Fungal contamination in rainbow trout eggs in Kermanshah province propagations with emphasis on Saprolegniaceae

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Received: May 2009 Accepted: July 2009

Abstract

Fungal infection in the eggs of freshwater fish is well known as a problematic disease. The aim of the present study was to isolate and identify parasitic and saprophytic fungi from affected eggs of rainbow trout at two fish hatchery in Kermanshah province. The sample were inoculated in culture media (SDA,CMA,GPA and stilled water with cotton seed culture) at room temperature (18-24°C).17 species of fungi isolated from the fungal eggs. Five fungi species that isolated in this study were belonged to the saprolegniaceae family including *Saprolegnia paraitica, Saprolegnia lapponica ,Saprolegnia ferax, Saprolegnia hypogyna* and *Saprolegnia diclina*. Another fungi that isolated in this study were, *Fusarium oxyparum*, *Fusarium npoa*, *Fusarium* sp., *Penicilium citrinium, Penicillium expansum, Aspergillus treuse, Aspergillus clavatus, Cladosporium* sp., *Alternaria* sp., *Helmintosporium* sp., *Pscilomyces* sp., Mocur. It seems that *Saprolegnia parasitica* with 26.8 percent of isolation was the most important fungal infestation of egg in kermanshah trout hatcheries. Mocur with 19.6% had the most frequency after *S. parasitic* and *Fusarium, Aspergillus* and *Pescillomyces* with 2.45% had the lowest frequency. In this study *S. ferax*, *S. hypogyna* and *S. diclina* are reported from Iran for the first time.

Keywords: *Saprolegnia*, Rainbow trout egg, Fungal infestation

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[DOR: 20.1001.1.15622916.2010.9.1.15.7]

Introduction

Over the past ten years, aquaculture has increased on average by 11% per year. Production has increased from 13 million tons of fish in year 1990 to 37.9 million tons in 2001 and 51.65 million tons in 2006 (West, 2006). The majority of global production (58%) comes from freshwater aquaculture (West, 2006). Aquaculture especially rainbow trout fish farming has been developed in Iran and fish culture now is becoming an economically important industry. Water mold infections cause losses of freshwater fish and their eggs in both nature and commercial fish farms (Bangyeekhun *et al*., 2001). Unfertilized fish eggs are susceptible to fungal infection particularly from the family Saprolegniaceae. During egg incubation, these fungi produce mycelia which grow and spread from the nonviable to the healthy eggs suffocating them and causing mortality (Mousavi, 2009). During recent years decreasing of rainbow trout eggs in propagation center of Iran have become common and evidences show that about half of the produced egg loses due to fungal infection (Ebrahimzadeh, 2006). Oomycets contain some of the most devastating pathogens of animals. Taxonomically Oomycetes are divided into three subclasses: Saprolegniomycetidae, Rhiomycetidae and Peronospormycetidae. Most fish and animal pathogen Oomycetides belong to the Saprolegniomycetidae which has two order: Saprolegniale and Leptomitales (West, 2006). Saproleniaceae genera particularly Saprolegnia and Achlya are generally opportunitic pathogen (Bruno & Wood, 1994) but some strains can be virulent and able to cause primary infection in fish and their eggs (Willoughby & Pickering, 1997). Identification of *Saprolegnia* species classically is based on morphology of the reproductive structures, i.e. antheridia, oogonia and oospores (Willoughby, 1978; Neish & Hughes, 1980). There are some reports about fungi isolation from fish and fish eggs from Iran (Ghiassi, 2007; Dakhili, 2009; Kazemi, 2009; Bozorgnia, 2009).

In this survey, for the first time, isolation of fungi along with emphasis of morphological characteristics of Saprolegniaceae from eggs of salmonid hatcheries in Kermanshah province was studied.

Materials and methods

400 infected rainbow trout eggs were collected from 2 hatcheries with temperature between 10.4-11.8°C and pH 7.5-7.8 in Kermanshah province, west of Iran during winter of 2008. Fungus-contaminated eggs were collected by sterile forceps and transferred to screw capped bottle contained sterilized tap water (STW). In laboratory the sample were washed 3 times with sterile distilled water and were placed in each egg sterilized Petri dishes containing three halves of sterilized cottonseeds and sterilized tap water, STW (volume 40ml) at then incubated at 18-24°C for 8 hours under natural light. From the growth fungi microscopic slide were taken and examined under compound microscope. In order to obtain sexual organs, some hyphae were aseptically taken out with the help of sterile

[DOR: 20.1001.1.15622916.2010.9.1.15.7]

needles and transferred to GPA (glucose peptone agar) containing 250µgr/ml penicillin and 250µgr/ml chloramphenicol for prevention of bacterial contamination at 18˚C for at least 48-72 hours (Willoughby *et al.*, 1984). All morphological characteristics measurements and observation under microscopic study were done. For isolation of another saprophytic fungi, Sabourodexteros agar (SDA) and Corn Meal Agar (CMA) media were used. Culture media after inoculation, incubated at 18-24˚C for 24-48 hours and from colonies wet smears were done with used from lactophenol methylen blue microscopic study were done. The identification were followed by methods described by Coker & Matthews (1937), Johnson (1956), Seymour (1970), Beakes *et al*. (1994) and Khulbe (2001). In table 2 some hatcheries condition during sampling were shown. Water temperature, salinity and pH were measured digitally (Multi 340i-WTW) and eggs number, infested eggs percent and infested broodstock percent were obtained manually (observation).

Results

Based on fungal morphological characterists 17 species of fungus were isolated from the infected eggs including *Penicillium citrinum, Penicillium expansum, fusarium poae, Fusarium oxysporum, Fusarium* sp. *Aspergillus clavatuse, Aspergillus treuse*, *Cladosporium* sp., *Alternaria* sp., *Helmintosporium* sp., *Pcscilomyces* sp. and *mocur* sp. (Table 1). In saprolegniaceae 5 isolated species were *S. parasitica, S. lapponica*, *S. diclina, S*. hypogyna and *Saprolenia ferax*. The morphological characteristics of the isolated Saprolegnia species are as fallow:

Withish cotton–like colonies were observed which stout hyphe especially in place of hyphe adhesion to clavate zoosporangia on GPagar. After 18 days of culture in STW on the cotton seeds at room temperature, sexual structures were formed. Lateral oogonia and spherical moderately thick, 35-45µm in diameter was observed. Antheridia did not develop in the entire culture (Fig. 1). Cotton-like whitish colony, hyphae slender, aseptate and cylindrical zoosporangia were formed on GPA. Sexual structure was formed after 8 days of culture on GPagar with cottonseeds. Terminal pyriform oogonia with centric oospore (45-70µm in diameter). Anthridia were present diclinous and laterally appresssed to the oogonial wall (Fig. 2). Whitish cotton-like colonies on GPagar, abundant cylindrical zoosporangia, terminal spherical and cylindrical oogonia (90-110µm in diameter) were formed on main hyphae. Anthridia (arrow) was observed (diclinous) (Fig. 3).

Whitish cotton-like colonies on GPA and CMA (Fig. 4), hyphae moderately stout, aseptate branched (30-75µm in diameter) were observed. Zoosporangium were abundant with different shape (cylindrical, pyriform and the other) sexual structure were not observed on cottonseeds culture in STW, gemmae abundant, variable in shape, spherical and pyriforme or irregular (Fig. 5). Hyphae slender, aseptate and branched, zoosporangia filiform or clavate; oogonia terminal, lateral spherical or pyriform (50-55µm in diameter) and oospores centric (Fig. 6).

[DOR: 20.1001.1.15622916.2010.9.1.15.7]

Figure1: Oogonia of *Saprolegnia lapponica* (100x)

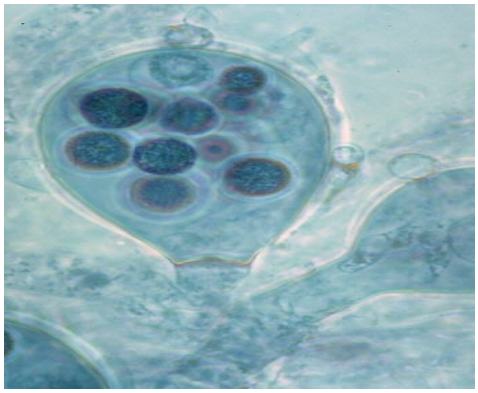

Figure 2: Oogonia of *Saprolegnia diclina* (100x)

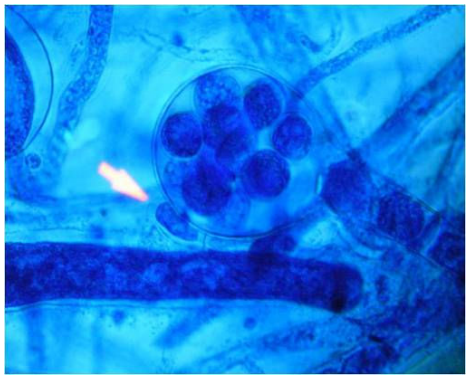

Figure 3: Oogonia of *Saprolegnia ferax* (100x)

Figure 4: Oogonia of *Saprolegnia hypogyna* (100x)

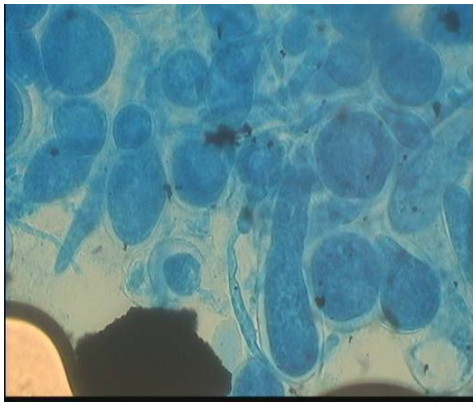

Figure 5: Pyriform zoosporangium of *S. parasitica* (40x)

Figure 6: Cotton like colony of *S. parasitica*

[DOR: 20.1001.1.15622916.2010.9.1.15.7]

Table 1: Absolute and relative frequency of isolated fungi from rainbow trout eggs

Fungi	Farm 1	Farm 2	frequency	Percentage
S. parasitica	7	4	11	26.8
S. lapponica	1	-	1	2.45
S. diclina	1	-	1	2.45
S. ferax	1	-	1	2.45
S. hypogyna	1	-	1	2.45
Fusarium oxyparum	1	1	2	4.89
Fusarium poa	-	1	1	2.45
Fusarium sp.	-	1	1	2.45
Penicilium citrinium	2	-	2	4.89
Penicillium expansum	-	2	2	4.89
Aspergillus treuse	-	1	1	2.45
Aspergillus clavatus	-	1	1	2.45
Cladosporium	3	2	5	12.2
Alternaria	1	-	1	2.45
Helmintosporium	1	-	1	2.45
Pscilomyces	1	-	1	2.45
Mocur	5	3	8	19.6
Absolute frequency	25	16	41	100
Relative frequency	61	39	100	100

Table 2: Some hatchery conditions during sampling

Broodstock age (year)	3-5
Crowding of eggs (Number/cm^2)	49- 65
Water temperature (°C)	10.4-11.5
Salinity	0.4-0.6
pH	7.5-7.8
Eggs infestation (%)	20-42
Broodstock infestation (%)	30-45
Water resource	Spring & river

[DOR: 20.1001.1.15622916.2010.9.1.15.7]

Discussion

In saprolegniacae family 5 isolates were *S. parasitica, S. laponica, S. diclina, S. hypogyna and S. ferax.* In the study in cultivated cyprinids in Iran, thirty-nine species of fungi were isolated from the gill lesions. The most important pathogenic fungi were *Branchiomyces*, *Saprolegnia*, *Fusarium*, *Phoma and Exophiala*. The most frequency isolated fungi were Fusarium (46.4%) and Saprolegnia (25%), whereas Exophiala (3.6%) was shown the lowest frequency (Firouzbakhsh *et al*., 2005). In this study, like previous ones, the highest rate of infestation was related to *Saprolegnia* genus with 36.6% frequency. Among different isolates from eggs, *S. parasitica* was the most important with 26.8% frequency. It has been established that the greatest losses on fish eggs, however, are due to *saprolegnia* species (Willoghby, 1970; Czeczuga & Kiziewicz, 1999 ; Hussein *et al*., 2001). *Saprolegnia ferax* was reported from different places under various ecological condition in the world (Khulbe, 2001). In this study *S. ferax*, *S. hypogyna* and *S. diclina* were reported from Iran for the first time. *S. frax* cause great losses in acipenserid hatcheries in Russia (Lartseva, 1986). Kitanchareon (1997) reported *S. ferax* and *S. hypogyna* in aquatic system and on infected salmonid fish and fish eggs from Japan. Ghiassi *et al.* (2007) reported *S. parasitica* from *Rutillus frisii kutum* in hatcheries in Mazandaran province, Iran. Kazemi (2009) reported five species of fungi were isolated from the *Acipenser persicus* larva that were included of *Alternaria* spp., *Cladosporium* spp., *paecilomyces* spp., *penicillium* spp. and *chrysosporium* spp.

In cases that Fusarium mostly were isolated, lower saprolegnia infestation were observed. The same result was reported by Ebrahimzadeh in rainbow trout propagation in Mazandaran province (Ebrahimzadeh *et al*., 2007). In the study by Ebrahimzadeh (2007) in Mazandaran province, twelve species of fungi isolated from the fungal salmonid eggs. Three of the isolated fungi were belonged to the saprolegniaceas' family including: *Saprolegnia parasitica*, *Saprolegnia* sp., *Achlya* sp. Other nine recognized fungi were: *Penicillium, Aspergillus, Paeciliomyces, Acremonium, Fusarium oxysporum, F. solani, Alternaria, Mucor* and *Helminthosporium.* In this study three species of pathogenic fungi in aquatic were isolated, including:

Saprolegnia parasitica, *Saprolegnia* sp., *Achlya* sp. It suggested that *saprolegnia parasitica* with 13.18% of isolation was the most important of fungal egg infestation Mazandaran salmonid hatcheries.

Mueller in a same study showed that in water culture media *Fusarium* prevents growth of *Saprolegnia* (Pickering *et al*., 1994). Totally in this study, 17 fungi species were isolated respectively with higher infestation by Saprolegnia. There were some noticeable differences between studied hatcheries. Frequency of fungal infestation in farm 1 and 2 were 61 and 39% respectively that the same ratio (2/1) was shown macroscopically. It can be

[DOR: 20.1001.1.15622916.2010.9.1.15.7]

concluded that collecting fungal eggs (can prevent from spread of contamination to healthy eggs), inhibiting high density of eggs in incubators, maintaining physical and chemical quality of water, brood stock age and health are the main factors to control fungal diseases in fish farms. Ecological differences resulting from different hatcheries conditions (chemical factors, age of bloodstock, eggs crowded, ect) may have played a role of the fungi that developed on rainbow trout eggs in the present study. Although environmental variables were not studied directly, they are known to influence the growth, reproduction and intensity of aquatic fungal infection (Richards & Pickering, 1978; Willoughby, 1994). Alabi (1971) observed that the occurrence of Saprolegniaceae correlated with some parameters of water (i.e., temperature, pH, ionic concentration and organic content). There is no doubt that ecological differences play an important role in the species diversity of the fungi that develop on both fish and eggs (Willoughby, 1986; Hussein *et al*., 2001). There are some report that showed water quality, crowded hatchery conditions, pollution and water temperature changes can causes of Saprolegniasis in fish and fish eggs (Pickering, 1994; Bruno, 1999; Snisezco, 1974; Beakes *et al.,* 1994). Higher infestation in farm 2 might be related to lower age of brood stocks but there was not any relation with infestation in eggs. It is note worthy that in farm 2 all infected eggs were collected daily but in farm 1 it was done once a week. So by keeping in mind that *Saprolegnia* genus was the most important one it can be concluded that Saprolegniacea is the pioneer in affecting rain bow trout eggs.

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[DOR: 20.1001.1.15622916.2010.9.1.15.7]

مطالعه آلودگیهای قارچی در تخمهای قزل‌آلای رنگین کمان مراکز تکثیر استان کرمانشاه با تاکید بر ساپرولگنیاسه

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تاریخ دریافت: اردیبهشت ۱۳۸۸ تاریخ پذیرش: تیر ۱۳۸۸

چکیده

عفونت‌های قارچی بعنوان یکی از بیماری‌های مهم در تخم ماهیان آب شیرین بخوبی شناخته شده است. هدف از این مطالعه جداسازی و شناسایی قارچهای ساپروفیت از تخمهای آلوده قزل‌آلای رنگین کمان در دو مرکز تکثیر استان کرمانشاه می‌باشد. در این بررسی از ۴۰۰ تخم قارچ زده نمونه‌برداری انجام شد و در محیط کشتهای سابورو دکستروز آگار، گلوکز پپتون آگار و کورن میل آگار به همراه پنبه دانه در دمای محیط (۲۴-۱۸ درجه سانتی‌گراد)کشت داده شد. در این مطالعه ۱۷ نوع قارچ براساس مشخصات ریخت‌شناسی جداسازی گردید که ۵ نوع آن متعلق به خانواده ساپرولگنیاسه‌آ بودند و عبارتند از: ساپرولگنیا پارازیتیکا، ساپرولگنیا لاپونیکا، ساپرولگنیا هایپوجینا، ساپرولگنیا دیکلینا و ساپرولگنیا فراکس. در این مطالعه دیگر قارچهای جدا شده عبارت بودند از: فوزاریوم اکسیسپاروم، فوزاریوم پوا، گونه فوزاریوم، پنی سیلیوم سنتریتوم، پنی‌سیلیوم اکسپانسوم، آسپرژیلوس ترئوس، آسپرژیلوس کلاواتوس، کلادوسپوریوم، آلترناریا، هلمینتوسپوریوم، پسیلومایسس و موکور. بنظر می‌رسد گونه ساپرولگنیا پارازیتیکا با درصد آلودگی ۲۶/۸ درصد مهمترین قارچ در تخمهای قارچ زده قزل‌آلای رنگین کمان در مراکز تکثیر استان کرمانشاه می‌باشد. موکور با ۱۹/۶ درصد بعد از ساپرولگنیا پارازیتیکا بیشترین موارد جداسازی و قارچهای فوزاریوم، آسپرژیلوس و پسیلومایسس با ۲/۴۵ درصد کمترین فراوانی را داشتند.

در این مطالعه برای اولین بار قارچهای ساپرولگنیا هایپوجینا، ساپرولگنیا دیکلینا و ساپرولگنیا فراکس از ایران گزارش می‌گردد.

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[DOR: 20.1001.1.15622916.2010.9.1.15.7]

کلمات کلیدی : ساپرولگنیا، تخم قزل‌آلای رنگین‌کمان، عفونت قارچی

[DOR: 20.1001.1.15622916.2010.9.1.15.7]