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A STUDY OF THE PATHOGENICITY OF TWO AQUATIC FUNGI ON CARP EGGS

AL-REKABI, S.A.W. *, AL-JUBORI, S.S. AND SHNESHIL, S.S.

Department of Biology Al Mustansiriyah University, Baghdad College of Science.

Key words: Carp fish, Aquatic fungi, pathogenic fungi.

ABSTRACT

Oomycetes Fungi are real Aquatic Fungi, they are widely spread in Aquatic systems, specially fresh and brackish water, few are present in saline water they are of a great importance to the Aquatic system due to thier Saprophytic nutrition which decompose non-living plants and animal bodies in water, In addition to that many were found to be parasitic to Fish and their eggs.In this study two Oomycetes were isolated from the Tigris river in the area of Baghdad, the aim of the work was to investigate if these two fungi are pathogenic to *Cyprinus Carpio* (Carp Fish) eggs or not. Using different degrees of temperature and using different baiting techniques, it was found that non-living fish eggs gave earlier growth 24hrs. compared to *Sesamum indicum* (sesame) seeds which took 72hrs. to attract the zoospores and establish growth.Test of the pathogenisity was carried out using living eggs. It was found that the eggs were penetrated by and fully colonized with each fungi seperatly after 20 hrs. at 20^{o}C.Enzymatic tests confirmed the ability of booth of *S. turfosa* and *Achlya* spp. to produce lipase, lecithinase and proteinase in addition to parasitic nature of these fungi to fish & their eggs, this would be confirmed in a future study.

INTRODUCTION

Mastigomycotina are real aquatic fungi due to their formation of swimming spores (zoospores) which enable them to spread in water.Oomycetes ,a major class belongs to Mastigomycotina spreads mainly through rivers, less in lakes and rarely present in sea (Chauvet, 1992 and Czeczuga, 1994) Being, Saprophytic, Oomycetes are of great role in decomposing plants, and animals remains.In addition to that it was found recently that a large number of species are parasitic to fish, fish eggs; algae and crustacea (Alderman & Polyase, 1986; Czeczuga *et al*,1999; Czeczuga and Orolowska 2000).Furthermore many species are important plant pathogens.

The parasitism includes first the attraction of fungal zoospores to the host by the process of chemotaxis (Rand &Munden 1993), secondly the adhesion to the host by secreting glycoproteins (Sing & Bartinikia-Garcia 1975), third the germination of the encysted spores and penetration of the host which includes secretion of enzymes (Peterson *et al*, 1997).

Czeczuga & Muszynska (1999) investigated the pathogenicity of many genera belonging to the family Saprolegniaceae (Oomycetes) towards 33 genera of fish belongs to the family Cyprinidae, it was found that the species *Saprolegnia terresteris, S.crustosa & Achlya megasperma* infected only *Cyprinus carpio* (Carp fish), on the other hand, *S.turfosa* infected the eggs of *Gobio albipinnatus* . The exoenzymes secreted by the fungi are important for both of nutrition and pathogenecity, because it enables the hyphae of fungi to pentrate the host (Hube *et al*, 1994).

This study was conducted to understand if the fungi isolated from Dijla river is pathogenic to carp eggs and if the

*Corresponding author

temperature is affecting this process. In addition, to that the enzymes secreted by these fungi are screened.

MATERIALS & METHODS

Fungal Cultures:

Water samples were collected from Dijala-river (Shawakka) in Baghdad region from three different sites in dark bottles.Baiting technique was used to isolate fungi from the water samples (Jones, 1971), one ml of chloramphenicol ($^{mg}/_{ml}$) was added to the culture to avoid bacterial contamination, Hemp seeds were replaced by Seseme seeds (Al-Rekabi *et al*, 1996), cultures were incubated at 20°C.

Water cultures were examined every 24hours for the growth of Aseptate hyphae and later on for the production Sporangia and Oogonia to be able to identify of growth according to Seymour(1970) and Cooker(1965), pure cultures were prepared by transferring a single Hypha into sterilized glass petridishes containing Corn Meal Agar (CMA).Stock cultures were maintained after Dick (1965).

Pathogenicity:

Mature living Carp Fish were obtained from the market, dissected rapidly, the eggs were removed into sterilized physiological saline then 6-8 eggs were separated gently, transferred into petridish containing 10ml of sterilized distilled water and Seseme seeds with a grown colony of the fungus.

To asses the effect of temperature on the infection of eggs the cultures were incubated at (15, 20, 25, 30) °C; three replicates for each treatment was prepared.

Enzymes Test:

The ability to produce Lipase was examined on Tween 80 according to Cowan (1986).Lecithinase production was tested on Lecithovitelline Agar according to Blazevic&Grace, (1984), production of proteinase was carried out on Milk Agar Media Cowan(1986), Chitinase examined on Chitine Agar Media.

RESULTS AND DISCUSSION

Cultures:

Different genera of Oomycetes were isolated.The most abundant were *Saprolegnia turfosa* and asexual *Achlya* spp. Figs 1, 2, 3 show two fungi were chosen to carry out this study.

Pathogenicity:

It was found that *S. turfosa* infected Carp eggs after 20hrs. at 15°C Fig. (4) and that is faster than *Achlya* spp which took 24hrs. to infect the eggs at the same Temp., this was also true at 20°C which indicated that *S. turfosa* more pathogenic to Carp eggs than *Achlya* spp. is in agreement with Czeczuga and Muszynska (1999) who found that *S. turfosa* is pathogenic to Carp fish and its eggs among other fish.

At 25°C the infection with *S. trufosa* took 26hrs., while *Achlya* spp. needed 30hrs. to infect eggs, this difference of the time of infection might be due to the difference in the time needed for germination of Zoospores. However, at 30°C there was delay in the time of infection which reached 24hrs. for both fungi which means that this temperature is much higher than the optimum for both fungi.

Enzymes Test:

The test for Lipase secretions was positive for both fungi Fig. (5), this explains the ability of both to penetrate the lipids in the fish body.

Proteinase was also produced by the two fungi.As Fig. (7) indicates this support the ability to infect the fish eggs which containes high protein content, this was pointed out by St-Leger *et al* (1997) which found that this enzyme is necessary to make the fungus able of attaching fish and their eggs.

The ability to produce Chitinase was positive for both fungi Fig. (8), but the result

delayed till the 5th day because the fungi utilized the simpler nutrients (Carbon source) then when it was exhausted it attacks Chitine which is a complex source of Carbon, the ability to produce Chitinase was also proved by Anderson, (2001).

The above work indicated the pathogenicity of the isolates of *S. turfosa* and *Achlya* spp isolated from Dijala river towards Carp eggs, however *S. turfosa* was faster, temperature plate an important rule in this process which is achieved by the presence of different enzymes in these fungi.

Fig. (6) shows that both genera produce Lecithinase, this is shown by the dark zone surrounding the colonies.

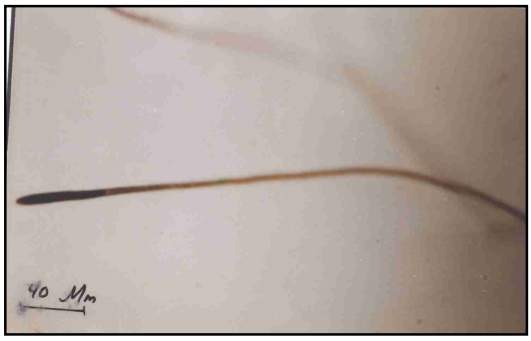


Fig.1: Sporangium of *S.turfosa*

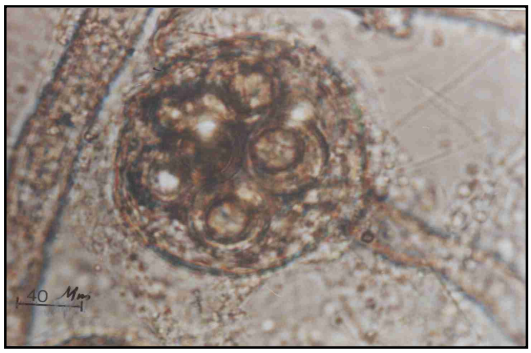


Fig.2: Oogonium of *S.turfosa*

Fig.3: Spore ball of *Achlya* spp.

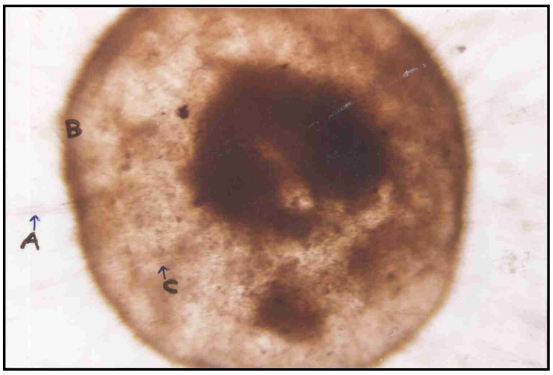


Fig. 4: Carp egg infected with *S. turfosa* A,C, Fungal hyphae. B, Egg wall.

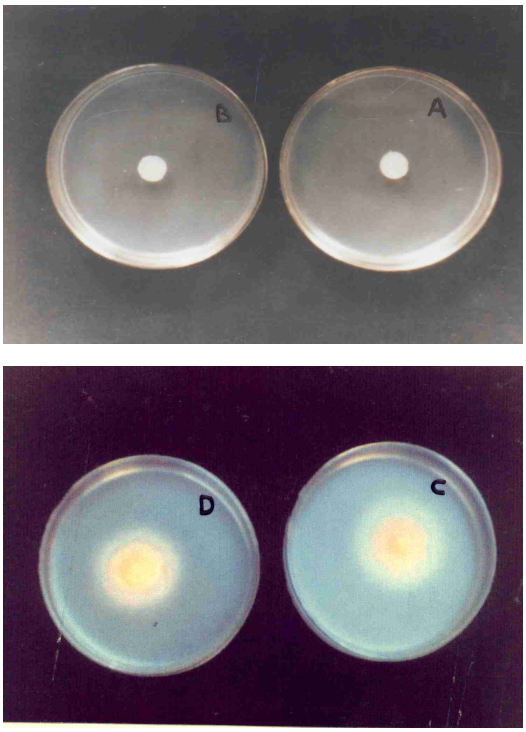


Fig. 5: Test for Lipase production

A- *S. turfosa*
C- *S. turfosa* after five days

B- *Achlya* spp.
D- *Achlya* spp. After five days

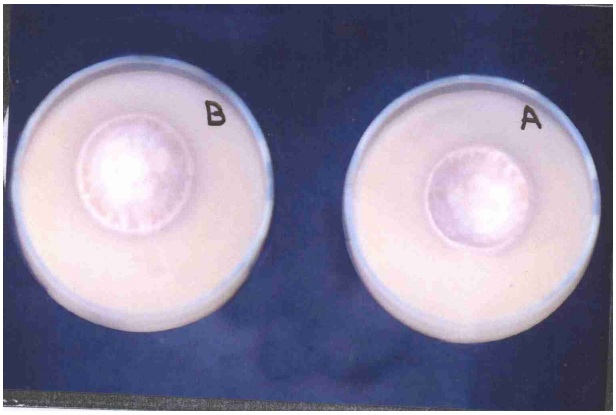
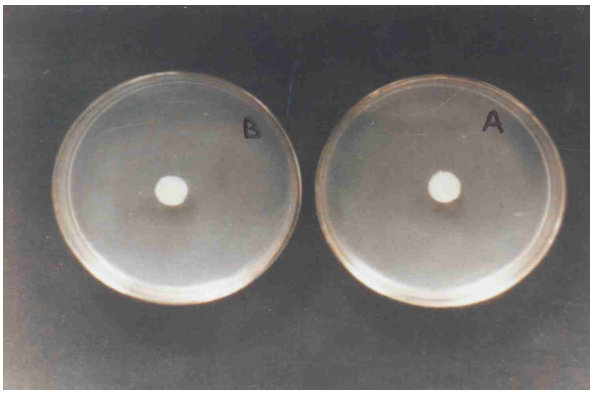


Fig. 6: Positive results for Lecithinase
A- *S. turfosa*
B- *Achlya* spp.



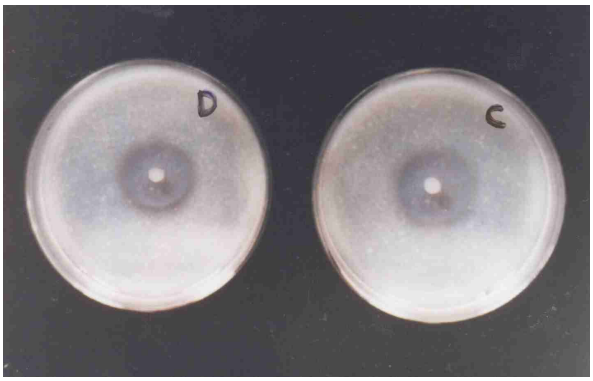


Fig.7: Test for Proteinase of:
A- ***S. turfosa***
B- ***Achlya*** spp.
C- Positive result of A
D- Positive result of B

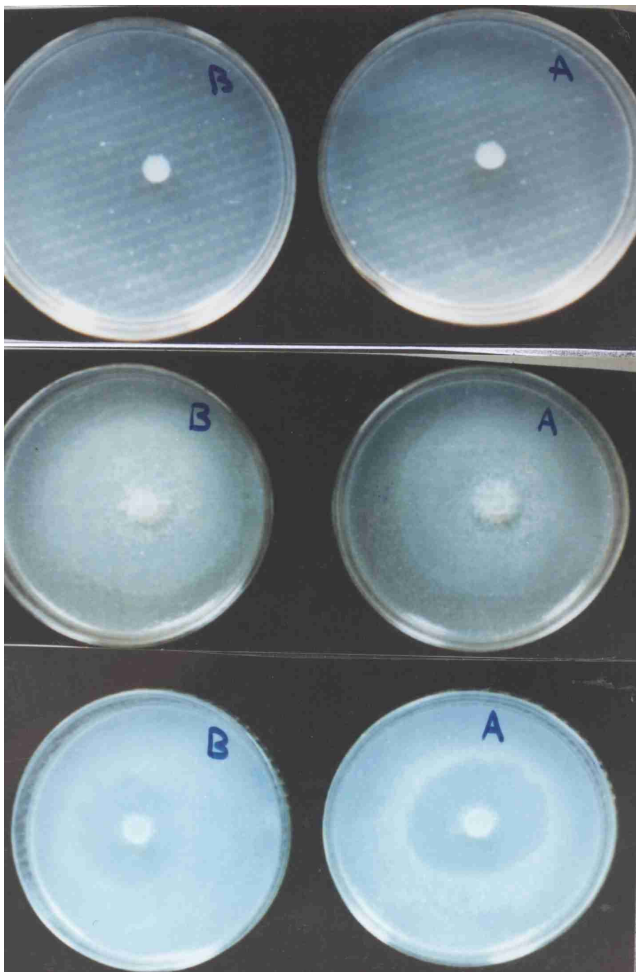


Fig. 8: Test for Chitinase for

B- *S. turfosa*

C- *Achlya* spp.

1. Before Incubation
2. Negative result after five days
3. Positive result after eight days

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