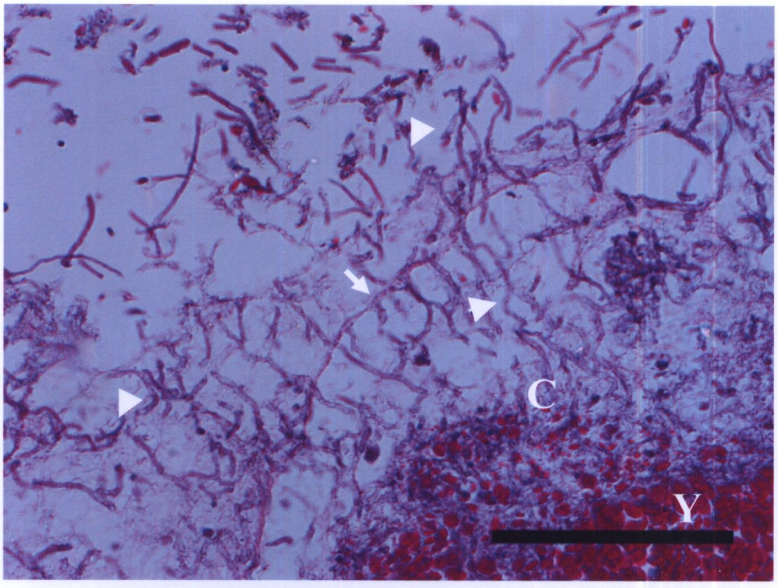


Figure 4.5 Cross-section of fungal infected egg showing highly invasive of fungal hyphae (▶) in cytoplasm (C). Note disintegrated egg envelope (➡) (Y=Yolk granule) (H&E, bar=50 μm).

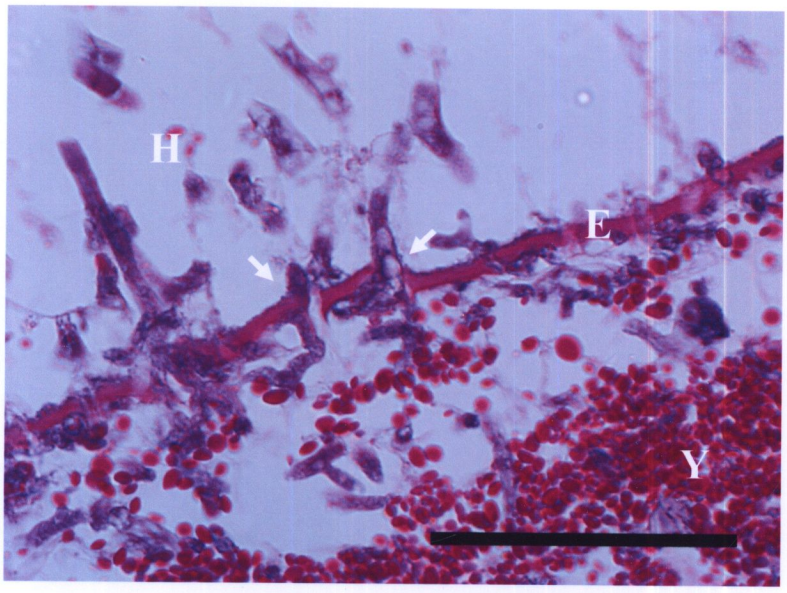


Figure 4.6 Cross-section of fungal infected egg showing fungal hyphae (➡) penetrated into the egg envelope (E) (Y=Yolk granule) (H&E, bar=50 μm).

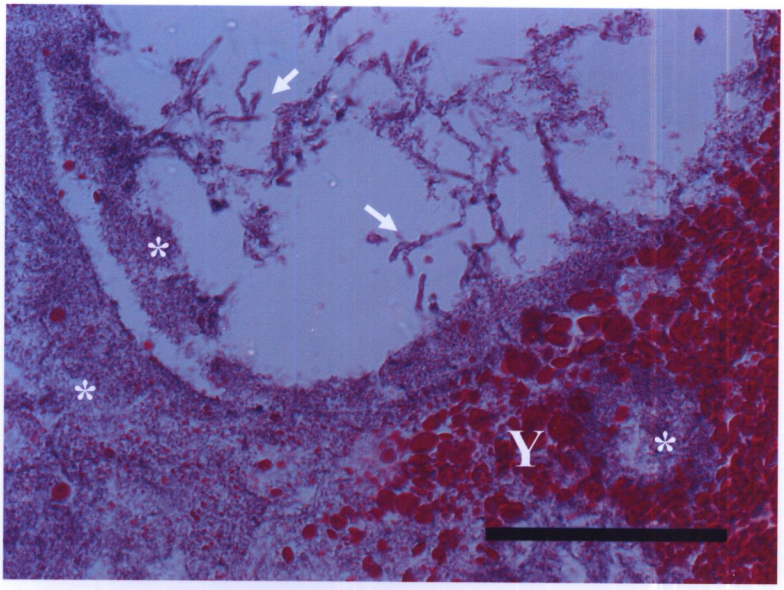


Figure 4.7 Cross-section of fungal infected egg showing hyphae (➡) and growing bacteria (*) deposited in degenerated yolk granules (Y) (H&E, bar=50 μm).

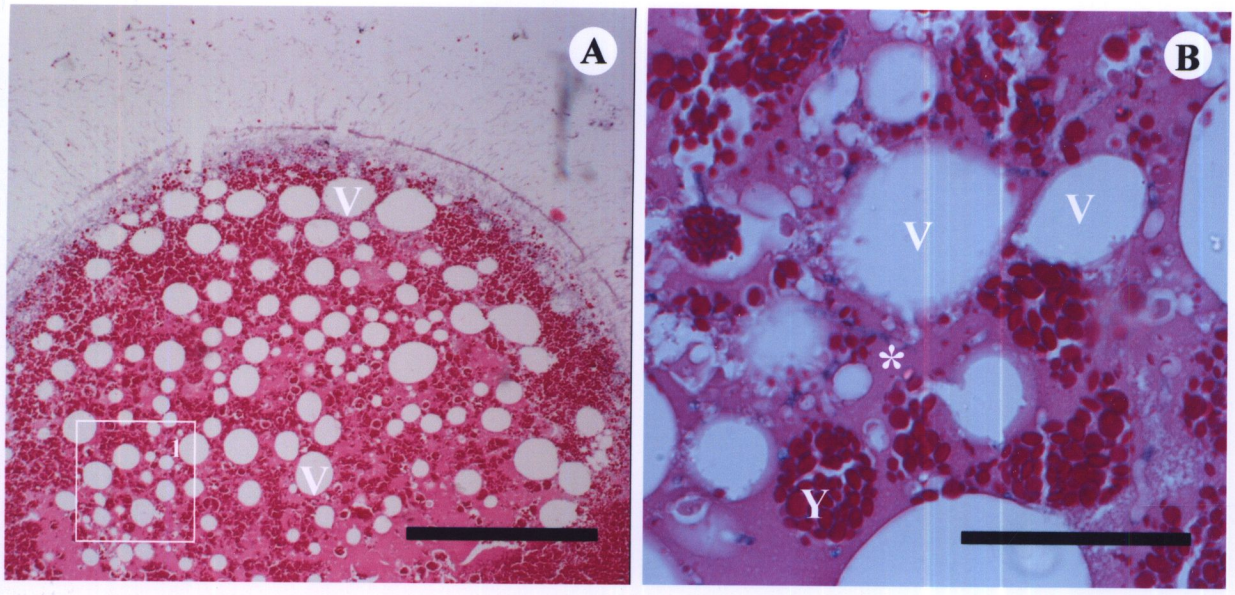


Figure 4.8 A. Low magnification of infected egg showing vacuoles (V) in cytoplasm (H&E, bar=400 µm) B. High magnification of area 1 seen in Figure 4.8A showing degenerated yolk granules (*) and vacuoles (V) (Y=Yolk granule) (H&E, bar=50 µm).

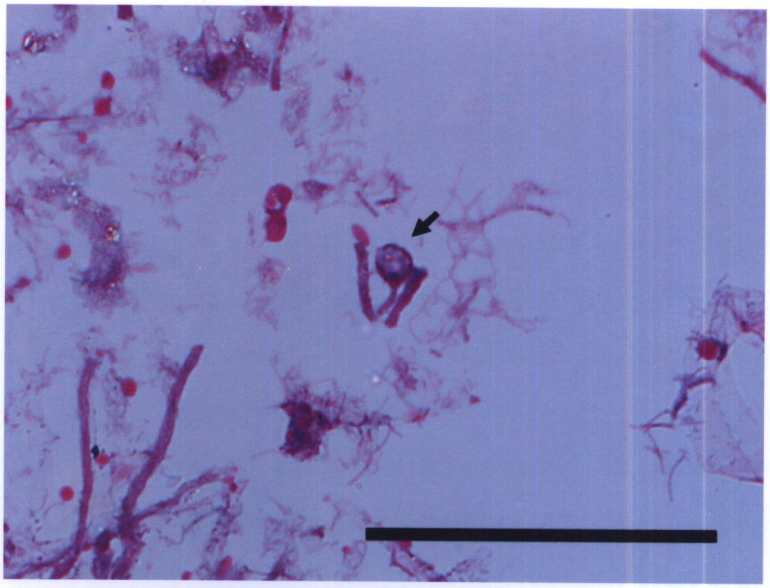


Figure 4.9 Cross-section of fungal infected eggs showing germination of zoospore (➡) (H&E, bar=50µm).

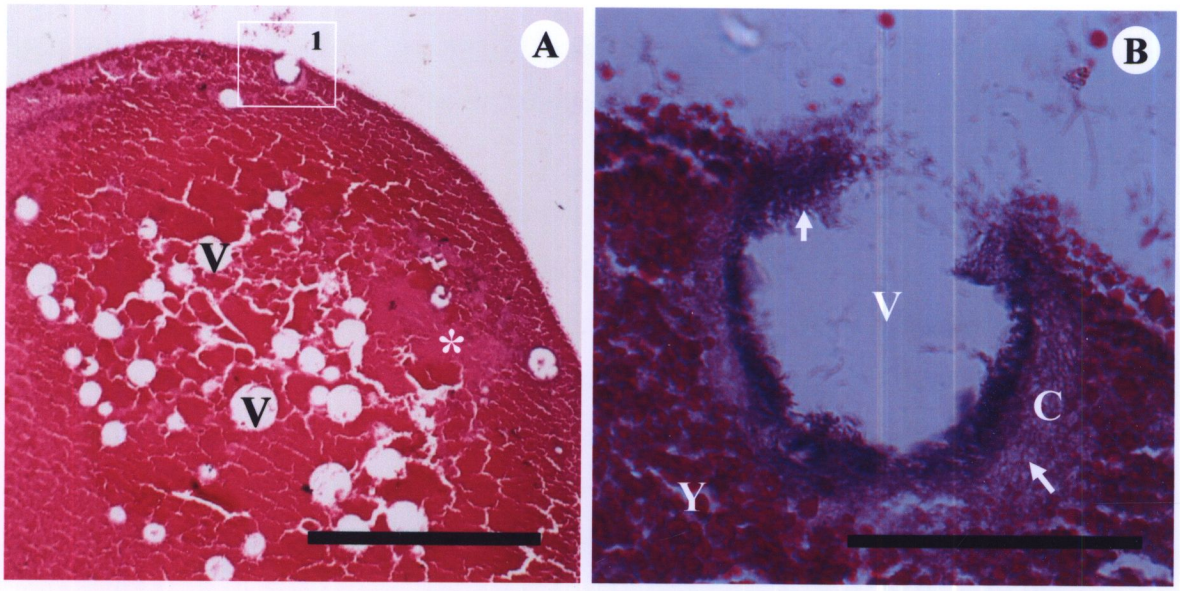


Figure 4.10 Cross-section of fungal infected egg with growing bacteria (H&E) A. Low magnification showing degenerated yolk granule (*) and vacuoles (V). (bar=400 μm) B. High magnification of area 1 seen in Figure 4.10A showing numerous bacteria (➡) inside vacuole (V) and cytoplasm (C) (bar=50 μm).

4. Discussion

The histopathological examination of fungal infected tilapia eggs showed numerous fungal hyphae surrounded and attached outer layer of egg envelope. Enormous hyphae penetrated through egg envelope by invasive pores. This result is similar to Rakmanee (2004) reported the histopathological structures of infected common carp eggs. This may be because fungal cells released digestive enzymes into surrounding to reduce organic matter into absorbable nutrients (Moore-Landecker, 1972 *cited by* Post, 1983) as well as Rand and Munden (1992) stated that fungi released enzymes to alter the integrity of the chorionic membrane by solubilizing structure polymers, therefore the hyphae could penetrate easily. According to Post (1983), Egusa (1992), Noga (1996) and Roberts (2001), dead fish eggs are suitable substrata for the fungi, fungal infection usually established itself first on unfertilized or other dead eggs and they rapidly spread to neighboring healthy eggs, which produce a suffocating mycelial growth over living eggs. Furthermore, surrounded

hyphae growth could reduce the water circulation around the live eggs and blocked oxygen transportation and thus contributed to death of the eggs (Kitancharoen and Hatai, 1997).

From the histopathological examination, zoospore germination was also observed at outer surface of fungal infected egg envelope (see Figure 4.9), this result may suggest that fungus can attack live tilapia eggs, either directly from the zoospore, or by mycelial attack from an adjacent dead egg.

Another finding in this study was enormous numbers of growing bacteria interspersed in area of hyphae deposited outside egg envelope (Figure 4.7) and accumulated beneath egg envelope and scattered among hyphae in egg cytoplasm (Figure 4.10). The occurrence of bacteria in this study may be associated with fungal infections on tilapia eggs.

However, member of Oomycetes are generally considered agents of secondary infection arising from conditions such as bacterial infections, poor husbandry, infestation by parasites and social interaction (Bruno and Wood, 1999). Suresh (2003) stated that secondary infections due to *Saprolegnia* are common in many fish species, including tilapias, and it is a common problem in hatcheries, affecting eggs and reducing hatch rates. Therefore, the pathogenicity of these fungi on fish eggs and individuals are required for further study.

CHAPTER V
ANTI-FUNGAL EFFECT OF SODIUM CHLORIDE (NaCl) AND POTASSIUM PERMANGANATE ($KMnO_4$) AND THE TOXICITY TO TILAPIA EGGS

1. Introduction

Aquatic fungi (Saprolegniales) are ubiquitous in natural water supplies of fish hatcheries and often cause serious disease problems for fish culturists (Schreck *et al.*, 1993; Rach *et al.*, 1997, 2005). In the past, this problem was solved with the extremely effective fungicidal, malachite green and formalin (Hansen, 2004). Due to their toxicity and carcinogenicity for fish, fish eggs and health of farmers, these two chemicals were canceled and discontinue using for fungal control on other edible aquatic animals (Schreck *et al.*, 1993).

Many chemicals and drugs were recommended for use in aquaculture for prevention and treatment of diseases and also for the improvement of water quality (Chinabut, 1997). The commonly treatment with sodium chloride (NaCl) and potassium permanganate ($KMnO_4$) are commonly used in aquaculture to treat external parasites (Klinger and Francis-Floyd, 2002; Marecaux, 2006). They have been also reported as anti-fungal agents for fish culture (Schreck *et al.*, 1993; Bruno and Wood, 1999).

The aims of present study were to examine the anti-fungal effects of sodium chloride and potassium permanganate on (1) the vegetative and zoosporic stages of 4 genera *Achlya*, *Aphanomyces*, *Saprolegnia* and *Pythium*, and (2) the toxicity to eyed tilapia eggs.

2. Materials and Methods

2.1 Fungi and culture condition

Due to *Achlya* sp. BKKU 0502, *A. ambisexualis* BKKU 0615, *A. bisexualis* BKKU 0616, *Aphanomyces* sp. BKKU 0508, *S. diclina* BKKU 0506 and *Pythium* sp. BKKU 0507 grew well and produced numerous zoospores at 25 °C (Table 3.3, Chapter III), therefore these isolates were selected as a representative. Six isolates of aquatic fungi as shown in Table 5.1 were used in this experiment. All fungal isolates were cultured on GY agar, incubated at 25 °C to obtain the vegetative stage and subcultured every month.

Table 5.1 Fungal species used in this study.

Isolates		Sites	Locations	Dates of sampling
Achlya sp.	BKKU 0502	II	Kalasin	June, 2005
A. ambisexualis	BKKU 0615	VI	Sakon Nakhon	March, 2006
A. bisexualis	BKKU 0616	VII	Sakon Nakhon	March, 2006
Aphanomyces sp.	BKKU 0508	VII	Sakon Nakhon	July, 2005
S. diclina	BKKU 0506	IV	Khon Kaen	June, 2005
Pythium sp.	BKKU 0507	IV	Khon Kaen	June, 2005

2.2 Source of eggs and incubation water

Samples of healthy eyed eggs were obtained from Khon Kaen Inland Fisheries Research and Development Center hatchery, Khon Kaen Province. The eggs were brought back to the Fish Diseases Laboratory, Department of Biology, Faculty of Science, Khon Kaen University. The healthy eggs were washed 3 times by sterilized tap water (STW) (Figure 5.1).

Source of incubation water from Khon Kaen Inland Fisheries Research and Development Center hatchery, Khon Kaen Province was used for this experiment. The water was filtered through filter paper No. 1 (Whatman, England) and autoclaved before using.

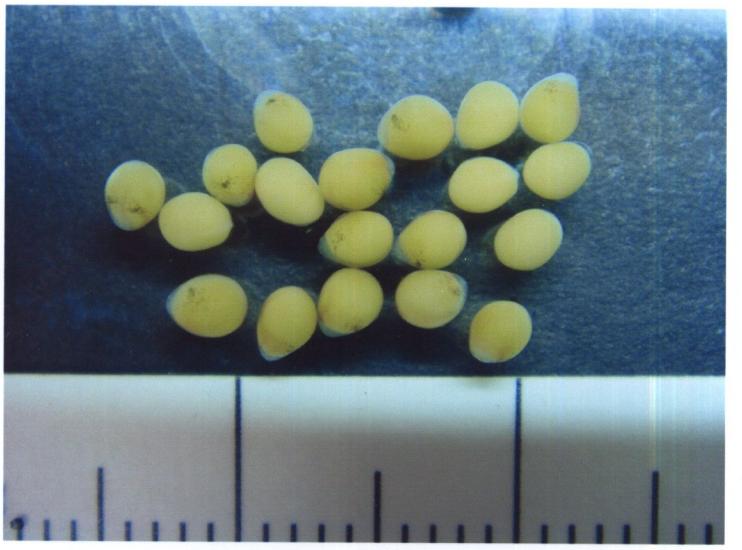


Figure 5.1 The eyed tilapia eggs used in the experiment (1 scale bar=1 mm).

2.3 Fungistatic effect of NaCl and $KMnO_4$

The minimum concentration of NaCl and $KMnO_4$ which inhibited the growth of fungi was verified to determine the proper dosages for fungistatic effect. Commercial grade of NaCl (Univar, Asia Pacific Specialty Chemicals Limited, Australia) and $KMnO_4$ (EIS, Inter-education Supply Inc. Ltd., Bangkok, Thailand) were used in the experiment and these two chemicals were prepared in various concentrations immediately before use.

The preparations of actively growing hyphae of 6 fungal isolates (as shown in Table 5.1) were the same procedure as described in Chapter III. Approximately the same amount of hyphae was put into small Petri dishes (50x15 mm) containing various concentrations of 10 mL 0.5, 2.5 and 5.0 % of NaCl or 50, 100, 200 and 400 µg/mL of $KMnO_4$. The hyphae of control groups were put into STW without NaCl (0 %) or $KMnO_4$ (0 µg/mL). The vegetative growth of treatment groups with NaCl and $KMnO_4$ comparing with the control group were observed by naked-eye after 1, 2 and 5 days inoculation. When no growth appeared after 5 days at 25 °C, the fungal hyphae were removed, washed several times by 20 mL STW and then put onto 20 mL GY agar plate for 2 days at 25 °C to observe fungal viability.

2.4 Fungicidal effect of NaCl and $KMnO_4$

The minimal concentrations of NaCl and $KMnO_4$ which were against the growth of fungi from Experiment 2.3 were verified to determine the proper dosages for treatment in fungicidal activity.

2.4.1 Fungicidal effect of NaCl and $KMnO_4$ on vegetative growth

Various concentrations of NaCl and $KMnO_4$ were prepared immediately before use. The preparations of actively growing hyphae of 6 fungal isolates were the same procedure as previously described in Chapter III. After that, approximately the same amount of hyphae was put into small Petri dishes (50x15 mm) containing various concentrations of 10 mL 1.5, 2.0, 2.5, 3.0 % of NaCl or 25, 50, 100, 150, 200 µg/mL of $KMnO_4$ for 30 minutes, 1, 2, 6 and 24 hours treatment. The hyphae of control groups were put into STW without NaCl (0 %) or $KMnO_4$ (0 µg/mL) for the same period as the treatment groups. Then, the mycelia were removed, washed several times by 20 mL STW and put onto 20 mL GY agar plate at 25 °C. The effect of NaCl and $KMnO_4$ against the vegetative growth was observed by determined fungal viability within 48 hours comparing with the control groups.

2.4.2 Fungicidal effect of NaCl and $KMnO_4$ on zoospore germination

Zoospore suspensions of each isolate were prepared by the same procedure as previously described in Chapter III, counted and adjusted to $1x10^3$ zoospores/mL. Concentrations of NaCl and $KMnO_4$ were prepared as same as in Experiment 2.4.1 immediately before use. Then 1 mL of NaCl or $KMnO_4$ solution of 10 times desired final concentration was added to 9 mL of zoospore suspension, and the mixture was incubated at 25 °C for 30 minutes, 1, 2, 6 and 24 hours. After that 120 µL portion of the mixture was inoculated into GY broth. The viability of zoospores was determined by observing the appearance of the colonies during 2 days with naked-eye. The control groups without NaCl (0 %) and $KMnO_4$ (0 µg/mL) treatment were also performed.

2.5 Toxicity of NaCl and $KMnO_4$ on hatching rate of tilapia eggs

Fifty eyed eggs were random sampled per batch. The experiments were divided into 3 trials as follows: (1) the control group contained of STW, (2) experimental group I contained of 2.0, 2.5, 3.0 % NaCl, and (3) the experimental group II composed of 25, 50, 100, 150, 200 μg/mL $KMnO_4$. The concentrations of NaCl and $KMnO_4$ used in this experiment were the anti-fungal concentrations against the vegetative and zoosporic stages as in Experiment 2.4.

The eggs of each group were held in 1,000 mL plastic beakers containing of 500 mL of solution with aeration at 27-29 °C and bathed for 1 and 24 hours, respectively. After that all eggs were washed by sterilized incubation water and transferred to sterilized incubation water for 7 days until they hatched. Three replicates of each experiment were conducted. Hatching rate was determined after bathed for 1 and 24 hours and calculated by formula of Barnes *et al.* (1998).

The percentage corrected mortality of eggs was calculated by Abbott's formula.

$$\text{Percentage of corrected mortality (Pt)} = \frac{Po - Pc}{100 - Pc} \times 100$$

Po = mortality of test group

Pc = mortality of control group

2.6 Statistical analysis

Comparison data of mean hatching rate and corrected mortality of fish eggs between the control and treatment groups were computed with the statistical software with One-Way ANOVA by SPSS 15.0 (SPSS, 1994).

3. Results

3.1 Fungistatic effect of NaCl and $KMnO_4$

As presented in Table 5.2, treatment with various concentrations of NaCl at 25 °C demonstrated that all isolates were able to grow in 0 and 0.5 % NaCl. The fungistatic dosage of NaCl were found to be 2.5 % for *Achlya* sp. BKKU 0502, *A. ambisexualis* BKKU 0615, *A. bisexualis* BKKU 0616, *Aphanomyces* sp. BKKU 0508, *S. diclina* BKKU 0506 and *Pythium* sp. BKKU 0507.

The results of treatment with $KMnO_4$ at 25 °C showed that the fungistatic dosage of each fungal species were 50 µg/mL for both *A. ambisexualis* BKKU 0615 and *S. diclina* BKKU 0506, 100 µg/mL for *Achlya* sp. BKKU 0502, *Aphanomyces* sp. BKKU 0508 and *Pythium* sp. BKKU 0507 and 200 µg/mL for *A. bisexualis* BKKU 0616 (Table 5.2).

Table 5.2 Fungistatic effect of NaCl and $KMnO_4$ on vegetative growth of fungal isolates at 25 °C at day 5.

NaCl concentrations (%)	*Achlya* sp. BKKU 0502	*A. ambisexualis* BKKU 0615	*A. bisexualis* BKKU 0616	*Aphanomyces* sp. BKKU 0508	*S. diclina* BKKU 0506	*Pythium* sp. BKKU 0507
0 (Control)	+	+	+	+	+	+
0.5	+	+	+	+	+	+
2.5	-*	-*	-*	-*	-*	-*
5.0	-	-	-	-	-	-
0 (Control)	+	+	+	+	+	+
50	+	-*	+	+	-*	+
100	-*	-	+	-*	-	-*
200	-	-	-*	-	-	-
400	-	-	-	-	-	-

+ vegetative growth detected, - no vegetative growth detected,

-* viability retained and vegetative growth appeared after 24 hours

3.2 Fungicidal effect of NaCl and $KMnO_4$

3.2.1 Fungicidal effect of NaCl and $KMnO_4$ on vegetative growth

As shown in Table 5.3, the fungicidal dosage of NaCl against the vegetative growth of *Achlya* sp. BKKU 0502, *A. ambisexualis* BKKU 0615, *A. bisexualis* BKKU 0616, *Aphanomyces* sp. BKKU 0508 and *S. diclina* BKKU 0506 was 2.5 % for 24 hours treatment except *Pythium* sp. BKKU 0507. Whereas 3.0 % NaCl was effective to kill all isolates for 24 hours exposure.

The fungicidal dosages of $KMnO_4$ on vegetative growth are presented in Table 5.4. The treatment with 50 μg/mL $KMnO_4$ was effective for hyphae of both *Achlya* sp. BKKU 0502 and *S. diclina* BKKU 0506 for 2 hours and *Aphanomyces* sp. BKKU 0508 for 24 hours exposure. The fungicide dosage of $KMnO_4$ was 150 μg/mL against *Pythium* sp. BKKU 0507 and *A. ambisexualis* BKKU 0615 for 1 and 24 hours treatment, respectively. The fungicide dosage of $KMnO_4$ was 200 μg/mL against *A. bisexualis* BKKU 0616 for 24 hours exposure.

Table 5.3 Fungicidal effect and exposure times of NaCl on vegetative stage of fungal isolates at 25 °C.

NaCl concentrations (%)	Exposure times	*Achlya* sp. BKKU 0502	*A. ambisexualis* BKKU 0615	*A. bisexualis* BKKU 0616	*Aphanomyces* sp. BKKU 0508	*S. diclina* BKKU 0506	*Pythium* sp. BKKU 0507
0 (Control)	30 min	+	+	+	+	+	+
	1 hour	+	+	+	+	+	+
	2 hours	+	+	+	+	+	+
	6 hours	+	+	+	+	+	+
	24 hours	+	+	+	+	+	+
1.5	30 min	+	+	+	+	+	+
	1 hour	+	+	+	+	+	+
	2 hours	+	+	+	+	+	+
	6 hours	+	+	+	+	+	+
	24 hours	+	+	+	+	+	+
2.0	30 min	+	+	+	+	+	+
	1 hour	+	+	+	+	+	+
	2 hours	+	+	+	+	+	+
	6 hours	+	+	+	+	+	+
	24 hours	+	+	+	+	+	+
2.5	30 min	+	+	+	+	+	+
	1 hour	+	+	+	+	+	+
	2 hours	+	+	+	+	+	+
	6 hours	+	+	+	+	+	+
	24 hours	(-)	(-)	(-)	(-)	(-)	+
3.0	30 min	+	+	+	+	+	+
	1 hour	+	+	+	+	+	+
	2 hours	+	+	+	+	+	+
	6 hours	+	+	+	-	-	+
	24 hours	-	-	-	-	-	(-)

\+ vegetative growth detected within 48 hours,

\- no vegetative growth detected within 48 hours

◯ fungicide dosages of each fungal isolate

Table 5.4 Fungicidal effect and exposure times of $KMnO_4$ on vegetative growth of fungal isolates at 25 °C.

$KMnO_4$ concentrations (µg/mL)	Exposure times	*Achlya* sp. BKKU 0502	*A. ambisexualis* BKKU 0615	*A. bisexualis* BKKU 0616	*Aphanomyces* sp. BKKU 0508	*S. diclina* BKKU 0506	*Pythium* sp. BKKU 0507
0 (Control)	30 min	+	+	+	+	+	+
	1 hour	+	+	+	+	+	+
	2 hours	+	+	+	+	+	+
	6 hours	+	+	+	+	+	+
	24 hours	+	+	+	+	+	+
25	30 min	+	+	+	+	+	+
	1 hour	+	+	+	+	+	+
	2 hours	+	+	+	+	+	+
	6 hours	+	+	+	+	+	+
	24 hours	+	+	+	+	+	+
50	30 min	+	+	+	+	+	+
	1 hour	+	+	+	+	+	+
	2 hours	(-)	+	+	+	(-)	+
	6 hours	-	+	+	+	-	+
	24 hours	-	+	+	(-)	-	+
100	30 min	+	+	+	+	+	+
	1 hour	+	+	+	+	+	+
	2 hours	-	+	+	+	-	+
	6 hours	-	+	+	+	-	+
	24 hours	-	+	+	-	-	+
150	30 min	+	+	+	+	+	+
	1 hour	+	+	+	-	-	(-)
	2 hours	-	+	+	-	-	-
	6 hours	-	+	+	-	-	-
	24 hours	-	(-)	+	-	-	-
200	30 min	+	+	+	+	+	+
	1 hour	+	+	+	-	-	-
	2 hours	-	+	+	-	-	-
	6 hours	-	+	+	-	-	-
	24 hours	-	-	(-)	-	-	-

+ vegetative growth detected within 48 hours,

- no vegetative growth detected within 48 hours

◯ fungicide dosages of each fungal isolate

3.4.2 Fungicidal effect of NaCl and $KMnO_4$ on zoospore germination

Tables 5.5-5.6 show the fungicidal dosages of NaCl on zoosporic stage were 2.0 % against *Achlya* sp. BKKU 0502, *A. ambisexualis* BKKU 0615, *Aphanomyces* sp. BKKU 0508 and *S. diclina* BKKU 0506 for 24 hours treatment and 2.5 % NaCl against *A. bisexualis* BKKU 0616 for 2 hours treatment.

The fungicide dosages of $KMnO_4$ on zoosporic stage was 25 μg/mL against *Achlya* sp. BKKU 0502, *A. ambisexualis* BKKU 0615, *A. bisexualis* BKKU 0616, *Aphanomyces* sp. BKKU 0508 and *S. diclina* BKKU 0506 for 30 minutes exposure.

Table 5.5 Fungicidal effect and exposure times of NaCl on zoospore germination of fungal isolates at 25 °C.

NaCl concentrations (%)	Exposure times	*Achlya* sp. BKKU 0502	*A. ambisexualis* BKKU 0615	*A. bisexualis* BKKU 0616	*Aphanomyces* sp. BKKU 0508	*S. diclina* BKKU 0506	*Pythium* sp. BKKU 0507
0 (Control)	30 min	+	+	+	+	+	ND
	1 hour	+	+	+	+	+	
	2 hours	+	+	+	+	+	
	6 hours	+	+	+	+	+	
	24 hours	+	+	+	+	+	
1.5	30 min	+	+	+	+	+	ND
	1 hour	+	+	+	+	+	
	2 hours	+	+	+	+	+	
	6 hours	+	+	+	+	+	
	24 hours	+	+	+	+	+	
2.0	30 min	+	+	+	+	+	ND
	1 hour	+	+	+	+	+	
	2 hours	+	+	+	+	+	
	6 hours	+	+	+	+	+	
	24 hours	(-)	(-)	+	(-)	(-)	
2.5	30 min	+	+	+	+	+	ND
	1 hour	+	+	+	+	+	
	2 hours	+	+	(-)	-	+	
	6 hours	+	+	-	-	+	
	24 hours	-	-	-	-	-	
3.0	30 min	+	+	+	+	+	ND
	1 hour	+	+	+	-	+	
	2 hours	+	+	-	-	+	
	6 hours	+	-	-	-	+	
	24 hours	-	-	-	-	-	

\+ vegetative growth detected within 48 hours,

\- no vegetative growth detected within 48 hours

◯ fungicidal dosages of each fungal isolate

ND=not detected

Table 5.6 Fungicidal effect and exposure times of $KMnO_4$ on zoospore germination of fungal isolates at 25 °C.

$KMnO_4$ concentrations (µg/mL)	Exposure times	*Achlya* sp. BKKU 0502	*A. ambisexualis* BKKU 0615	*A. bisexualis* BKKU 0616	*Aphanomyces* sp. BKKU 0508	*S. diclina* BKKU 0506	*Pythium* sp. BKKU 0507
0 (Control)	30 min	+	+	+	+	+	ND
	1 hour	+	+	+	+	+	
	2 hours	+	+	+	+	+	
	6 hours	+	+	+	+	+	
	24 hours	+	+	+	+	+	
25	30 min	-	-	-	-	-	ND
	1 hour	-	-	-	-	-	
	2 hours	-	-	-	-	-	
	6 hours	-	-	-	-	-	
	24 hours	-	-	-	-	-	
50	30 min	-	-	-	-	-	ND
	1 hour	-	-	-	-	-	
	2 hours	-	-	-	-	-	
	6 hours	-	-	-	-	-	
	24 hours	-	-	-	-	-	
100	30 min	-	-	-	-	-	ND
	1 hour	-	-	-	-	-	
	2 hours	-	-	-	-	-	
	6 hours	-	-	-	-	-	
	24 hours	-	-	-	-	-	
150	30 min	-	-	-	-	-	ND
	1 hour	-	-	-	-	-	
	2 hours	-	-	-	-	-	
	6 hours	-	-	-	-	-	
	24 hours	-	-	-	-	-	
200	30 min	-	-	-	-	-	ND
	1 hour	-	-	-	-	-	
	2 hours	-	-	-	-	-	
	6 hours	-	-	-	-	-	
	24 hours	-	-	-	-	-	

\+ vegetative growth detected within 48 hours,

\- no vegetative growth detected within 48 hours

◯ fungicidal dosages of each fungal isolate

ND=could not detected

3.3 Toxicity of NaCl and $KMnO_4$ on hatching rate of tilapia eggs

As presented in Table 5.7, the untreated control trial (0 % NaCl) showed the highest mean percent hatching rate 96.6 % for both 1 and 24 hours exposure. The treatment groups with 3 selected concentrations of NaCl (2.0, 2.5 and 3.0 %) for 1 hour showed decrease mean percent hatching rate 86.6, 80.6 and 70.6 %, respectively. Whereas the treatment groups exposed with 2.0, 2.5 and 3.0 % NaCl for 24 hours showed decrease mean percent hatching rate 71.7, 37.3 and 0 %, respectively. The results showed decrease mean percent hatching rate corresponding with the increase salt concentrations and there was a significant difference ($P<0.05$, One-way ANOVA) between the control group and the treatment groups.

$KMnO_4$ treatment, the results showed that 25, 50, 100, 150 and 200 µg/mL $KMnO_4$ were effective to cause 0 % hatching rate after 1 and 24 hours exposure (Table 5.8).

Table 5.7 Mean percent hatching rate and mortality of the eyed eggs after exposed to various concentrations of NaCl at 27-29 °C for 1 and 24 hours.

NaCl concentrations (%)	Mean percent			
	1 hour		24 hours	
	Hatching rate	Corrected mortality	Hatching rate	Corrected mortality
0 (Control)	96.6[a]	0[a]	96.6[a]	0[a]
2.0	86.6[b]	10.3[b]	71.3[b]	26.1[b]
2.5	80.6[c]	16.5[c]	37.3[c]	61.3[c]
3.0	70.6[d]	26.9[d]	0	100.0[d]

Different letters in the same vertical column means statistically significant difference between treatments ($P<0.05$, One-way ANOVA).

Table 5.8 Mean percent hatching rate and mortality of the eyed eggs after exposed to various concentrations of $KMnO_4$ at 27-29 °C for 1 and 24 hours.

$KMnO_4$ concentrations (µg/mL)	Mean percent			
	1 hour		24 hours	
	Hatching rate	Corrected mortality	Hatching rate	Corrected mortality
0 (Control)	96.6	0	96.6	0
25	0	100.0	0	100.0
50	0	100.0	0	100.0
100	0	100.0	0	100.0
150	0	100.0	0	100.0
200	0	100.0	0	100.0

4. Discussion

Fungal infections on eggs are prevalent in the hatchery rearing of many fish species. The management of fungal infections traditionally depended on prophylactic or therapeutic administration of chemicals. Sodium chloride has been recognized as a safe treatment for *Saprolegnia* on eggs of fall chinook salmon (*Oncorhynchus tshawytscha*) (Waterstrat and Marking, 1995). The present study, fungicidal effect of NaCl on zoospore germination of 6 isolates was 2.5 %, whereas concentration of 3.0 % NaCl was toxic to the vegetative growth for 24 hours treatment. The fungicidal dosage which effective in killing zoosporic stage of fungi was lower than vegetative stage. This may be due to the zoosporic stage was more sensitive to chemicals than the vegetative stage (Muller-Breban *et al.*, 1995). According to Kitancharoen *et al.* (1997), 2.5 % NaCl for exposed 1 hour twice a week was best for fungal control without affecting the egg condition, and Marking *et al.* (1994) reported that 3.0 % NaCl for 1 hour every other day inhibited fungal infection and increased hatching rate, but concentrations above 3.0 % NaCl were toxic to eggs. From this study, it showed that percent hatching rate of the treatment groups with 2.0, 2.5 and 3.0 % NaCl for 1 and 24 hours exposure were significantly different ($P<0.05$) between the treatment groups and the untreated control group. Especially, 3.0 % NaCl treatment within 24 hours caused 0 % hatching rate. Fungal infection was controlled at 2.5 % NaCl but 3.0 % NaCl killed hatched eggs.

Therefore, percent hatching rate of tilapia eggs was strongly affected by concentrations of salt and exposure times. This result was similar to the report of Edgell *et al.* (1993) that 2.5 % NaCl or higher concentrations also killed pre-eyed eggs. According to Martinez-Palacios *et al.* (2004), it is possible that high salinities have an inhibitory action on the movement of the fish embryo due to the high osmotic impact on the perivitelline layer. Yamagami (1988, *cited by* Martinez-Palacios *et al.*, 2004) stated that hatching success is principally affected by level of chorionase activity and movement of the embryo. In this study, a longer chemical exposure period (24 hours) could be harmful for all eggs. A shorter chemical exposure period may have reduced the stress to the egg and allowed for a safe chemical application (Rach *et al.*, 1997; 2000a, 2000b).

Potassium permanganate has been used to treat external pathogens including fungus, bacteria and some parasites (Noga, 1996; Francis-Floyd and Klinger, 2002). From this study, fungicidal effect of $KMnO_4$ on the vegetative and zoosporic stages was varied among the isolates and exposure times. It showed that 25 μg/mL $KMnO_4$ and shorter chemical exposure period (30 minutes) had stronger effective on zoosporic stage than vegetative stage. The concentration of 200 μg/mL $KMnO_4$ at 24 hours was effective to control both vegetative and zoosporic stages of all fungal isolates. This result was not similar to the report of Yuasa *et al.* (2000) that 200 μg/mL $KMnO_4$ was toxic to *Achlya*, *Aphanomyces* and *Pythium* for 6 hours and toxic to genus *Saprolegnia* for 12 hours treatment. Marking *et al.* (1994) reported that $KMnO_4$ was fungicide for inhibition of cultured fungi (*in vitro*) at 50 μg/mL and toxicity on eggs at 150 μg/mL in 1 hour exposure at 12 °C. However, treatment of eyed eggs with 25, 50, 100, 150 and 200 μg/mL $KMnO_4$ within 1 and 24 hours showed 0 % hatching rate.

Therefore, it may be possible to use 3.0 % sodium chloride or higher concentrations to prevent fungal infection on tilapia eggs by bath less than 1 hour exposure.

CHAPTER VI
CONCLUSIONS

The morphological characteristics of fungal isolates in this study are described and classified as families *Saprolegniaceae* and *Pythiaceae*. Family *Saprolegniaceae* is composed of *Achlya* spp., *A. ambisexualis*, *A. bisexualis*, *Aphanomyces* spp., *Saprolegnia diclina* and family *Pythiaceae* is composed of *Pythium* sp. *Achlya* is a common genus, found from almost hatcheries and occurred throughout the year. Water quality as salinity and water supply for eggs incubation may have effected on the occurrence and distribution of fungi.

Biological characteristic studies showed that *Achlya* and *Aphanomyces* grew well at 25-35 °C, while *Saprolegnia* and *Pythium* grew well at 20-35 °C, respectively. They produced numerous zoospores at 25 °C, except *Pythium* sp. did not produce zoospores at all temperature tested. All isolates of *Achlya*, *Aphanomyces*, *Saprolegnia* and *Pythium* grew well in pH 6.0-8.0, 6.0-9.0, 7.0-9.0 and 6.0-7.0, respectively, and they grew up to 1.5, 2.0, 3.0 and 2.0 % sodium chloride, respectively. Therefore, the biological characteristics of isolates from this study can be used to classify fungi in the genus.

The histopathological examination of fungal infected tilapia eggs from the samplings showed numerous hyphae spread over surface of egg envelopes. The hyphae penetrated egg envelope and accumulated in cytoplasm and degenerated yolk granules. Enormous numbers of growing bacteria were also seen beneath the egg envelope and scattered throughout the necrotic yolk granules.

The anti-fungal effect of sodium chloride on zoosporic stages of 6 fungal isolates was 2.5 % for 2 hours treatment, while concentration of 3.0 % sodium chloride was toxic to the vegetative stage for 24 hours treatment. It was found that 25 and 200 μg/mL potassium permanganate were effective in killing the zoosporic and vegetative stages at 30 minutes and 24 hours, respectively.

Toxicity of 2.0, 2.5 and 3.0 % sodium chloride was strongly affected on eyed eggs, which decreased percent hatching rate of the eggs. Whereas 25, 50, 100, 150,

200 μg/mL potassium permanganate were effective toxic to the eggs resulted in 0 % hatching rate.

Therefore, it may be possible to use 3.0 % sodium chloride or higher concentrations to prevent fungal infection on tilapia eggs by bath less than 1 hour exposure.

Comments

1. To remove fungal infected eggs as soon as possible to prevent the fungi from spreading further.
2. To isolate fungi from eggs incubation water.
3. To study some biochemical test of fungi.
4. The pathogenicity of fungi is required for further investigation.

REFERENCES

Alexopoulos, C.J. 1962. **Introductory Mycology**. Department of Botany, State University of Iowa, USA.

Barnes, M.E., Ewing, D.E., Cordes, R.J. and Young, G.L. 1998. Observations on hydrogen peroxide control of *Saprolegnia* spp. during rainbow trout egg incubation. **The Progressive Fish-Culturist** 60: 67-70.

Benli, A.C.K. and Yildiz, H.Y. 2004. Blood parameters in Nile tilapia (*Oreochromis niloticus* L.) spontaneously infected with *Edwardsiell tarda*. **Aquaculture Research** 35: 1388-1390.

Bittencourt, N.D.L.R., Molinari, L.M., Scoaris, D.D.O., Pedroso, R.B., Nakamura, C.V., Ueda-Nakamura, T., Filho, B.A.D.A. and Filho, B.P.D. 2003. Haematological and biochemical values for Nile tilapia *Oreochromis niloticus* cultured in semi-intensive system. **Acta Scientiarum Biological Science** 25(2): 385-389.

Bruno, D.W. and Wood, B.P. 1999. *Saprolegnia* and other Oomycetes. In: **Fish Diseases and Disorders Volume 3 Viral, Bacterial and Fungal Infections.** Woo, P.T.K. and Bruno, D.W. (Eds.), pp. 599-659. CABI Publishing, USA.

Chapman, F.A. 2000. **Culture of Hybrid Tilapia: A Reference Profile**[1]. Document No. CIR716, Institute of Food and Agricultural Science, University of Florida. 5 pp.

Chaudhuri, H., Kochhar, P.L., Lotus, S.S., Banerjee M.L. and Khan, A.H. 1947. *cited in* El-Hissy, F.T. and Khallil, A.R.M. (1991). **A Hand Book of Indian Water moulds. Part I**. Publication on Botany, University of Punjab, India,

Chinabut, S. 1997. **Lethal concentration of some chemicals and drugs used in aquaculture.** The Aquatic Animal Health Research Institute (AAHRI) Newsletter Article, Thailand. 12 pp.

_____, Limsuwan, C. and Kitsawat, P. 1991. **Histology of the Walking Catfish, *Clarias batrachus***. The Aquatic Animal Health Research Institute (AAHRI), Thailand.

Chinabut, S., Roberts, R.J., Willoughby, G.R. and Pearson, M.D. 1995. Histopathology of snakehead, *Channa striatus* (Bloch), experimentally infected with the specific *Aphanomyces* fungus associated with epizootic ulcerative syndrome (EUS) at different temperatures. **Journal of Fish Diseases** 18: 41-47.

Chukanhom, K. 2004. **Study on fungal diseases of freshwater fishes in Southeast Asia**. Ph.D. Thesis of Fish diseases department, Nippon Veterinary and Life Science University, Japan.

_____ and Hatai, K. 2004. Freshwater fungi isolated from eggs of the common carp (*Cyprinus carpio*) in Thailand. **Mycoscience** 45: 42-48.

Czeczuga, B. and Woronowicz, L. 1993. Aquatic fungi developing on the eggs of certain fresh-water fish species and their environment. **ACTA Ichthyologica Et Piscatoria** 23(1): 39-57.

_____ and Muszynska, E. 1997. Aquatic fungi growing on the eggs of andromous fish species of the family Clupeidae. **ACTA Ichthyologica Et Piscatoria** 27(1): 83-93.

_____, Kiziewicz, B. and Danilkiewicz, Z. 2002. Zoosporic fungi growing on the specimens of certain fish species recently introduced to Polish waters. **ACTA Ichthyologica Et Piscatoria** 32(2): 117-125.

Dix, N.J. and Webster, J. 1995. **Fungal Ecology**. Chapman & Hall, UK.

Edgell, P., Lawseth, D., Mclean, W.E. and Britton, E.W. 1993. The use of salt solutions to control fungus (*Saprolegnia*) infestations on Salmon eggs. **The Progressive Fish-Culturist** 55: 45-52.

Egusa, S. 1992. **Infectious Diseases of Fish**. A.A. Balkema publishers, Brookfield. USA.

El-Hissy, F.T. and Khallil, A.R.M. 1991. Distribution and seasonal occurrence of aquatic phycomycetes in water and submerged mud in El-Ibrahimia canal (Upper Egypt). **Journal of Islamic Academy of Sciences** 4(4): 311-316.

_____, Khallil, A.M. and Abdel-Raheem, A.A. 1992. Occurrence and distribution of zoosporic fungi and aquatic hyphomycetes in Upper Egypt. **Journal of Islamic Academy of Sciences** 5(3): 173-179.

Francis-Floyd, R. and Klingker, R.E. 2002. **Use of Potassium Permanganate to Control External Infections of Ornamental Fish[1].** Document No. FA37, Institute of Food and Agricultural Science, University of Florida. 4 pp.

Gbankoto, A. Pampoulie, C., Marques, A. and Sakiti, G.N. 2001. Occurrence of myxosporean parasites in the gills of two tilapia species from Lake Nokoué (Bénin, West Africa): effect of host size and sex, and seasonal patterns of infection. **Diseases of Aquatic Organisms** 44: 217-222.

Griffin, D.H. 1994. **Fungal Physiology**. 2nd ed. A John Wiley and Sons, New Yolk.

Grizzle, J.M. and Rogers, W.A. 1976. **Anatomy and Histology of the Channel Catfish**. Auburn University Agricultural Experiment Station, Auburn, Alabama.

Groman, D.B. 1982. **Histology of Striped Bass**. American Fisheries Society, Bethesda, Maryland.

Gupta, A.K. and Mehrotra, R.S. 1989. Seasonal periodicity of aquatic fungi in tanks at Kurukshetra, India. **Hydrobiologia** 173: 219-229.

Hansen, E.P.E. 2004. **Prevention of *Saprolegnia* on rainbow trout eggs.** Bachelor of Science Thesis in Biology, University of the Faroe Island.

Hatai, K. and Egusa, S. 1979. Studies on the pathogenic fungus of mycotic granulomatosis III. Development of the medium for egg-fungus. **Fish Pathology** 13: 147-152.

Humason, G.L. 1972. **Animal Tissue Techniques**. 3rd ed. W.H. Freeman and Company, San Francisco.

Hussein, M.M.A., Hatai, K. and Nomura, T. 2001. Saprolegniosis in salmonids and their eggs in Japan. **Journal of Wildlife Disease** 37(1): 204-207.

Jalabert, B. and Zohar, Y. 1982. Reproductive physiology in Cichlid fishes, with particular reference to *Tilapia* and *Sarotherodon*. In: **The Biology and Culture of Tilapias**. Pullin, R.S.V. and McConnell, R.H.L. (Eds.), pp. 129-140. International Center for Living Aquatic Resources Management (ICLARM), Manila, Philippines.

Johnson, T.W. 1956. **The Genus *Achlya*: Morphology and Taxonomy**. The University of Michigan Press, Ann Arbor.

Kiryu, Y., Shields, J.D. and Vogelbein, W.K. 2002. Induction of skin ulcers in Atlantic Menhaden by injection and aqueous exposure to the zoospores of *Aphanomyces invadens*. **Journal of Aquatic Animal Health** 14: 11-24.

Kitancharoen, N., Hatai, K., Ogihara, R. and Aye, D.N.N. 1995. A new record of *Achlya klebsiana* from snakehead, *Channa striatus*, with fungal infection in Myanmar. **Mycoscience** 36, 235-238.

_____, Yuasa, N. and Hatai, K. 1996. Effect of pH and temperature on growth of *Saprolegnia diclina* and *S. parasitica* isolated from various sources. **Mycoscience** 37: 385-390.

_____, and Hatai, K. 1997. Aquatic fungi developing on eggs of Salmonids. **Journal of Aquatic Animal Health** 9: 314-316.

_____, Hatai, K. and Yamamoto, A. 1997. Aquatic fungi developing on eggs of salmonids. **Journal of Aquatic Animal Health** 9: 314-316.

Klingker, R.E. and Francis-Floyd, R. 2002. **Introduction to Freshwater Fish Parasites**. Document No. CIR716, Institute of Food and Agricultural Science, University of Florida. 13 pp.

Laoprasert, T., Bangyekhun, E., Supaporn, T., Lapsongpol, T. and Chinabut, S. 2005. Identification and some biological characteristics of water mould isolated from ornamental fish. **Thai Fisheries Gazette** 58(5): 395-399. (in Thai)

Lawhavanit, O., Chukanhom, K. and Hatai, K. 2002. Effect of *Tetrahymena* on the occurrence of achlyosis in the guppy. **Mycoscience** 43: 27-31.

Lio-Po, G.D. and Lim, L.H.S. 2002. Infectious diseases of warm water fish in fresh water. In: **Diseases and Disorders of Finfish in Cage Culture**. Woo, P.T.K., Bruno, D.W. and Lim, L.H.S. (Eds.), pp. 231-281. Biddles Ltd, UK.

Lucas, J.S. and Southgate, P.C. 2003. **Aquaculture Farming Aquatic Animals and Plants**. Fishing News Books a Blackwell Publishing company, UK.

Luzur, A.M. 2002. **The Use of Potassium Permanganate in Fish Ponds**. Document No. FA23, Institute of Food and Agricultural Science, University of Florida. 3 pp.

Marecaux, E.N. 2006. **Effect of potassium permanganate on the sailfin molly, *Poecilia latippinna*, at varying salinity levels.** Master of Science Thesis, University of Florida.

Marking, L.L., Rach, J.J. and Schreier, T.M. 1994. Evaluation of antifungal agents for fish culture. **The progressive Fish-Culturist** 56: 225-231.

Martinez-Palacios, C.A., Morte, J.C., Tello-Ballinas, J.A., Toledo-Cuevas, M. and Ross, L.G. 2004. The effects of saline environments on survival and growth of eggs and larvae of *Chirostoma ester ester* Jordan 1880 (Pisces: Atherinidae). **Aqualculture** 238: 509-522.

Mer, G.S., Sati, S.C. and Khulbe, R.D. 1980. Occurrence, distribution and seasonal periodicity of some aquatic fungi of Sal-Tal (Nainital), India. **Hydrobiologia** 76: 201-205.

Misra, J.K. 1982. Occurrence, distribution and seasonality of aquatic fungi as affected by chemical factors in six alkaline ponds of India. **Hydrobiologia** 97: 185-191.

Moore-Landecker, E. 1972. *cited in* Post, 1983. **Fundamentals of the fungi.** Prentice-Hall, Inc. Englewood Cliffs, Nj.

Muller-Breban, F., Berger, B. and Yegen, O. 1995. Chemical composition and fungitoxic properties of phytopathogenic fungi of essential oils of selected aromatic plants growing wild in Turkey. **Journal Agricultural Food Chemical** 43: 2262-2266.

Munchan, C. 2003. **Some biological and pathological characteristics of *Saprolegnia* and *Achlya* isolated from ornamental fishes.** Master of Science Thesis in Biology, Khon Kaen University, Thailand. (in Thai)

Nejadsattari, T. 2000. Occurrence and distribution of aquatic Saprolegniaceae in Northwest and South of Tehran. **Iranian International Journal of Science** 2: 91-98.

Noga, E.J. 1996. **Fish Disease: Diagnosis and Treatment**. Walsworth publishing Co., USA.

Ogbonna, C.I.C. and Alabi, R.O. 1991. Studies on species of fungi associated with mycotic infections of fish in a Nigerian freshwater fish pond. **Hydrobiologia** 220: 131-135.

Olàh, J. and Farkas, J. 1978. Effects of temperature, pH, antibiotics, formalin and malachite green on the growth and survival of *Saprolegnia* and *Achlya* parasitic on fish. **Aquaculture** 13: 273-288.

Paxton, C.G. and Willoughby, L.G. 2000. Resistance of perch eggs to attack by aquatic fungi. **Journal of Fish Biology** 57: 562-570.

Pillay, T.V.R. 1993. **Aquaculture Principles and Practices**. A division of Blackwell Scientific Publications Ltd, USA.

Piper, R.J., McElwin, I.B., Orme, L.E., McCraren, J.P., Fowler, L.G. and Leonard, J.R. 1982. *cited in* Rach, J.J., Schreier, T.M., Howe, G.E. and Redman, S.D. 1997. **Fish hatchery management**. U.S. Fish and Wildlife Service, Washington, D.C.

Popma, T. and Masser, M. 1999. **Tilapia Life History and Biology**. SRAC Publication No. 283, Southern Regional Aquaculture Center. 4 pp.

Post, G. 1983. **Textbook of Fish Health**. TFH Publications, Inc.Ltd, Neptune City, USA.

Rach, J.J., Howe, G.E. and Schreier, T.M. 1997. Safety of formalin treatments on warm and cool water fish eggs. **Aquaculture** 149: 183-191.

_____, Gaikowski, M.P. and Ramsay, R.T. 2000a. Efficacy of hydrogen peroxide to control mortalities associated with bacterial gill disease infections on hatchery reared salmonids. **Journal of Aquatic Animal Health** 12: 119-127.

_____, Gaikowski, M.P. and Ramsay, R.T. 2000b. Efficacy of hydrogen peroxide to control parasitic infestations on hatchery-reared fish. **Journal of Aquatic Animal Health** 12: 267-273.

_____, Schreier, T., Gaikowski, M.P. and Schleis, S.M. 2005. Efficacy of formalin and hydrogen peroxide to increase survival of channel catfish infected with Saprolegniasis. **North American Journal of Aquaculture** 67: 312-318.

Rakmanee, C. 2004. **Some morphological and biological characteristics of fungi isolated from eggs of African catfish (*Clarias gariepinus* Burch) and common carp (*Cyprinus carpio* L.).** Master of Science Thesis in Biology, Khon Kaen University, Thailand. (in Thai)

Rakmanee, C., Hanjavanit, C. and Kitancharoen, N. 2004. Effect of temperature, pH and sodium chloride on the growth of *Saprolegnia* and *Achlya* isolated from infected eggs of African catfish (*Clarias gariepinus* Burch) and common carp (*Cyprinus carpio* L.). **The 6th Symposium on Graduate Research, KKU.** 18 February 2004. Saisuree Jittikul convention hall, Faculty of Education, Khon Kaen University.

Rand, T.G. and Munden, D. 1997. Enzyme involvement in the invasion of brook char, *Saivelinus fontinalis* (Mitchill), eggs by *Saprolegnia diclina* (Oomycotina: Saprolegniaceae). **Journal of Fish Diseases** 15: 91-94.

Roberts, R.J. 2001. **Fish Pathology.** 3rd ed. W.B. Saunders, London.

Roberts, R.E. 1963. *cited in* Dix, N.J. and Webster, J. 1995. A study of the distribution of certain members of the Saprolegnials. **Transactions of the British Mycological Society** 46: 213-224.

Schmitt, J.A. and Beneke, E.S. 1962. Aquatic fungi from South bass and neighboring Islands in Western Lake Erie. II. Additional biflagellate and uniflagellate phycomycetes. **The Ohio Journal of Science** 62(1): 11-12.

Schreck, C.B., Fitzpatrick, M.S., Marking, L.L., Rach, J.J. and Schreier, T.M. 1993. **Research to Identify Effective Antifungal Agents**. Annual Report 1992 of Bonneville Power Administration (BPA) No. 89-054. 30 pp.

Scott, W.W. 1961. **A monograph of the genus *Aphanomyces***. Virgenia Agricultural Experimental Station Technical Bulletin 151.

Seymour, R.L. 1970. **The genus *Saprolegnia*.** Nova Hewigia. 19: 1-125.

Silva, S.S.D., Subasinghe, R.P., Bartley, D.M. and Lowther, A. 2004. **Tilapia as Alien Aquatics in Asia and the Pacific: a Review. FAO Fisheries Technical Paper 453**. Food and Agriculture Organization of the United Nations, Rome.

SPSS. 1994. SPSS Inc. 444N. Michigan Avenue Chicago, Illinois.

Srisakuntiew, P. 2000. Status of tilapia in aquaculture of Thailand. **Khon Kaen Agriculture Journal** 28: 173-181. (in Thai)

Srivastava, R.C. 1980. Fungal parasites of certain freshwater fishes of India. **Aquaculture** 21: 387-392.

Srivichai, S. and Boonyene, N. 2004. Freshwater fungi. In: **Thai Fungal Diversity**. Jones, E.B.G., Tantichareon, M. and Hyde, K.D. (Eds.), pp. 95-106. National Center for Genetic Engineering and Biotechnology (BIOTEC), Thailand.

Steciow, M.M. 2001. *Achlya fuegiana*, A new species from Tierra Del Fuego Province (Argentina). **Mycologia** 93(6): 1195-1199.

Suresh, V. 2003. Tilapias. In: **Aquaculture, Farming Aquatic Animals and Plants**. Lucas, J.S. and Southgate, P.C. (Eds.), pp. 321-345. Blackwell Publishing, USA.

Tawaratmaneekul, P. and Tungtrongpiroj, M. 1993. A guideline for development of the Nile tilapia culturing. **Thai Fisheries Gazette** 46(4): 309-313. (in Thai)

Van Der Plaats-Niterink, A.J. 1981. **Monograph of genus *Pythium* studies in mycology No.21.** Centraalbureau Vor Schimmelcultures, Netherlands.

Wada, S., Hatai, K. and Ishii, H. 1993. Mycotic gastritis of juvenile Ayu (*Plecoglossus altivelis*) caused by *Saprolegnia diclina* type I. **Journal of Wildlife Diseases** 29(4): 587-590.

Waterstrat, P.R. and Marking, L.L. 1995. Clinical evaluation of formalin, hydrogen peroxide and sodium chloride for the treatment of *Saprolegnia parasitica* on fall chinook salmon eggs. **The Progressive Fish-Culturist** 57: 287-291.

Willoughby, L.G. 1985. Rapid preliminary screening of *Saprolegnia* on fish. **Journal of Fish Diseases** 8: 473-476.

_____ and Copland, J.W. 1984. Temperature-growth relationships of *Saprolegnia* pathogenic to fish, especially eels cultivated in warm water. **Nova Hedwigia** 39: 35-55.

_____ and Lilley, J.H. 1992. The ecology of aquatic fungi in Thailand and the fish diseases relationship. **AAHRI Newsletter Article** 1(1): 5-60.

Wiwattanachaiseat, Y. 1996. Is the tilapia culture do easily and resistant to disease? **Thai Fisheries Gazette** 49(6): 529-536. (in Thai)

Wohlfarth, G.W. and Hulata, G. 1983. **Applied Genetics of Tilapias**. International Center for Living Aquatic Resources Management, Manila.

Woo, P.T.K. and Bruno, D.W. 1999. **Fish diseases and Disorder, Volumn 3 Viral, Bacterial and Fungal Infections**. CABI Publishing, UK.

Wood, S.E., Willoughby, L.G. and Beakes, G.W. 1986. Preliminary evidence for inhibition of *Saprolegnia* fungus in the mucus of brown trout, *Salmo trutta* L. following experimental challenge. **Journal of Fish Diseases** 9: 557-560.

Yamagami, K. 1988. *cited in* Martinez-Palacios, C.A., Morte, J.C., Tello-Ballinas, J.A., Toledo-Cuevas, M. and Ross, L.G. 2004. Mechanisms of hatching in fish. In: **Fish Physilogy. Part A. Eggs and Larvae, vol. XI**. Hoar, W.S., Randall, D.J. (Eds.), 546 pp. Academic Press, London.

Yuasa, N. Chaloakpantharat, P. and Teabsree, S. 2000. Fungal diseases in economically important fishes and disease prevention. **KKU fund for research report.** Khon Kaen University. 25 pp. (in Thai)

APPENDICES

Appendix A

Fungal medium

Fungal medium (Hatai and Egusa, 1979)

Glucose Yeast extract Agar (GYA)

D (+)-glucose anhydrous	1.0	mg
Yeast extract	0.25	mg
Agar	1.4	mg
Distill water	100	mL

Glucose Yeast extract Broth (GYB)

D (+)-glucose anhydrous	1.0	mg
Yeast extract	0.25	mg
Distill water	100	mL

Appendix B

Data table of physical and chemical parameters of incubation water

Appendix Table B-1: Means of physical and chemical parameters from various sites.

Water parameters	Durations	I	II	III	IV	V	VI	VII
Temperature (°C)	Jun.-Jul.05	32.0	32.9	26.8	27.6	24.6	28.1	27.3
	Oct.-Nov.05	26.5	25.7	25.8	26.6	26.7	24.2	25.8
	Mar.-Apr.06	25.3	27.4	27.8	24.6	25.9	20.9	23.5
DO (mg/L)	Jun.-Jul.05	4.3	3.6	4.1	3.3	5.4	4.1	4.5
	Oct.-Nov.05	4.7	5.1	3.8	4.9	4.5	5.4	5.0
	Mar.-Apr.06	5.0	3.7	4.9	4.3	4.4	4.6	3.8
pH	Jun.-Jul.05	7.60	6.9	7.2	7.3	7.4	7.5	7.3
	Oct.-Nov.05	7.5	7.3	7.7	7.6	7.5	7.3	8.0
	Mar.-Apr.06	7.8	7.7	6.9	6.8	8.1	7.9	7.8
TDS (mg/L)	Jun.-Jul.05	215.5	186.7	1165.7	206.2	509.0	3870.0	534.0
	Oct.-Nov.05	221.7	1174.3	1351.0	222.0	268.0	3530.0	731.0
	Mar.-Apr.06	272.0	1963.3	1664.0	385.0	1060.0	1666.0	212.7
Electro-conductivity (µs/cm)	Jun.-Jul.05	324.3	280.3	1749.0	308.3	763.0	5800.0	801.0
	Oct.-Nov.05	326.3	1760.0	2030.0	337.3	366.3	5290.0	1097.3
	Mar.-Apr.06	373.0	2953.3	2496.7	578.3	1591.0	2503.3	320.0
Salinity (%)	Jun.-Jul.05	0	0	0	0	0	0.02	0
	Oct.-Nov.05	0	0	0	0	0	0.03	0
	Mar.-Apr.06	0	0	0.01	0	0	0.01	0

Appendix C

Data table of mean fungal colony radius on various temperatures

Appendix Table C-1: Effect of various temperatures on means of hyphal growth of 16 fungal isolates from tilapia eggs incubated for 7 days.

Isolates	Days	Temperatures (°C)							
		5	10	15	20	25	30	35	40
Achlya spp.									
BKKU 0501	1	0	4.6	8.4	9.9	10.7	17.6	14.3	0
	2	0	5.3	14.9	17.1	21.0	29.7	25.7	0
	3	0	5.5	21.0	23.3	30.8	39.1	34.9	0
	4	0	5.8	24.7	30.6	39.9	45.0	45.0	0
	5	0	6.1	28.1	36.2	45.0	45.0	45.0	0
	6	0	6.5	30.8	40.9	45.0	45.0	45.0	0
	7	0	6.9	31.9	43.1	45.0	45.0	45.0	0
BKKU 0502	1	0	3.9	7.3	9.3	9.5	12.3	12.6	0
	2	0	5.7	15.3	15.9	20.0	22.9	25.3	0
	3	0	8.4	21.9	23.2	29.9	32.6	38.7	0
	4	0	8.8	28.3	29.0	38.1	39.9	45.0	0
	5	0	9.9	32.6	36.5	45.0	45.0	45.0	0
	6	0	10.9	36.6	40.5	45.0	45.0	45.0	0
	7	0	12.1	40.8	43.5	45.0	45.0	45.0	0
BKKU 0503	1	0	3.3	4.5	9.0	10.1	12.3	14.4	0
	2	0	4.3	6.8	17.7	21.2	23.6	28.6	0
	3	0	4.8	9.2	25.6	31.7	34.0	41.3	0
	4	0	4.8	11.6	32.9	40.8	42.7	45.0	0
	5	0	5.3	14.2	38.1	45.0	45.0	45.0	0
	6	0	5.4	15.4	42.5	45.0	45.0	45.0	0
	7	0	5.9	18.0	44.8	45.0	45.0	45.0	0
A. ambisexualis									
BKKU 0614	1	0	4.6	4.7	10.6	12.4	13.1	14.3	0
	2	0	5.9	7.5	21.0	25.4	26.4	28.8	0
	3	0	6.7	10.9	31.5	36.7	39.4	41.2	0
	4	0	7.4	14.4	40.4	45.0	45.0	45.0	0
	5	0	8.1	18.1	45.0	45.0	45.0	45.0	0

Note: 0 = no growth

Appendix Table C-1: Effect of various temperatures on means of hyphal growth of 16 fungal isolates from tilapia eggs incubated for 7 days (Cont.).

Isolates	Days	Temperatures (°C)							
		5	10	15	20	25	30	35	40
BKKU 0614	6	0	10.4	22.0	45.0	45.0	45.0	45.0	0
	7	0	11.6	25.3	45.0	45.0	45.0	45.0	0
BKKU 0615	1	0	4.2	5.0	10.5	10.3	14.6	14.6	0
	2	0	5.4	7.6	20.4	21.9	28.3	28.3	0
	3	0	5.9	10.7	29.4	33.7	40.3	40.3	0
	4	0	6.5	13.7	38.0	45.0	45.0	45.0	0
	5	0	6.7	17.0	44.3	45.0	45.0	45.0	0
	6	0	7.7	20.3	45.0	45.0	45.0	45.0	0
	7	0	8.2	23.5	45.0	45.0	45.0	45.0	0
A. bisexualis									
BKKU 0504	1	0	3.6	8.5	8.2	10.9	12.5	12.8	0
	2	0	4.2	15.4	16.6	21.5	24.2	23.9	0
	3	0	5.3	23.2	24.8	31.4	34.4	35.9	0
	4	0	6.2	29.1	31.3	40.7	41.9	41.8	0
	5	0	7.3	34.0	38.3	45.0	45.0	45.0	0
	6	0	8.2	38.7	43.7	45.0	45.0	45.0	0
	7	0	9.1	42.0	45.0	45.0	45.0	45.0	0
BKKU 0509	1	0	3.7	11.2	9.6	10.4	17.8	15.2	0
	2	0	4.6	15.8	16.5	21.2	31.7	27.6	0
	3	0	5.9	23.2	22.9	31.2	42.3	37.1	0
	4	0	6.9	28.2	29.5	39.8	45.0	44.2	0
	5	0	7.8	36.2	34.7	45.0	45.0	45.0	0
	6	0	8.8	35.9	39.4	45.0	45.0	45.0	0
	7	0	9.9	37.8	42.0	45.0	45.0	45.0	0
BKKU 0510	1	0	4.2	7.3	8.2	10.7	12.2	13.3	0
	2	0	5.1	16.6	16.2	22.2	25.1	25.7	0
	3	0	5.7	23.1	23.2	32.2	37.2	39.4	0
	4	0	7.0	29.9	29.4	41.0	43.1	45.0	0
	5	0	8.0	34.1	35.8	45.0	45.0	45.0	0

Note: 0 = no growth

Appendix Table C-1: Effect of various temperatures on means of hyphal growth of 16 fungal isolates from tilapia eggs incubated for 7 days (Cont.).

Isolates	Days	Temperatures (°C)							
		5	10	15	20	25	30	35	40
BKKU 0510	6	0	9.0	37.8	40.2	45.0	45.0	45.0	0
	7	0	11.4	40.5	42.3	45.0	45.0	45.0	0
BKKU 0511	1	0	3.8	6.4	8.6	10.8	13.4	12.8	0
	2	0	5.6	12.9	15.7	21.5	27.3	26.2	0
	3	0	7.5	19.0	23.1	30.2	41.0	38.9	0
	4	0	9.6	27.6	29.1	39.1	45.0	43.9	0
	5	0	11.7	29.3	36.6	45.0	45.0	45.0	0
	6	0	13.0	39.6	43.7	45.0	45.0	45.0	0
	7	0	14.1	36.9	45.0	45.0	45.0	45.0	0
BKKU 0612	1	0	0	3.7	10.1	10.5	13.6	13.1	0
	2	0	0	6.1	19.0	21.5	25.8	26.6	0
	3	0	0	8.8	26.8	32.0	37.5	39.5	0
	4	0	2.4	10.2	32.8	40.6	45.0	45.0	0
	5	0	2.6	14.3	38.1	45.0	45.0	45.0	0
	6	0	3.3	17.7	42.9	45.0	45.0	45.0	0
	7	0	3.6	19.6	45.0	45.0	45.0	45.0	0
BKKU 0613	1	0	4.5	4.2	9.4	10.6	12.8	14.0	0
	2	0	4.8	6.5	18.4	22.1	22.8	27.4	0
	3	0	5.3	8.0	27.5	32.2	32.3	40.2	0
	4	0	5.2	10.4	35.8	41.0	41.1	45.0	0
	5	0	5.2	12.9	40.3	45.0	45.0	45.0	0
	6	0	6.6	15.4	43.7	45.0	45.0	45.0	0
	7	0	6.9	18.0	45.0	45.0	45.0	45.0	0
BKKU 0616	1	0	3.9	4.2	10.2	10.8	13.4	13.7	0
	2	0	4.1	6.5	19.4	20.2	27.1	26.2	0
	3	0	4.4	8.8	28.2	30.4	41.3	39.5	0
	4	0	4.4	11.7	35.9	39.4	45.0	45.0	0
	5	0	4.7	14.6	41.7	45.0	45.0	45.0	0

Note: 0 = no growth

Appendix Table C-1: Effect of various temperatures on means of hyphal growth of 16 fungal isolates from tilapia eggs incubated for 7 days (Cont.).

Isolates	Days	Temperatures (°C)							
		5	10	15	20	25	30	35	40
BKKU 0616	6	0	4.8	17.6	45.0	45.0	45.0	45.0	0
	7	0	5.3	19.9	45.0	45.0	45.0	45.0	0
Aphanomyces spp.									
BKKU 0505	1	0	4.2	6.9	8.8	11.6	17.2	19.2	0
	2	0	5.8	15.3	18.0	24.0	34.0	37.6	0
	3	0	7.3	20.7	27.1	33.8	45.0	45.0	0
	4	0	9.9	25.5	34.8	41.9	45.0	45.0	0
	5	0	11.9	29.8	42.8	45.0	45.0	45.0	0
	6	0	13.6	34.0	45.0	45.0	45.0	45.0	0
	7	0	15.6	37.1	45.0	45.0	45.0	45.0	0
BKKU 0508	1	0	4.1	9.2	8.9	13.7	15.9	16.0	0
	2	0	5.9	15.8	17.3	26.5	30.0	30.9	0
	3	0	7.6	22.7	24.3	38.0	42.7	42.5	0
	4	0	9.3	27.1	29.6	45.0	45.0	45.0	0
	5	0	10.9	32.0	35.8	45.0	45.0	45.0	0
	6	0	12.3	37.1	40.7	45.0	45.0	45.0	0
	7	0	14.1	40.4	44.4	45.0	45.0	45.0	0
S. diclina									
BKKU 0506	1	0	7.1	8.7	19.6	23.1	24.7	24.4	0
	2	0	11.3	17.0	39.8	45.0	44.5	45.0	0
	3	0	15.3	25.7	45.0	45.0	45.0	45.0	0
	4	0	20.2	34.2	45.0	45.0	45.0	45.0	0
	5	0	28.1	42.0	45.0	45.0	45.0	45.0	0
	6	0	31.4	45.0	45.0	45.0	45.0	45.0	0
	7	0	34.6	45.0	45.0	45.0	45.0	45.0	0

Note: 0 = no growth

Appendix Table C-1: Effect of various temperatures on means of hyphal growth of 16 fungal isolates from tilapia eggs incubated for 7 days (Cont.).

Isolates	Days	Temperatures (°C)							
		5	10	15	20	25	30	35	40
Pythium sp.									
BKKU 0507	1	0	7.7	11.3	13.0	16.1	19.0	15.4	0
	2	0	10.5	23.8	24.9	32.3	37.0	25.9	0
	3	0	16.0	33.5	36.8	45.0	45.0	36.7	0
	4	0	20.0	38.3	45.0	45.0	45.0	45.0	0
	5	0	23.0	45.0	45.0	45.0	45.0	45.0	0
	6	0	25.9	45.0	45.0	45.0	45.0	45.0	0
	7	0	28.7	45.0	45.0	45.0	45.0	45.0	0

Note: 0 = no growth

Appendix D

Data table of mean fungal colony radius on various pHs

Appendix Table D-1: Mean colony radius on effect of various pHs on hyphal growth of 16 fungal isolates incubated for 5 days.

Isolates	Days	pHs									
		3	4	5	6	7	8	9	10	11	12
Achlya spp.											
BKKU 0501	1	0	11.1	15.8	19.4	19.0	18.6	16.1	7.3	0	0
	2	0	18.1	27.8	31.8	32.0	33.9	31.4	10.2	0	0
	3	0	23.4	37.0	41.7	42.2	45.0	44.4	13.6	0	0
	4	0	28.3	44.1	49.3	49.1	51.6	52.6	18.3	0	0
	5	0	32.5	50.4	56.3	56.9	59.6	64.0	21.8	0	0
BKKU 0502	1	0	4.1	5.5	14.5	15.5	16.4	13.0	4.7	3.7	0
	2	0	5.0	8.2	20.6	23.4	25.8	20.4	7.7	5.0	0
	3	0	6.0	10.7	28.1	32.4	34.0	29.1	11.9	5.8	0
	4	0	7.3	26.4	35.4	4.4	42.1	36.4	14.6	6.6	0
	5	0	8.3	15.1	40.9	48.1	48.3	43.3	18.4	7.5	0
BKKU 0503	1	0	12.1	16.6	18.4	19.8	19.8	17.5	6.6	0	0
	2	0	17.7	25.9	29.4	35.2	35.2	32.9	10.5	0	0
	3	0	22.3	34.8	40.5	45.6	45.6	43.8	13.8	0	0
	4	0	27.4	42.1	49.0	52.4	52.4	52.4	21.1	0	0
	5	0	31.4	50.9	59.2	64.0	59.0	59.5	26.2	0	0

Note: 0 = no growth

Appendix Table D-1: Mean colony radius on effect of various pHs on hyphal growth of 16 fungal isolates incubated for 5 days (Cont.).

Isolates	Days	pHs									
		3	4	5	6	7	8	9	10	11	12
A. ambisexualis											
BKKU 0614	1	0	12.1	18.4	19.6	21.2	22.0	14.8	7.6	3.2	0
	2	0	18.6	32.0	36.1	37.6	39.6	29.1	10.1	5.7	0
	3	0	24.5	41.9	47.1	50.2	50.5	42.2	12.6	9.6	0
	4	0	29.3	51.5	56.0	59.3	61.5	55.2	16.9	12.8	0
	5	0	53.5	64.0	64.0	64.0	64.0	64.0	19.7	15.0	0
BKKU 0615	1	0	8.5	12.4	16.8	18.3	14.2	7.1	3.7	3.4	0
	2	0	10.4	18.7	29.4	30.3	25.4	11.6	6.1	4.8	0
	3	0	12.5	23.7	39.7	41.3	36.2	15.8	8.2	7.3	0
	4	0	14.2	26.8	47.7	50.7	45.3	20.1	10.3	9.6	0
	5	0	17.2	33.3	55.3	59.9	56.4	25.4	12.1	11.8	0
A. bisexualis											
BKKU 0504	1	0	6.4	11.1	15.0	18.4	15.9	16.2	8.8	5.0	0
	2	0	9.0	15.4	20.9	29.8	22.1	25.2	10.5	5.7	0
	3	0	11.0	20.5	28.9	37.6	32.3	35.8	13.9	6.9	0
	4	0	13.5	26.0	36.3	43.0	40.6	44.6	17.2	8.0	0
	5	0	15.8	31.0	41.5	49.8	45.7	52.9	24.0	9.8	0

Note: 0 = no growth

Appendix Table D-1: Mean colony radius on effect of various pHs on hyphal growth of 16 fungal isolates incubated for 5 days (Cont.).

Isolates	Days	pHs									
		3	4	5	6	7	8	9	10	11	12
BKKU 0509	1	0	5.7	11.6	13.8	17.2	18.2	15.3	6.3	4.8	0
	2	0	8.9	16.9	24.7	35.7	28.7	26.7	9.8	6.7	0
	3	0	11.3	23.5	31.6	44.5	36.6	36.3	12.4	8.2	0
	4	0	13.5	28.2	39.1	51.4	42.8	44.1	15.1	10.4	0
	5	0	15.4	32.1	48.2	56.2	51.0	51.4	21.8	12.3	0
BKKU 0510	1	0	7.4	10.9	16.4	17.2	17.3	11.6	6.0	5.6	0
	2	0	9.3	15.5	24.4	25.6	27.6	23.5	9.6	6.5	0
	3	0	11.9	19.6	31.3	34.4	36.2	32.5	12.0	7.3	0
	4	0	13.8	24.1	40.0	43.7	44.4	40.3	15.1	7.9	0
	5	0	15.9	26.4	46.9	52.0	51.0	48.1	18.6	8.9	0
BKKU 0511	1	0	7.6	11.8	16.2	16.8	17.4	12.4	5.8	3.9	0
	2	0	10.2	16.2	21.3	24.3	25.7	21.0	8.7	6.4	0
	3	0	12.1	22.1	28.0	31.6	35.6	30.3	12.6	7.6	0
	4	0	14.7	27.5	36.1	39.8	43.1	38.7	17.6	8.9	0
	5	0	16.5	31.2	41.3	45.4	50.1	47.0	25.6	10.1	0
BKKU 0612	1	0	9.3	11.6	16.2	18.4	17.4	14.8	7.3	6.8	0
	2	0	11.4	16.2	22.7	35.4	29.5	25.2	9.4	8.0	0

Note: 0 = no growth

Appendix Table D-1: Mean colony radius on effect of various pHs on hyphal growth of 16 fungal isolates incubated for 5 days (Cont.).

Isolates	Days	pHs									
		3	4	5	6	7	8	9	10	11	12
BKKU 0612	3	0	13.2	22.6	31.2	43.5	39.0	34.9	12.3	9.7	0
	4	0	15.8	26.0	37.6	48.8	46.3	43.0	16.6	10.9	0
	5	0	18.1	31.3	45.6	55.7	54.0	51.3	24.0	12.2	0
BKKU 0613	1	0	7.1	11.6	16.3	16.8	17.2	14.4	5.0	5.0	0
	2	0	9.2	15.3	22.6	28.1	30.2	22.8	8.7	6.6	0
	3	0	11.6	19.2	29.8	38.4	40.8	32.2	12.7	8.3	0
	4	0	13.9	23.7	35.8	46.8	47.5	41.9	18.0	9.8	0
	5	0	16.9	26.7	44.2	54.7	55.3	51.5	26.5	11.6	0
BKKU 0616	1	0	5.0	11.3	15.3	16.9	15.3	12.8	3.6	4.9	0
	2	0	7.3	16.3	29.9	34.6	27.5	25.3	4.8	6.6	0
	3	0	13.8	21.0	26.8	43.2	36.0	35.0	6.6	7.7	0
	4	0	14.4	25.9	32.3	51.3	44.1	44.8	8.0	8.1	0
	5	0	15.8	30.8	37.4	57.9	50.9	51.3	9.3	9.1	0
Aphanomyces spp.											
BKKU 0505	1	0	0	7.7	11.2	12.5	11.9	10.3	7.5	4.1	0
	2	0	0	15.5	20.6	21.4	21.6	19.1	14.2	5.5	0
	3	0	0	24.3	32.5	34.5	32.4	30.5	23.4	7.7	0

Note: 0 = no growth

Appendix Table D-1: Mean colony radius on effect of various pHs on hyphal growth of 16 fungal isolates incubated for 5 days (Cont.).

Isolates	Days	pHs									
		3	4	5	6	7	8	9	10	11	12
BKKU 0505	4	0	0	32.1	42.6	45.3	45.0	44.6	35.2	9.7	0
	5	0	0	38.7	55.1	56.6	55.0	54.1	44.7	12.3	0
BKKU 0508	1	0	0	7.5	12.0	13.4	13.1	13.1	6.4	4.6	0
	2	0	0	13.7	20.6	23.7	23.4	19.6	15.9	7.2	0
	3	0	0	23.4	30.2	35.2	33.4	28.0	25.5	11.2	0
	4	0	0	32.9	39.0	46.6	44.8	36.3	34.2	14.5	0
	5	0	0	41.4	47.1	55.8	58.7	42.9	43.6	18.7	0
S. diclina											
BKKU 0506	1	0	0	7.5	12.0	13.4	13.1	13.1	6.4	4.6	0
	2	0	0	13.7	20.6	23.7	23.4	19.6	15.9	7.2	0
	3	0	0	23.4	30.2	35.2	33.4	28.0	25.5	11.2	0
	4	0	0	32.9	39.0	46.6	44.8	36.3	34.2	14.5	0
	5	0	0	41.4	4 7.1	55.8	58.7	42.9	43.6	18.7	0
Pythium sp.											
BKKU 0507	1	0	0	13.3	18.6	18.7	17.1	17.0	12.0	0	0
	2	0	0	23.1	32.2	33.7	32.2	32.7	22.0	0	0
	3	0	0	33.8	45.0	49.7	46.3	45.8	31.6	0	0

Note: 0 = no growth

Appendix Table D-1: Mean colony radius on effect of various pHs on hyphal growth of 16 fungal isolates incubated for 5 days (Cont.).

Isolates	Days	pHs									
		3	4	5	6	7	8	9	10	11	12
BKKU 0507	4	0	0	43.5	57.9	59.8	54.9	50.7	39.2	0	0
	5	0	0	53.4	64.0	64.0	64.0	56.8	47.3	0	0

Note: 0 = no growth

Appendix E

Data table of mean fungal colony radius on various salinity of sodium chloride

Appendix Table E-1: Mean colony radius on effect of NaCl on hyphal growth of 16 fungal isolates from tilapia eggs incubated for 7 days.

Isolates	Days	NaCl (%)								
		0	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0
Achlya spp.										
BKKU 0501	1	10.7	10.4	5.0	2.9	0	0	0	0	0
	2	21.0	17.5	8.1	3.4	0	0	0	0	0
	3	30.8	24.4	11.4	3.8	0	0	0	0	0
	4	39.9	30.7	14.4	4.3	0	0	0	0	0
	5	45.0	36.7	17.8	4.7	0	0	0	0	0
	6	45.0	45.0	21.2	4.8	0	0	0	0	0
	7	45.0	45.0	24.0	5.0	0	0	0	0	0
BKKU 0502	1	9.5	9.0	3.6	2.9	0	0	0	0	0
	2	20.0	14.9	5.0	3.3	0	0	0	0	0
	3	29.9	21.1	7.0	3.6	0	0	0	0	0
	4	38.1	26.8	9.3	4.1	0	0	0	0	0
	5	45.0	32.7	12.0	4.4	0	0	0	0	0
	6	45.0	38.6	14.3	4.7	0	0	0	0	0
	7	45.0	45.0	16.2	5.3	0	0	0	0	0
BKKU 0503	1	10.1	8.9	3.7	2.7	0	0	0	0	0
	2	21.2	15.6	5.2	2.9	0	0	0	0	0
	3	31.7	22.3	6.9	3.4	0	0	0	0	0
	4	40.8	28.1	9.1	3.9	0	0	0	0	0
	5	45.0	33.6	11.6	4.3	0	0	0	0	0
	6	45.0	37.9	13.8	4.7	0	0	0	0	0
	7	45.0	42.5	16.8	5.4	0	0	0	0	0
A. ambisexualis										
BKKU 0614	1	12.4	9.6	4.5	2.9	0	0	0	0	0
	2	25.5	15.6	7.6	3.8	0	0	0	0	0
	3	36.7	21.5	10.8	4.5	0	0	0	0	0
	4	45.0	28.3	14.0	5.5	0	0	0	0	0
	5	45.0	34.6	17.1	6.4	0	0	0	0	0

Note: 0 = no growth

Appendix Table E-1: Mean colony radius on effect of NaCl on hyphal growth of 16 fungal isolates from tilapia eggs incubated for 7 days (Cont.).

Isolates	Days	NaCl (%)								
		0	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0
BKKU 0614	6	45.0	38.8	19.9	7.4	0	0	0	0	0
	7	45.0	43.8	23.7	8.3	0	0	0	0	0
BKKU 0615	1	10.3	9.2	4.8	2.9	0	0	0	0	0
	2	21.9	15.8	7.6	3.3	0	0	0	0	0
	3	33.7	22.1	10.4	3.9	0	0	0	0	0
	4	45.0	28.6	14.3	4.4	0	0	0	0	0
	5	45.0	33.9	17.3	5.4	0	0	0	0	0
	6	45.0	39.4	20.5	6.0	0	0	0	0	0
	7	45.0	45.0	23.9	6.9	0	0	0	0	0
A. bisexualis										
BKKU 0504	1	10.9	8.9	4.9	3.1	0	0	0	0	0
	2	21.5	15.4	8.8	3.5	0	0	0	0	0
	3	31.4	22.2	12.2	3.8	0	0	0	0	0
	4	40.7	28.5	15.9	4.3	0	0	0	0	0
	5	45.0	35.9	20.5	4.7	0	0	0	0	0
	6	45.0	41.2	24.5	5.0	0	0	0	0	0
	7	45.0	45.0	28.0	5.6	0	0	0	0	0
BKKU 0509	1	10.4	9.6	5.3	3.1	0	0	0	0	0
	2	21.2	15.8	8.3	3.6	0	0	0	0	0
	3	31.2	21.9	11.6	4.0	0	0	0	0	0
	4	39.8	27.4	15.2	4.5	0	0	0	0	0
	5	45.0	33.3	19.6	4.7	0	0	0	0	0
	6	45.0	38.7	22.8	5.2	0	0	0	0	0
	7	45.0	45.0	25.5	5.4	0	0	0	0	0
BKKU 0510	1	10.7	7.3	4.4	3.5	0	0	0	0	0
	2	22.2	9.0	7.4	4.4	0	0	0	0	0
	3	32.2	18.8	10.7	5.4	0	0	0	0	0
	4	41.0	25.0	14.1	6.3	0	0	0	0	0
	5	45.0	29.3	17.4	7.2	0	0	0	0	0

Note: 0 = no growth

Appendix Table E-1: Mean colony radius on effect of NaCl on hyphal growth of 16 fungal isolates from tilapia eggs incubated for 7 days (Cont.).

Isolates	Days	NaCl (%)								
		0	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0
BKKU 0510	6	45.0	34.3	20.3	8.0	0	0	0	0	0
	7	45.0	38.0	24.2	9.2	0	0	0	0	0
BKKU 0511	1	10.8	8.4	3.7	2.7	0	0	0	0	0
	2	21.5	14.1	5.1	3.1	0	0	0	0	0
	3	30.2	19.9	6.7	3.3	0	0	0	0	0
	4	39.1	26.2	9.0	4.1	0	0	0	0	0
	5	45.0	32.1	11.3	4.4	0	0	0	0	0
	6	45.0	37.6	13.2	4.9	0	0	0	0	0
	7	45.0	45.0	16.0	5.7	0	0	0	0	0
BKKU 0612	1	10.5	9.8	5.0	3.2	0	0	0	0	0
	2	21.5	16.9	8.4	3.5	0	0	0	0	0
	3	32.0	25.1	11.7	3.8	0	0	0	0	0
	4	40.7	32.2	14.5	4.3	0	0	0	0	0
	5	45.0	39.3	17.8	4.7	0	0	0	0	0
	6	45.0	45.0	21.0	5.1	0	0	0	0	0
	7	45.0	45.0	27.0	5.7	0	0	0	0	0
BKKU 0613	1	10.6	7.3	4.2	2.9	0	0	0	0	0
	2	22.1	12.9	6.9	3.2	0	0	0	0	0
	3	32.2	18.1	9.9	3.7	0	0	0	0	0
	4	41.0	24.2	13.1	4.1	0	0	0	0	0
	5	45.0	28.6	16.5	4.6	0	0	0	0	0
	6	45.0	34.0	19.8	5.2	0	0	0	0	0
	7	45.0	38.6	23.4	5.9	0	0	0	0	0
BKKU 0616	1	10.8	8.5	4.8	3.7	0	0	0	0	0
	2	20.2	14.2	8.4	4.0	0	0	0	0	0
	3	30.4	19.2	11.5	4.1	0	0	0	0	0
	4	39.4	24.9	15.5	4.7	0	0	0	0	0
	5	45.0	29.8	18.6	5.2	0	0	0	0	0
	6	45.0	34.6	21.4	5.7	0	0	0	0	0

Note: 0 = no growth

Appendix Table E-1: Mean colony radius on effect of NaCl on hyphal growth of 16 fungal isolates from tilapia eggs incubated for 7 days (Cont.).

Isolates	Days	NaCl (%)								
		0	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0
BKKU 0616	7	45.0	40.2	25.4	6.3	0	0	0	0	0
Aphanomyces spp.										
BKKU 0505	1	11.6	11.2	5.7	3.5	0	0	0	0	0
	2	24.0	18.8	9.3	4.6	0	0	0	0	0
	3	33.8	26.8	12.8	5.9	0	0	0	0	0
	4	41.9	34.2	15.3	7.1	0	0	0	0	0
	5	45.0	40.5	18.3	8.3	0	0	0	0	0
	6	45.0	43.5	21.1	9.2	0	0	0	0	0
	7	45.0	45.0	23.6	10.0	0	0	0	0	0
BKKU 0508	1	13.7	11.4	6.4	3.8	2.6	0	0	0	0
	2	26.5	18.9	10.4	5.4	3.0	0	0	0	0
	3	38.0	25.5	14.3	6.7	3.5	0	0	0	0
	4	45.0	31.9	18.1	7.6	4.1	0	0	0	0
	5	45.0	37.6	21.8	8.3	4.4	0	0	0	0
	6	45.0	42.3	25.1	9.3	4.9	0	0	0	0
	7	45.0	45.0	27.8	10.2	5.3	0	0	0	0
S. diclina										
BKKU 0506	1	23.1	24.9	20.3	14.2	10.8	3.4	2.9	0	0
	2	45.0	45.0	38.4	29.7	19.1	7.5	4.2	0	0
	3	45.0	45.0	45.0	41.5	27.8	12.0	5.5	0	0
	4	45.0	45.0	45.0	45.0	36.2	16.5	7.2	0	0
	5	45.0	45.0	45.0	45.0	45.0	20.9	9.1	0	0
	6	45.0	45.0	45.0	45.0	45.0	25.2	11.0	0	0
	7	45.0	45.0	45.0	45.0	45.0	29.9	12.2	0	0
Pythium sp.										
BKKU 0507	1	16.1	16.8	13.5	9.9	5.6	3.7	0	0	0
	2	32.3	27.3	21.7	16.4	8.8	3.7	0	0	0
	3	45.0	37.2	29.6	22.7	12.1	3.7	0	0	0

Note: 0 = no growth

Appendix Table E-1: Mean colony radius on effect of NaCl on hyphal growth of 16 fungal isolates from tilapia eggs incubated for 7 days (Cont.).

Isolates	Days	NaCl (%)								
		0	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0
BKKU 0507	4	45.0	45.0	37.0	29.5	15.1	3.7	0	0	0
	5	45.0	45.0	45.0	35.9	18.6	3.7	0	0	0
	6	45.0	45.0	45.0	41.3	21.6	3.7	0	0	0
	7	45.0	45.0	45.0	45.0	30.2	3.7	0	0	0

Note: 0 = no growth

RESEARCH PUBLICATIONS

Panchai, K., Hanjavanit C. and Kitancharoen, N. Characteristics of *Achlya bisexualis* isolated from eggs of Nile tilapia (*Oreochromis niloticus* Linn.). **KKU Research.** In press.

PRESENTATIONS

Panchai, K., Hanjavanit, C. and Kitancharoen, N. 2005. **Some morphological and biological characteristics of *Achlya ambisexualis* isolated from tilapia fry, *Oreochromis niloticus* Linn.** Presented at 31st Congress on Science and Technology of Thailand, 18-20 October 2005, Technopolis, Suranaree University of Technology, Nakhon Ratchasima, Thailand. (Poster) (Sheet1)

Panchai, K., Hanjavanit, C. and Kitancharoen, N. 2006. **Characteristics of *Achlya bisexualis* isolated from eggs of tilapia (*Oreochromis niloticus* Linn.).** Presented at The First International Conference on Science and Technology for Sustainable Development of the Greater Mekong Sub-region, 15-16 August 2006, Pienvichit Building, Faculty of Engineering, Khon Kaen University, Khon Kaen, Thailand. (Poster) (Sheet2)

Panchai, K., Hanjavanit, C. and Kitancharoen, N. 2006. **Some biological characteristics of *Achlya ambisexualis* isolated from tilapia eggs (*Oreochromis niloticus* Linn.).** Presented at 10th BRT Annual Conference, 8-11 October 2006, Maritime Park & Spa Resort, Krabi, Thailand. (Poster) (Sheet3)

Panchai, K., Hanjavanit, C. and Kitancharoen, N. 2006. **Characteristics of *Saprolegnia diclina* isolated from eggs of tilapia (*Oreochromis niloticus* Linn.).** Presented at 32st Congress on Science and Technology of Thailand, 10-12 October 2006, Venue: Queen Sirikit National Center (QSNCC), Bangkok, Thailand. (Poster) (Sheet4)

Sheet 1

IC_{50} (□g/ml) values, were evaluated from a plot of percent cytotoxicity against the sample concentrations. DNA fragmentation were detected by agarose gel electrophoresis and ethidium bromide staining method. The essential oil samples that gave the highest cytotoxic activity (lowest IC_{50} values) on P388 and HeLa cell lines were Hairy Basil oil at 42.38 □g/ml and 80.0 □g/ml, respectively. Low molecular weight DNA fragmentation was observed at low concentration of 25 □g/ml of Hairy Basil and Sweet Basil oil on P388, but not on HeLa cell line. Further study of which mechanism involved the program cell death was needed. The results from this study can be applied for the development of these oil samples for pharmaceutical or nutraceutical products.

B0105-SOME MORPHOLOGICAL AND BIOLOGICAL CHARACTERISTICS OF *ACHLYA AMBISEXUALIS* ISOLATED FROM TILAPIA FRY, *OREOCHROMIS NILOTICUS* LINN

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Abstract: Infected tilapia fry (*Oreochromis niloticus* Linn.) with fungal hyphae covered on body surface were collected from a fish farm, Tambon Ta Pra, Muang District, Khon Kaen Province in November 2004. The objectives of the study were to isolate and identify the fungi from the infected fish and to study some biological characteristics of the fungi. The results showed that the hyphae were non-septate, stout with sharp tips. The typical zoosporangia were filiform and fusiform, and zoospore discharged was achlyoid type. The typical oogonia were spherical, oogonial wall were only pitted at the point of attachment of antheridial cells, and oogonial stalk was long and straight. Oospores were subcentric, antheridial branches were diclinous and typical gemmae were filiform. The morphological characteristics of the fungi were identified as *Achlya ambisexualis*. The optimum temperature for vegetative growth of *A. ambisexualis* was 15-35°C, with maximum growth at 35°C. The vegetative growth of *A. ambisexualis* was able to tolerate up to 10 ppt NaCl with maximum growth at 0-5 ppt NaCl.

B0106-EFFECT OF TESTOSTERONE ON VARIOUS BEHAVIORS OF ZEBRA DOVE *(Geopelia striata Linn.)*

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Abstract: The effect of testosterone on various behaviors of Zebra Dove with separate and group raising was studied during the morning and afternoon periods. It was found that testosterone had no effect on leg or wing stretching , scratching , feather licking and wing snapping. However, the hormone induced more crowing , more in the morning than in the afternoon. Individual or group raising also effected crowing. The bird in the group raising responded to testosterone better by causing the dove crowed more frequent and quicker than the bird in separate raising.

B0107-PURIFICATION AND CHARACTERIZATION OF SERINE HYDROXYMETHYLTRANSFERASE OF *PLASMODIUM VIVAX*

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Abstract: Serine hydroxymethyltransferase (SHMT), an enzyme in dTMP cycle, is a pyridoxal-5-phosphate (PLP) dependent enzyme catalyzing the reversible conversion of serine and tetrahydrofolate to glycine and 5, 10-methylenetetrahydrofolate. Because of its important role in cell survival, the enzyme has been proposed to be an antimalarial drug target. Our research group proposes to carry out the structure-function study of SHMT from a malarial parasite *Plasmodium vivax*. In order to obtain sufficient amount of enzyme for characterization, methodology in molecular biology to clone and express the gene encoding *pv*SHMT in *Escherichia coli* system was used. The recombinant enzyme has been purified to homogeneity by diethylaminoethyl (DEAE) anion exchange and hydroxyapatite chromatographies. The enzyme activity of SHMT has been determined by an enzyme coupling assay using Methylenetetrahydrofolate reductase (MTHFR) enzyme. The molar absorbance coefficient (ε) of enzyme-bound PLP is 8,075 $M^{-1}cm^{-1}$. Steady-state kinetic of the purified enzyme is being in progress.

Sheet 2

The First International Conference on Science and Technology for Sustainable Development of the Greater Mekong Sub-region, Khon Kaen, Thailand, 15-16 August 2006

Poster Presentation

CHARACTERISTICS OF *ACHLYA BISEXUALIS* ISOLATED FROM EGGS OF TILAPIA (*OREOCHROMIS NILOTICUS* LINN.)

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Fungal infected tilapia eggs (*Oreochromis niloticus* Linn.) were collected from fish hatcheries in Kalasin, Khon Kaen and Sakon Nakhon provinces. The sample collections were conducted in three periods, June-July, October-November 2005 and March-April 2006. Several fungal species have been isolated and identified from the infected eggs. Effects of temperature, pH and NaCl on mycelial growth were studied. Among fungal isolates, the species that had been frequently occurred is *Achlya bisexualis*. Biological characteristic examination of the fungal species showed that the optimum temperature and pH for mycelial growth were 15-35°C and 4-11, respectively. The vegetative growth of *A. bisexualis* was able to tolerate up to 15 ppt NaCl.

Keywords: *Achlya bisexualis*, tilapia eggs.

Sheet 3

ลักษณะทางชีววิทยาบางประการของราน้ำ *Achlya ambisexualis* ที่แยกจากไข่ปลานิล (*Oreochromis niloticus* Linn.)

Some biological characteristics of *Achlya ambisexualis* isolated from Tilapia Eggs (*Oreochromis niloticus* Linn.)

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Fungal infected tilapia eggs (Oreochromis niloticus Linn.) were collected from private fish farms (Kalasin, Khon Kaen and Sakon Nakhon provinces), an Inland Fisheries Station (Kalasin province) and Inland Fisheries Research and Development Centers (Khon Kaen, Mahasarakham and Sakon Nakhon provinces) during June-July, October-November 2005 and March-April 2006. Several fungal species have been isolated and identified. The effects of temperature, pH and NaCl on mycelial growth were studied. Achlya ambisexualis was isolated from fish hatcheries (Khon Kaen and Sakon Nakhon provinces). Biological characteristic examination of the fungus showed that the optimum temperature and pH for mycelial growth were 15-35°C and 4-11, respectively A. ambisexualis could grow on glucose yeast extract agar containing various concentrations of NaCl and it was able to tolerate up to 15 ppt NaCl.

Sheet 4

structure of elements were found. Two types of subsongs were found for contact and mobbing, and four types of calls for alert, alarm, excitement and begging.

B1_B0027 The Toxicity of Leave Crude Extracts from Neem Tree (*Azauirachta indica* Juss.) and Barlic (*Allium sativom* L.) on Mortality rate of Glogen Apple Snail (*Pomocea* sp.)

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Abstract: The Toxicity of Leave Crude Extracts from Neem Tree(Azauirachta indica Juss.) and Barlic(Allium sativom L.) on Mortality rate of Golden Apple Snail(Pomocea sp.) were studied. At concentration 50, 250, 500, 750 and 1,000 milligrams per liter were tested on Golden Apple Snail for 3 replications.High concentration of Neem Tree 1,000 milligrams per liter killed Golden Apple Snail 95.83% in 96 hours and high concentration of Barlic 1,000 milligrams per liter killed Golden Apple Snail 91.66% in 96 hours as compare to distill water control. Therefore, Neem Tree and Barlic are applicable for biological control of Golden Apple Snails.

B1_B0029 CHARACTERISTICS OF *SAPROLEGNIA DICLINA* ISOLATED FROM EGGS OF TILAPIA (*OREOCHROMIS NILOTICUS* LINN.)

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Abstract: Tilapia eggs (*Oreochromis niloticus* Linn.) with fungal hyphae were collected from Inland Fisheries Research and Development Center, Muang District, Khon Kaen province during June-July 2005. Fungal species have been isolated and identified. Biological characteristics such as effects of temperature, sodium chloride (NaCl) and pH on mycelial growth were studied. From the morphological study, the fungus was identified as *Saprolegnia diclina*. Biological characteristic examination of the fungal species showed that the optimum temperature for mycelial growth was 25°C and it was able tolerate up to 30 ppt NaCl. In addition, the vegetative growth of S. *diclina* could grow well at pH range of 7.0-9.0.

B1_B0034 ROLE OF 20-HYDROXYECDYSONE HORMONE ON THE DEVELOPMENT OF WING IMAGINAL DISKS IN *OMPHISA FUSCIDENTALIS* IN VITRO.

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Abstract: The final instar larvae of the bamboo borer (*Omphisa fuscidentalis* : Pyralidae ; Lepidoptera) are in a period of developmental arrest called larval diapause. The previous studies showed that topically applied 20-hydroxyecdysone (20E) induced pupation of the diapause larvae. Accordingly, we determined the effect of application 20E on the size of the wing imaginal disks and protein amounts in individual disks (in vitro). Culture of the disks in Grace's medium containing various amounts of 20E (0.05, 0.1, 0.25 and 0.5 µg/1 ml) revealed that 20E induced morphological changed of the disks. In conclusion, 20E is capable of inducing the development of disks both in vivo and in vitro.

B1_B0041 HORMONAL EFFECTS ON CELL CYCLE IN CULTURED WING IMAGINAL DISC OF SILKWORM, Bombyx mori.

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Abstract: This study was undertaken to find the factors which effect on wing imaginal disc differentiation in lepidopteran wing discs in vitro. Wing discs of silkworm were cultured in medium containing an optimal concentration of 20-hydroxyecdysone (20E) and/or bombyxin, FBS (fetal bovine serum) including the oxygen supply. After 24 h in culture, they were observed as S and M phase cell by immunocytochemistry and their numbers were counted by NIH image. The results showed that the number of S and M phase cells increased in the 20E alone and 20E with Bombyxin on the late fourth instar larvae and the highest increase on the early fifth instar larvae. FBS and oxygen supply also served to maintain the imaginal disc differentiation. These results suggest that the the number of S and M phase cells depend on the developmental stage and optimal concentration of 20E and bombyxin including growth factors can induce cell proliferation followed by imaginal differentiation.

VITAE

The author, Mr. Kwanprasert Panchai, was born on February 16, 1984 in Muang District, Sakon Nakhon Province, Thailand. He obtained a high school (Mathayomsuksa certificate) at Sakolrajwithayanukul School, Muang District, Sakon Nakhon Province during 1994-2000 and a Bachelor degree in Science (Biology) from Faculty of Science, Khon Kaen University during 2000-2004. He enrolled the Master of Science in Biology, Khon Kaen University during 2004-2007 and financial supported from Biodiversity Research and Training Program (BRT) grant No. BRT T_648003 during 2005-2006.