Original Article

Investigation and Analysis of Fungi Causing Cutaneous Infections in Fish Farms in Eastern Mazandaran Province

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A B S T R A C T

The genus Saprolegnia is one of the most important pathogenic aquatic fungi in farmed and wild fish. In this paper, 100 samples were prepared from 10 fish ponds in Sari, Neka and Behshahr. After cultivation, purification and macroscopic and microscopic examinations, we were able to isolate the fungi Saprolegnia parasitka, Saprolegnia and Aclia. Their prevalence was 48% Parasitica, 38% Saprolegnia, 36% Aclias and 15% other fungi, respectively. Their frequencies were 48 Parasitica, 38 Saprolegnia, 36 Aclias and 15 other fungi, respectively. Also, in the study of other parameters and their effect on the presence of Saprolegnia, it was observed that the ambient temperature of the water was suitable for the growth of this fungus and also the pH and salinity of the water also affected the presence of Saprolegnia.

Introduction

From all the various issues and problems that are always faced by human beings, it can be said that nutrition has always been his most important intellectual concern and among various foods, protein is more needed by human beings than other substances [1]. Today, the increase in population and the need to provide the protein needed by human societies, has stimulated the development of various systems for raising livestock, poultry, and aquatic animals. In this regard, the aquaculture sector is one of the most basic sectors that has been widely developed, but it should be noted that many factors will play a role in the development of this industry, one of which is to reduce fish disease and prevent it [2-5]. The possibility of infectious diseases can be minimized by choosing a suitable site, observing the desired density, proper transportation, and handling. In general, there are two categories of diseases in cage farming systems, the first of which is the disease that affects fish when they are selected for cage farming and storage, before being transferred to cages., and are the second category of diseases that occur either due to unfavourable living conditions or due to structural defects in

the cage. Due to the fact that disease control is very difficult in farming systems with water flow (sea currents) and the elimination of pathogens in the water column is impossible, epidemiological studies are of particular importance in reducing the incidence of disease and mortality [6-8]. Today, in many parts of the world, more than 130 species of fish and about 12 species of shrimp are bred in enclosed environments. In Iran, since 1993, fish farming has started in enclosed environments. The purpose of fish farming in cages is a part of seawater, mirage, water behind the dam, etc., which is surrounded by different tools such as nets with different springs around the floor, and in that enclosed environment, fish are raised [9-12]. Fish growth and the factors that affect the growth process are of paramount importance to the fish farmer. Because maximum growth of fish in a minimum period of time with a minimum amount of food is his main goal. Fish are cold-blooded animals and do not have to expend energy to maintain their body temperature. In this regard, a fish is more efficient and talented than a warm-blooded animal in terms of converting food into body protein [13-16]. Feeding the fish the energy needed by the fish comes from food; however, this energy is originally produced from the sun. Solar energy is converted into food by plants that use energy to make carbohydrates. Animals eat these plants and use the stored energy to carry out their activities. Therefore, plants are known as the primary producers of food, which is converted into other forms and then given to the fish [17]. Fungi can be distinguished from algae due to the absence of chlorophyll. The lack of chloroplasts in fungi prevents them from producing the energy they need through photosynthesis. Therefore, to survive, they have to live a parasitic or saprophytic life. The number of fungi that are considered as fish parasites is very small, but some fungi still do not have a specific category. Most of them are easily divided into two groups: Those with transverse walls, which are called transverse wall fungi and those that do not have a transverse wall, which are called transverse wall fungi [18-20].

In this paper, an attempt has been made to determine the fungi that cause skin infections in fish farms in eastern Mazandaran province (Sari Neka, Behshahr). Also, the causes of pollution have been be determined by pool, city, and its type [21].

Time and Place of Study

This study was performed in the Rakhsh laboratory of Behshahr city. The research population was 100 farmed salmon from 10 pounds in the east of Mazandaran province in the spring and winter seasons

Preparation of Sabrodextrose Agar Medium Containing Chloramphenicol

64 g of the powder was weighed with a scale and poured into a 2000 ml Erlenmeyer flask and 1000 ml of distilled water was added to it. It was then placed on the flame to dissolve well. After dissolving, it was transferred to an autoclave to be sterilized. To prevent bacterial growth in the medium, 50 mg of chloramphenicol powder was weighed and dissolved in 10 ml of alcohol and sterilized with a 0.22-micron needle filter. After completing the autoclave, the culture medium was removed and placed in the medium until the temperature reached about 45 °C, so that no burning was felt when handling it. At this time, Sterilized chloramphenicol solution was added to it. And then the good environment was shaken to be fully distributed throughout the environment. Then, under sterile conditions and next to the gas lamp flame, the culture media were divided into disposable plates. After closing the culture medium in the plates, three of them were transferred to the incubator to ensure that they were sterile; the rest were stored in the refrigerator.

Preparation of Peptone Agar Glucose Culture Medium

70 grams of this powder in one litter of cold distilled water was suspend, then heated to boiling point and sterilized by autoclave at 121 °C for 15 minutes.

Sampling

This study was performed by random sampling of farmed fish before and after transfer to the breeding cage on a monthly basis. 10 samples were taken from each pool.

After recording the specifications, the samples were transferred to the laboratory. In the laboratory, under sterile conditions, a piece of skin and gills (1 cm²) was removed with a sterile scalpel and scissors and washed 3 to 5 times with sterile distilled water.

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Figure 1 Sampling of pool fish

Cultivation of Samples

Samples prepared under sterile conditions were cultured under the hood, by the flame, and by sterile loop on plates containing

Sabrodextrose agar and Peptone agar glucose media. The plates containing the samples were then incubated for 25 hours at 25 °C to grow. To purify, the grown fungi were transferred to secondary environments.

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Figure 2 Incubators for sample growth

Microscopic Observation of Samples

After spore formation, the sample was fixed with a drop of methyl alcohol and stained with Lactophenol Catechin blue dye. Also, after pouring a drop of sterile water on the slide, along with the flame and under the hood, with the help of a sterile ounce, some samples were taken from the plates containing the samples and transferred to the water next to the flame. The samples were studied in terms of

transverse wall, sexual organ structure, spore size, and arrangement, etc.

Macroscopic Observation of Samples

Examination of 100 suspected specimens of farmed fish in terms of appearance such as skin injury, bleeding and abnormal conditions, especially in the tray fins and gills were examined. Also, the culture media after the growth of the prepared samples were

examined. The fungi were examined for color, shape, growth, etc.

Results of Microscopic Examination of Samples

In macroscopic examinations of the samples, fungal contamination was reported in all 10 pools. Also, out of 100 suspicious fish samples, all of them were detected as skin infected.

Saprolegnia Parasitica was grown on peptone agar glucose medium as a white cotton. After about a week, the whole culture medium was filled at room temperature. Saprolegnia grew on cotton in peptone agar glucose medium. Aclia swelled on peptone glucose agar medium and turned white, which filled the entire culture medium after about 5 days.
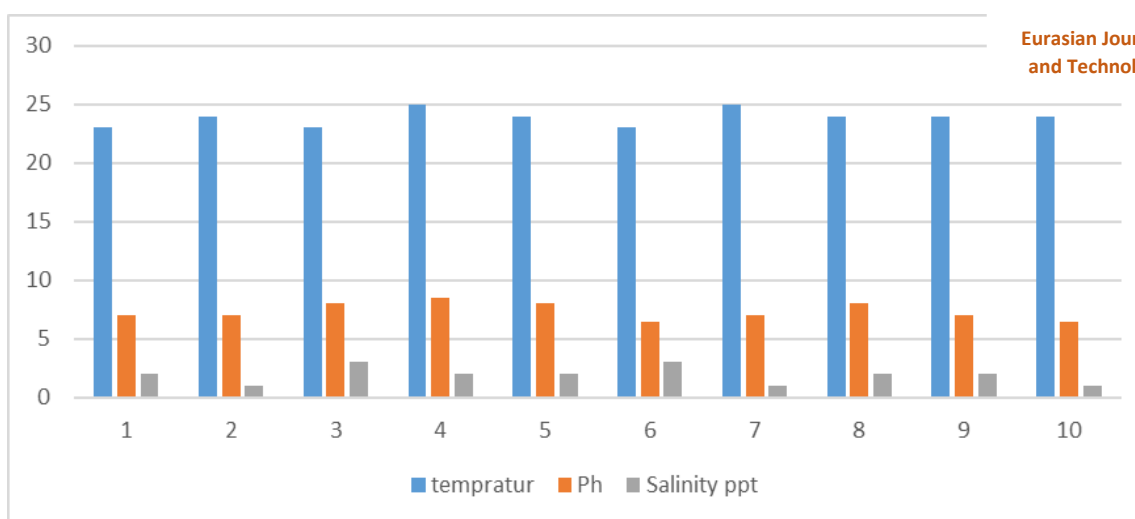
Figure 3 Microscopic observation of Saprolegnia

Table 1 Physical and chemical parameters of pool sets

Pool set (10 samples from each pool)	Salinity PPT	PH	Temperature
1	2	7	23
2	1	7	24
3	3	8	23
4	2	8.5	25
5	2	0.8	24
6	3	6.5	23
7	1	7	25
8	2	8	24
9	2	7	24
10	1	6.5	24

Figure 4 Diagram of physical and chemical parameters of pool sets

Table 2 Number and frequency of fungal contamination of pool complexes

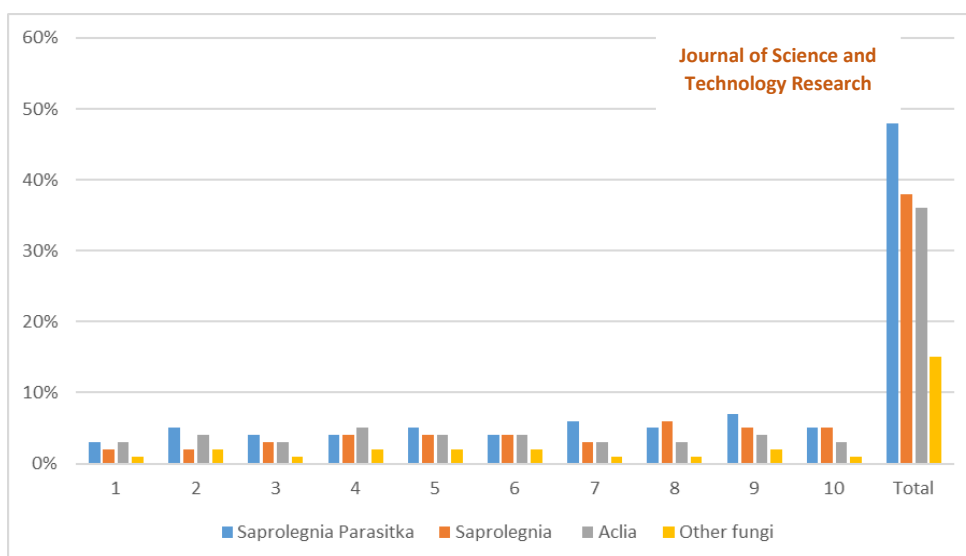| sample	Other fungi		Aclia		Saprolegnia		Saprolegnia Parasitka	
	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number
1	1%	1	3%	3	2%	2	3%	3
2	2%	2	4%	4	2%	2	5%	5
3	1%	1	3%	3	3%	3	4%	4
4	2%	2	5%	5	4%	4	4%	4
5	2%	2	4%	4	4%	4	5%	5
6	2%	2	4%	4	4%	4	4%	4
7	1%	1	3%	3	3%	3	6%	6
8	1%	1	3%	3	6%	6	5%	5
9	2%	2	4%	4	5%	5	7%	7
10	1%	1	3%	3	5%	5	5%	5
Total	15%	15	36%	36	38%	38	48%	48

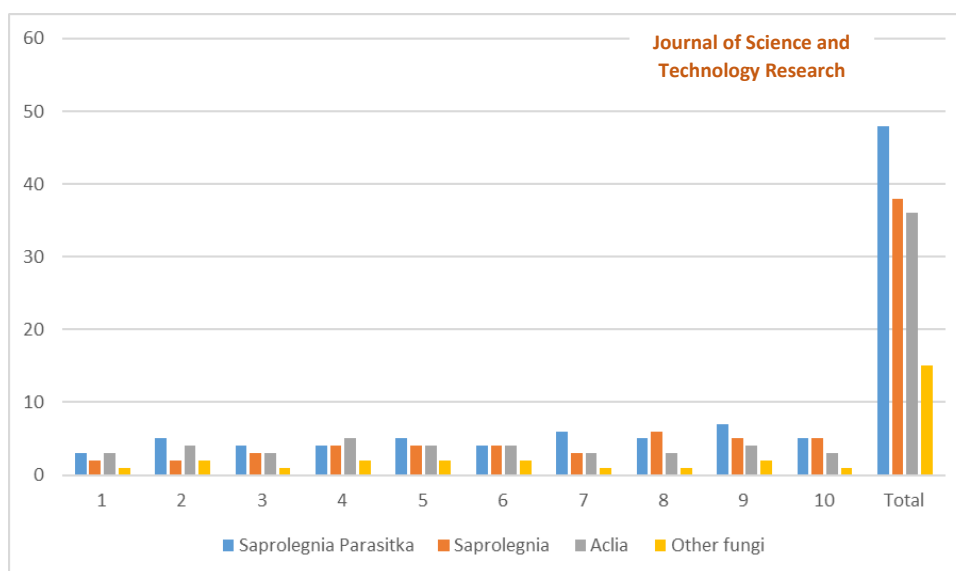**Figure 5** Percentage of fungal contamination of pool complexes

Figure 6 Diagram of the frequency of fungal contamination of pool complexes

Table 3 Number and frequency of fungal infections in fish breeding ponds by cities

Sample	Other fungi		Aclia		Saprolegnia		Saprolegnia Parasitka	
	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number
Sari (40 samples)	7%	7	16%	16	15%	15	%19	19
Neka (30 samples)	4%	4	10%	10	16%	16	17%	17
Behshahr (30 samples)	4%	4	10%	10	7%	7	12%	12
Total	%15	15	%36	36	%38	38	%48	48
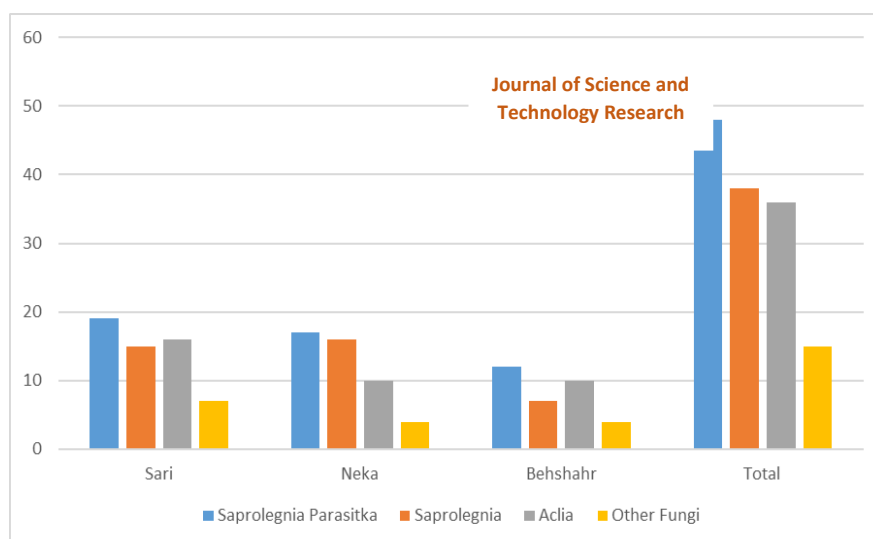
Figure 7 Frequency chart of fungal infections of fish breeding ponds by cities

After observing the skin and eye infections and detecting the appearance of the fish, they were examined for the identification of Saprolegnia and their isolation. As can be seen in the reports, the highest rate of infection was related to Sapr*olegnia Parasitka. Saprolegnia* and Aclia species showed the highest levels of infection, respectively. Considering the sizes of different environmental parameters that were done at the time of sampling, it can be concluded that salinity and pH have a direct effect on the amount of pollution in the collection. The incidence of Parasitica is 48%, Saprolegnia 38% and Aclia 36%. Also, at neutral pH, the level of contamination was higher.

Conclusion

Fungi are of special importance in fish health and should be considered as a key factor in determining the health status of industrial and ornamental fish farms. The number of fungi that are parasites of fish is very small. Most fish fungi are opportunistic and are considered as secondary pathogens. Numerous factors can cause fungal infections, and this highlights the importance of studying the fungal flora and identifying pathogenic fungi, as well as identifying treatment methods and prevention. Infectious and fungal diseases are among the problems of the aquaculture industry, which with the increase of dense aquaculture causes a lot of damage to producers. Aquatic fungi are among the harmful factors to the aquaculture industry. Fish skin is an organ to fight against pathogens and parasites. There is no cuticle in fish, which is compensated for by the secretion of mucus from the cells of the epithelial tissue. Fish skin differs significantly from other vertebrates on its surface, where living epidermal cells are in contact with the water environment and have no cuticle secretion but contain mucus.

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