EFFECT OF TEMPERATURE, PH, SODIUM CHLORIDE AND ANTIBIOTICE ON THE GROWTH OF *SAPROLGNIA* SP. AND *ACHLYA* SP. ISOLATED FROM INFECTED COMMON CARP (*Cyprinus carpio* L.)

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ABSTRACT

***Saprolegnia* sp. and *Achlya* sp. were isolated from infected common carp (*Cyprinus carpio*). Effect of temperature, pH, sodium chloride and antibiotic on the mycelia growth of *Saprolegnia* sp. and *Achlya* sp. were studied. The optimum temperatures for mycelia growth of *Saprolegnia* sp. and *Achlya* sp. were 25° C and 25-30° C respectively. The optimum pH for mycelia growth was 7-9 in *Saprolegnia* sp. and 6-8 in *Achlya* sp. While, the grow ability of fungi in glucose yeast agar contained of sodium chloride showed that *Saprolegnia* sp. was able to tolerate up to 30-35 ppt but *Achlya* sp. was exhibited grow and tolerate up to 10-15 ppt. Sensitivity and survival were determined for antibiotics, out of the 14 antibiotics tested only nalidixic exerted a significant growth inhibiting effect. From this study we can suggest that effect of temperature, pH, sodium chloride and antibiotic on the growth of fungi were contrastly in *Saprolegnia* sp. and *Achlya* sp.**

KEYWORDS: *Saprolegnia sp., Achlya sp., sodium chloride, common carp (Cyprinus carpio), nalidixic exerted.*

INTRODUCTION

Saprolegniasis is a fungal disease of fishes and fish eggs caused by a member of the family Saprolegniaceae. *Saprolegnia* and *Achlya* were common fungal obtained from fishes and fish eggs (Paxton & Willoughby, 2000). Classification of fungi, in case of mycotic disease of fishes, is an important part of the diagnosis (Stueland *et al*., 2005). Identification of genus and species is difficult and mostly uncertain because fungal classification is bases on the life cycle, morphology of the hypha, morphology of the reproductive units and the type of spore produced (Hussein *et al*., 2001). However, biological characteristics, such as growth temperature and pH, have also been brought to an attention for species determination (Al-Niaeem & Al-Yassein, 2009). Moreover, biological difference may be resulted in difference degree of pathogenicity and host specificity(Kitancharoen & Hatai, 1996).
The objective of the present study is to investigate the effect of temperature, pH, sodium chloride and antibiotic on the growth of Saprolegnia and AchIya isolated from naturally infected common carp, which may offer some useful data supporting generic or specific determination of the fungi.

MATERIALS AND METHODS

Fungal isolation.

Saprolegnia sp. and *Achlya* sp. isolates used in this experiment were collected from infected common carp at fish pond of Basrah University during the period from October 2010 till February 2011. Fish with fungal mycelia covered were washed with sterilized tap water and incubated on glucose yeast (GY) agar (10 glucose, 0.25 % yeast extract, 1.5 % agar) (Nilubol *et al*., 2000) and incubated at 25° C. For inhibited of bacteria growth, addition of ampicillin and streptomycin sulphate into the medium was required. For morphological observation, a GY agar block cut from an actively grow thing edge of a colony was put into 20 ml of glucose yeast broth (1 % glucose, 0.25 % yeast extract) and incubated at 25° C for 24-48 hours to grow of mycelia. The mycelia were washed twice with sterilized tap water and kept at 10-25° C. The fungal isolates were identified according to Woo & Bruno (1999).

Effect of temperature on mycelia growth

The advancing edges of the fungal colonies cultured at 25° C for 2 days were cut with a no. 2 cork borer (5.5 mm in diam) and placed onto the center of 100x20 mm petri dish containing 20 ml of GY agar, then incubated at various temperatures between 10-40° C. the radial growth of the colonies was measured for 7 days.

Effect of pH on mycelia growth

GY broth was autoclaved, pH of GY broth was adjusted to various values in the range of 312 with 1N NaOH, 5 N NaOH or concentrate HCI, then GY broth was filtered through 0.2° m millipore filter paper. Then 10 ml of each pH level of GY broth was poured into small petri dishes. The agar blocks with mycelia of each isolate were cut off with a no. 2 cork borer, and placed into the petri dishes containing GY broth various pH levels. The dishes were incubated at 25° C for 3 days and the colony diameter at each pH of GY broth was measured.

Effect of NaCI on mycelia growth

An agar block of each isolate was placed on GY agar plates containing various concentrations of (0, 5, 10, 15, 20, 25, 30, 35, 40) ppt NaCI and incubated at 25° C for 7 days. The radius colony was measured.

Effect of antibiotic on mycelia growth

Sensitivity to antibiotic was examined using 14 antibiotic: nalidixic, fucidin, linkomycin, cloxacillin, nystasin, gentamycin, vancomycine, kanamycine, penicilln, streptomycine, tetracycline, neomycin, erythromycin, nitrofurantion. The surfaces of GY agar plates were inoculated and in each petri dish five discs of different antibiotics content were placed on the agar plate. After the colony had developed, the diameter of the inhibition zone was measured (incubated at 25° C for 7 days).

RESULTS

Effect of temperature on mycelia growth

Saprolegnia sp. was able to grow in the temperature range of 10-30° C, with maximal growth at 25° C. *Achlya* sp. showed similar growth at 15-30° C, with maximal growth at 25° C. *Saprolegnia* sp. and *Achlya* sp. showed growth and could survive with normal hyphae occurrence after day 7 (Fig. 1).

Effect of pH on mycelia growth

As showed in Fig. (2), *Saprolegnia* sp. and *Achlya* sp. were able to grow in GY broth in the pH range 4-11. The optimum pH for mycelia growth of *Saprolegnia* sp. and *Achlya* sp. were 7-9 and 6-8 respectively.

Effect of NaCI on mycelia growth

The *Saprolegnia* sp. was able to tolerate up to 30-35 ppt of NaCI and no grow was observed on the GY agar containing 40 ppt of NaCl whereas *Achlya* sp. was able to grow and tolerate to 10-15 ppt of NaCI and no grow was observed on the GY agar containing 20 ppt of NaCl (Fig. 3).

Effect of antibiotics on mycelia growth

On the basis of the 14 antibiotic tested, only nalidixic was significant inhibition against *Saprolegnia* sp. and Achlya sp. Tetracycline and its derivatives had smaller effect (Fig. 4).

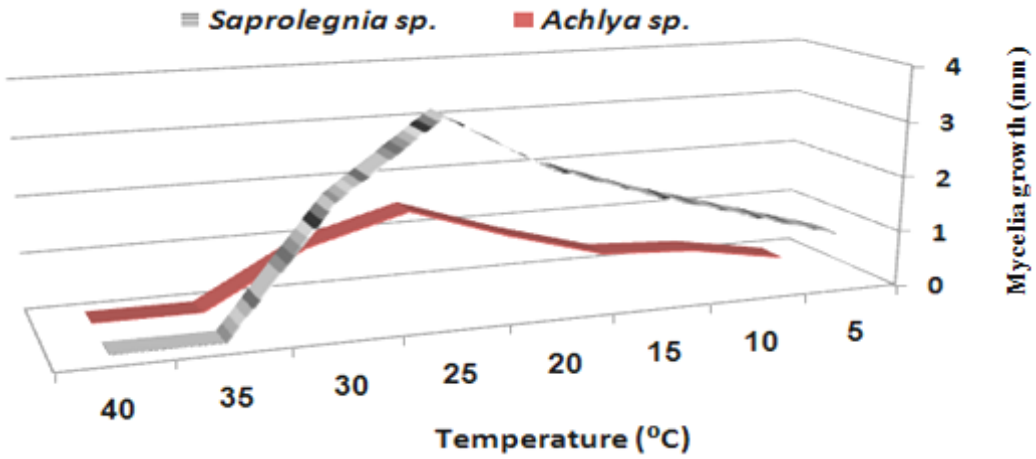

Figure (1): Effect of temperature on mycelia growth on *Saprolegnia* sp. and *Achlya* sp. for 7 day.

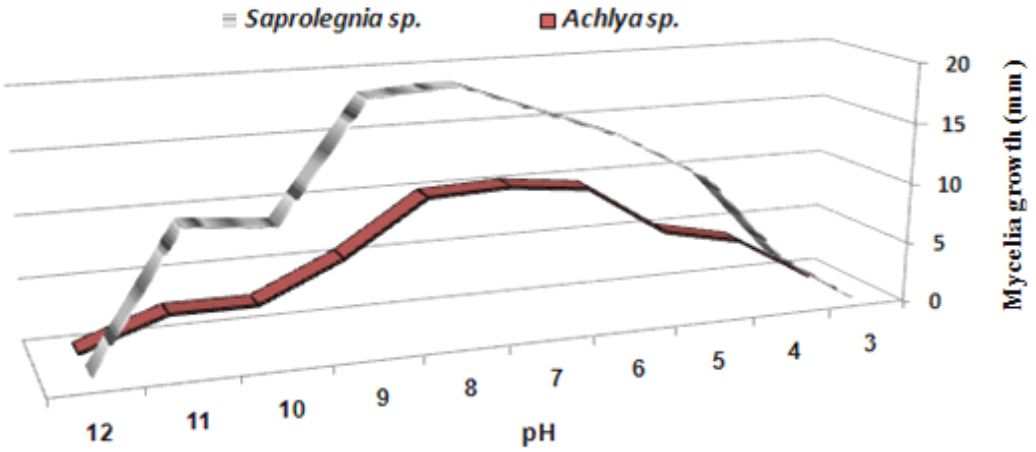

Figure (2): Effect of pH on mycelia growth on *Saprolegnia* sp. and *Achlya* sp. for 7 day.

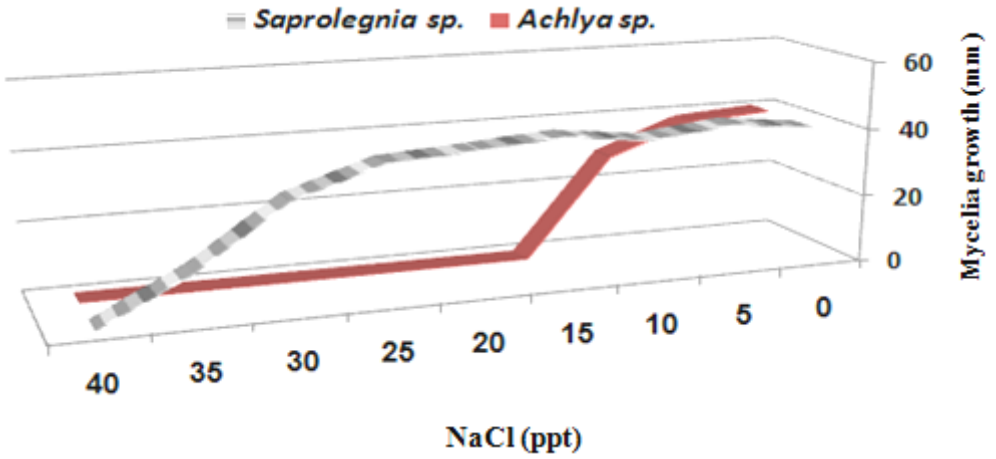

Figure (3): Effect of NaCl on mycelia growth on *Saprolegnia* sp. and *Achlya* sp. for 7 day.

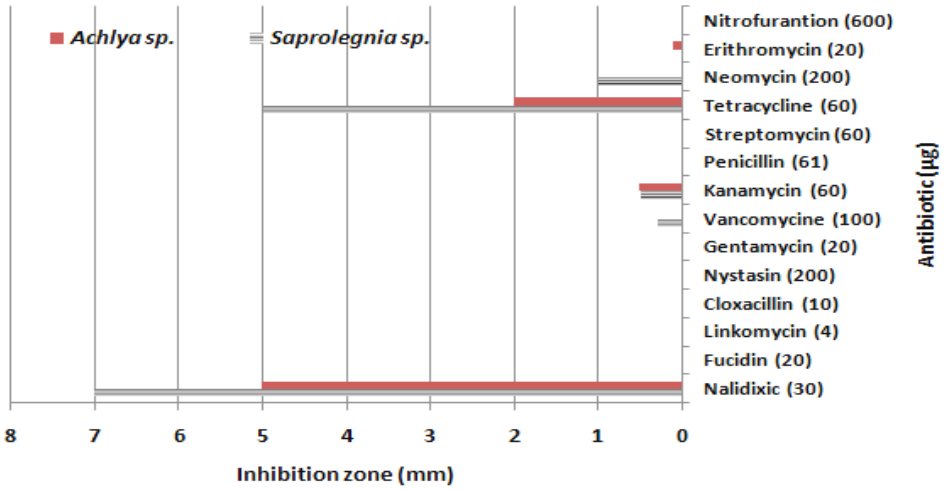

Figure (4): Effect of Antibiotic on mycelia growth on *Saprolegnia* sp. and *Achlya* sp. for 7 day.

DISCUSSION

It is generally known that fish diseases, like Saprolegniosis and Achlyosis, occur in cold water periods, primarily in early spring and a lesser extent in late autumn. Fungal attack and the subsequent development of powder-puff-like colonies on fish is also related in addition to the poorer condition of the fish and to mechanical injuries after wintering healthy fish are not usually infected by fungi.

Early observations on quantitative and seasonal occurrence of *Saprolegnia* sp. and *Achlya* sp. species reported maximum in early spring (Noga, 1993 and Anderson *et al*, 2006), but later investigators found a maximum in early spring and another in autumn (Quiniou *et al*., 1998; Bangyeekhun *et al*., 2003; Al-Niaeem, 2006). Winter and summer species, as well as species which occurred all year round, were distinguished on the basis of their quantitative distribution (Noga, 1993). We do not know, however, of any work in which the relationship between growth and temperature was examined, in- vitro with pure culture. Because of this, the temperature demands of the various species can be assessed only on the basis of their seasonal occurrences.

The present study, *Saprolegnia* sp. was able to grow at temperature range of 10-30°C, whereas *Achlya* sp. exhibited hyphal growth at a narrow temperature of 15-30°C, which is similar to Nilubol *et al*., (2000). The optimum temperature on maximal hyphae growth of *Saprolegnia* sp. and *Achlya* sp. were 25 and 25-30°C respectively (Robertson., 2006). The fungal disease in fishes caused by the *Saprolegnia* sp. and *Achlya* sp. tened to be seasonal occurance (Stueland *et al*., 2005). From the data obtained, it showed that either maximal mycelial growth production was occurred at 25°C for *Saprolegnia* sp. and 25-30°C for *Achlya* sp. which was corrected to the temperature of fish pond water that was usually around 25-30°C and never exceeded to 35°C.

That *Saprolegnia* sp. is more mesophilic in their temperature range (25-40°C), while the *Achlya* sp. is more thermophilic (more 40°C) (Yuasa & Hatai, 1995). Although the occurrences in early spring and late autumn, and the time of infection do not coincide with the optimal temperatures for growth, it is evident that it is only during these periods that, owing to their lower temperature demands. At higher water temperatures, the faster growing bacteria are always more successful in the competition for easily assailable organic materials, in poor supply in waters. *Saprolegnia* sp., on the other hand, can grow well at low a temperature and in early spring and appears in large numbers in the cold water, spores attached to injured fish, grow and develop their powder-puff-like colonies (Torto-Alalibo *et al*., 2005).

On the basis of occurrence data, it has been recognized by many authors that pH plays an important role in the growth and spread of aquatic fungi (Nilubol *et al*., 2000). Nevertheless, a survey of the

sporadic floristically data showed that different authors' data concerning optimum pH ranges of different species, assessed on the basis of their occurrences, did not agree (Torto-Alalibo *et al*., 2005).

According to Stueland *et al*. (2005) and Nilubol *et al*. (2000), the most suitable pH for *Saprolegnia* sp. tended to be weak acidic to weak alkaline for mycelia growth, whereas the optimum pH for *Achlya* sp. tended to weak acidic to weak alkaline for mycelia growth. The present study found that the optimum pH for mycelia growth of *Saprolegnia* sp. and *Achlya* sp. was neutral to weak alkaline conditions and weak acidic to weak alkaline conditions, respectively, which was similar to those reported by (Robertson, 2006; Nilubol *et al*., 2000). It may explain that the hypha growth of fungal taxonomic group is correlated with pH values.

In addition, it appeared that *Saprolegnia* sp. and *Achlya* sp. were able to grow on the GY agar containing 30-35 and 10-15 ppt NaCl, respectively. Khomvilai *et al*. (2006); Andrew & Phillips (2006) found that fungi in the genera *Saprolegnia* generally only grew in freshwater. It may be concluded that these.two genera of fungi were adapted to freshwater and *Achlya* sp. was more sensitive to NaCI than *Saprolegnia* sp.

On the basis of the 14 antibiotics tested, it can be stated that the majority is entirely ineffective against *Saprolegnia* sp. and *Achlya* sp. The scale of antibiotic sensitivity is arbitrary, since the smallest inhibition zone was taken into consideration (Fig. 4). According to that scale, significant inhibition was produced only by nalidixic, tetracycline and its derivatives had smaller effects. The 14 antibiotics examined are not suitable for therapeutic treatment (particularly as there are cheaper and more effective agents available) (Woo & Bruno, 1999).

REFERENCES

Al-Niaeem, K.S.K. (2006). Infection distribution of fish parasites in Basrah province and pathological effects of *Saprolegnia* sp. and its susceptibility to some plant extracts. Ph. D. Thesis Coll. Agric., Univ. Basrah: 172pp.

Al-Niaeem, K.S.K. & Al-Yassein, R. N. (2009). Treatment of Mosqutto fish (*Gaambusia affinis*) infected with fungi of the genus *Saprolegnia* by dipping with some plant extracts. *J. Coll. Basic Edu*., 15 (1): 899-910. (In Arabic).

Anderson, V.; Ssecombes, C. J.; Van West, P. (2006). Developing a vaccine against the fish pathogen *Saprolegnia parasitica* in salmonids. Retrieved May 4, 2008, from http://pmgn.vbi.vt.edu

Andrew, J.& Phillips, A. J. (2006). Molecular studies of the *Saprolegnia*-fish interaction. Retrieved February 9, 2009, from http://pmgn.vbi.vt.edu.

Bangyeekhun, E; Pylkkoe, P.; Vennerstroem, P.; Kuronen, H.; Cerenius, L. (2003). Prevalence of a single fish pathogenic *Saprolegnia* sp. clone in Finland and Sweden. *Dis. Aquat.* Org., 53: 47-53.

Hussein, M. M. A.; Hatai, K.; Nomura T. (2001). Saprolegniasis in salmonids and their eggs in Japan *J. Wildl. Dis*., 37: 204-207.

Kitancharoen, N. & Hatai, K. (1996). Experimental infection of Saprolgnia spp. in rainbow trout egg. *Fish Pathol*., 31: 49-50.

Khomvilai, C.; Kashiwagi, M.; Sangruang, C.; Yoshioka, M. (2006). Preventive efficacy of sodium hypochlorite against water mold infection on eggs of chum salmon *Oncorhynchus keta*. *Fish. Sci*., 72(1): 28-36.

Nilubol, Y.; Prapat, C.; Sanok. S. (2000). Study report, fungal disease in economically important fishes and disease prevention. Department of fisheries, Faculty of Agriculture, Khon Kaen. 25 pp.

Noga, E. J. (1993). Water mold infections of freshwater fish: Recent advances. *Ann. Rev. Fish Dis*., 3: 291-304.

Paxton, C. G. & Willoughby, L. G. (2000). Resistance of perch eggs to attack by aquatic fungi. *J. Fish Biol*., 57: 562-570.

Quiniou, S. M. A.; Bigler, S.; Clem, L. W.; Bly, J. E. (1998). Effects of water temperature on mucous cell distribution in channel catfish epidermis: A factor in winter Saprolegniasis. *Fish Shellfish Immunol*., 8(1): 1-11.

Robertson., J. E. (2006). Functional characterisation of a Kazal-like protease inhibitor from the fish pathogen *Saprolegnia parasitica.* Retrieved February 13, 2009, from http://pmgn.vbi.vt.edu

Stueland, S.; Hatai, K.; Skaar, I. (2005). Morphological and physiological characteristics of *Sarolegnia* spp. strains pathogenic to Atlantic salmon, *Salmo salar* L. *J. Fish Dis*., 28: 445-449.

Torto-Alalibo, T.; Tian, M.; Gajendran, K.; Mark, E. W; West, P. V.; Kamoun, S. (2005). Expressed sequence tags from the oomycete fish pathogen *Saprolegnia parasitica* reveal putative virulence factors. Retrieved August 4, 2010, from http://www. biomedcentral.com/ 1471-2180/5/46

Woo, P.T.K. & Bruno, D.W.(1999). Fish disease and disorder volume 3. Viral, Bacteria and Fungal infections. CABI Publishing. UK. 599-660.

Yuasa, K. & Hatai, K. (1995). Relationship between pathogenicity of *Saprolegnia* spp. isolates to rainbow trout and their biological characteristics. *Fish Pathol*., 30: 101-106.