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Isolation and Identification of Fungal Species Associated with the Skin and Gills of Siganus rivulatus (Forsskal, 1775)

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عزل وتحديد أنواع الفطريات المرتبطة بجلد وخياشيم سمكة البطاطا البيضاء (فورسكال، 1775)

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Abstract:
This study aimed to isolate and identify fungal species associated with the skin and gills of Siganus rivulatus (Forsskal, 1775) collected from the Qaminis region, west of Benghazi, Libya. Thirty (n = 30) specimens were examined to determine the prevalence and distribution of fungal infections. Samples were cultured on Sabouraud Dextrose Agar (SDA), and fungal isolates were identified based on morphological and microscopic characteristics. Fungal growth was detected in 39.31% of samples, with Aspergillus and Penicillium spp. representing the predominant genera. Skin infections (ecto-fungi) accounted for 67.2% of isolates, while gill infections (endo- fungi) represented 32.8%. Aspergillus niger was the most prevalent species, followed by Penicillium sp. The findings suggest that S. rivulatus may serve as a potential host for opportunistic fungal pathogens, highlighting the importance of routine mycological surveillance in marine and aquaculture environments.

Keywords: Siganus rivulatus, Fungal isolation, Aspergillus niger, Penicillium spp, Aquatic Mycology, Libya.

الملخص
هدفت هذه الدراسة إلى عزل وتحديد أنواع الفطريات المرتبطة بجلد وخياشيم سمكة البطاطا البيضاء (فورسكال، 1775) التي جُمعت من منطقة قمينس، غرب بنغازي، ليبيا. تم فحص ثلاثين عينة (ن = 30) لتحديد مدى انتشار وتوزيع العدوى الفطرية. زُرعت العينات على وسط أجار سابورو ديكستروز (SDA) ، وتم تحديد الفطريات المعزولة بناءً على خصائصها المورفولوجية والمجهرية. تم الكشف عن نمو فطري في 39.31% من العينات، حيث مثلت أنواع الاسبيرجيلس والبنسيلين الأجناس السائدة. شكلت التهابات الجلد (الفطريات الخارجية) 67.2% من الفطريات المعزولة، بينما مثلت التهابات الخياشيم (الفطريات الداخلية) 32.8%. كان الاسبيرجيلس النوع الأكثر انتشارًا، يليه البنسيلين. تشير النتائج إلى أن سمكة

S. rivulatus قد تكون مضيفًا محتملاً لمسببات الأمراض الفطرية الانتهازية، مما يسلط الضوء على أهمية المراقبة الفطرية الروتينية في البيئات البحرية والاستزراع المائي.

الكلمات المفتاحية: سمكة البطاطا البيضاء، عزل فطريات، فطر الاسبيرجيلس، فطر البنسيلين، علم الفطريات المائية، ليبيا.

Introduction:

Fungal infections are among the most significant causes of economic loss in aquaculture, second only to bacterial diseases [1]. Diseases are the primary cause of loss of revenue in the aquaculture industry, and throughout the past 20 years, fungal infections have grown in significance. In aquaculture, fungus spores are the main cause of infection. Virulence factors are necessary for a fungal pathogen to be able to cause infections. The vulnerable host can be harmed by fungus virulence agents such as glycosyl hydrolase, protease, subtilizing, kinases, and kinase inhibitors.

Nevertheless, there is still a lack of comprehensive information regarding the onset of illnesses linked to fungi [2]. Numerous fish species are susceptible to fungal infections, which can be lethal if left untreated. As a result, aquaculture bio-security programs that address aquatic animal diseases and pathogens have gained significant attention from the aquaculture sector. Around the globe, profitable and environmentally conscious aquaculture industries have been compromised by disease outbreaks [3]. The majority of fungus that have been shown to cause clinical illness in fish belong to the kingdoms of Eumycota, Protoctista, and Chromista (real fungi).

Previously classified under the kingdom Protoctista, oomycetes are now regarded as belonging to the new kingdom Chromista (Stramenopiles), which also includes brown algae, kelps, and diatoms [4]. Organisms near the animal/fungal evolutionary boundary make up the class Mesomycetozoea, another new taxonomic group in the kingdom Protista [5]. Among these "fungi" are Ichthyophonus hoferi, Dermocystidium, and the rosette agent, which was recently suggested to be a species of Sphaerothecum destruens, gen. and species nov [6].

Common fungal diseases of fish:

Aquatic fungi are commonly thought of as secondary tissue invaders that follow infectious pathogens, traumatic injuries, or environmental factors like low quality of water or low temperatures. Because they consume decaying waste products, fungus is prevalent in aquatic settings. The following table shows the most important fungal disease that affect fish and their important characteristics:

Table 1: The most important fungal disease that affect fish and their important characteristics.

Fish Fungal Diseases	Characteristics	Aqueous Medium
The water molds	The mycelium is the filamentous network of cells (hyphae) that have a cottony appearance.	Freshwater and brackish water
Saprolegniasis	Usually, cottony white, gray, or brown symptoms are seen in saprolegniasis. Lumps that are red or greenish on fish gills or skin. The fungi may initially just affect tiny portions of the body, but they can quickly expand and cover the majority of it. Although an event may be acute, its persistence is frequently persistent.	freshwater and brackish water
Aphanomyces invadans	The main cause of ulcerative disease epizootics in both wild and cultivated fish worldwide, it causes hemorrhages, ascites, and scale loss. Its zoospores infect fish via breaches in the epidermal barrier, where after the hyphae invade the skin, musculature and internal organs.	Brackish water
Branchiomycosis	known as gill rot, and it is believed that the fungus spreads via the digestive tract or damaged gills. flourish in organic matter that has decomposed. Mortalities from this disease may be as high as 50%, and it causes respiratory issues and gill tissue destruction.	Freshwater
Ichthyophoniasis	Unpredictable swimming patterns, enlarged bellies, anemia, and appetite loss are all signs of infection in fish. White nodules are a common internal symptom indicating swelling of important organs such as the liver, kidneys, and spleen. The afflicted fish's muscles also exhibit these whitish nodules.	Sea and freshwater

Material and methods:

The Sample of Study:

The fish chosen for this study, Siganus rivulatus, belongs to the genus Siganus. It is a ray-finned fish belonging to the Siganidae family that is sociable and mostly herbivorous. The Mediterranean Sea

was colonized by Lessepsian migration through the Suez Canal from its natural range, which includes the western Indian Ocean and the Red Sea as shown in Figure 1.

Figure 1: Siganus rivulatus

Sample collection:

Thirty fresh specimens (n = 30) of S. rivulatus with an average length of 18.5 cm and average weight of 110 g were collected from the fish market in Benghazi city, which was caught in the Qaminis area located west of Benghazi. Samples were collected in a sterile manner, Samples were transported to the laboratory under refrigerated conditions (4 °C) and processed within 24 hours. Each fish was first disinfected with a cotton swab dipped in 70% ethyl alcohol, then skin scrapings and gill swabs were collected by lifting the operculum covering the gills and wrapping a sterile swab around it. The skin scrapings and gill swabs were collected on sterile, labeled Petri dishes.

Fungi Isolation:

Equipment:

Water bath, petroleum jelly, sterile Pasteur pipettes, toothpicks, sterile Petri dishes, filter paper, forceps, inoculating loop and needle, sterile U-shaped bent glass rods, a thermometer, a dissecting microscope, a Bunsen burner, concave glass slides, coverslips, and a beaker filled with 95% ethyl alcohol.

Procedure:

Sabouraud Dextrose Agar (SDA) supplemented with chloramphenicol (0.05 g/L) to inhibit bacterial growth were grown for seven to ten days at 25°C, and the fungal growth was measured. Morphological and microscopic traits were analyzed to identify the fungal isolates according to (Dugan) [7]. The Cappuccino and Sherman method was applied in order to detect positive fungal colonies under a microscope [8]. The microscopic characteristics of fungi, such as conidia arrangements, hyphae, and conidial heads, were observed. To determine the type of growth, each mold plate was examined under a dissecting microscope at both low and high power.

Statistical analysis:

A non-parametric rank test (the Wilcoxon signed-rank test) has been used to analyze and compare the results of the two samples.

Results and discussion:

39.31% Fungal growth was detected in 27 of the samples in this investigation. (Table 1), where there was growth on all plates except samples numbered 7, 24, and 29, which showed no growth. Even though fungi have been isolated from these fish, there are no ulcers or other disease-related symptoms on their bodies, which seem healthy maybe their excellent immune system works hard to keep these invaders from injuring their tissues and muscles. and thus the appearance of signs of the disease, this is consistent with a study conducted by Afzali et al. in 2013 [9].

The proportion of ecto (skin fungus) was greater than that of endo (gill fungi), 67.2 and 32.8, respectively, which is consistent with the study conducted by Solomon and Olawale [10]. investigation.61 fungal strains from two genera, Aspergillus niger and Penicillium sp and genera with the highest infection rate were Aspergillus then Penicillium. The ratios of Exo (skin) to Endo (gills) were 0.71, 0.35 for Aspergillus and 0.69, 0.31 for Penicillium sp. Our findings are in agreement with previously reported data. [11], [12], [13], [14], [15] that Aspergillus niger represents the most common Aspergillus spp isolated.

Analysis of the results using Wilkinson's test showed that there is a statistically significant difference between the two variables at the 0.05 significance level; this reflects a significant difference in measurement between the two studied samples. This finding underscores the need to interpret the difference in the biological variables under investigation as presented in Figures 2,3,4, and 5.

Table 2: The number of fungi per colony –forming unit in 1 ml

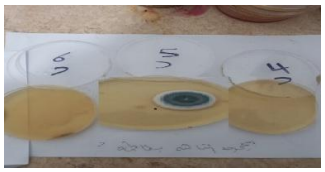
Sample number (SN).	Number of fungi per colony – forming unit in 1 ml		Total (No.)	Percentage (%)
	Exo (skin)	Endo (gills)		

1	2	No growth	2	3.28
2	2	No growth	2	3.28
3	4	No growth	4	6.55
4	No growth	No growth	-	-
5	1	No growth	1	1.63
6	1	No growth	1	1.63
7	No growth	No growth	-	-
8	1	1	2	3.28
9	4	2	6	9.83
10	4	No growth	4	6.55
11	1	1	2	3.28
12	3	1	4	6.55
13	No growth	1	1	1.63
14	1	1	2	3.28
15	No growth	1	1	1.63
16	No growth	1	1	1.63
17	No growth	2	2	3.28
18	1	1	2	3.28
19	No growth	1	1	1.63
20	1	No growth	1	1.63
21	1	1	2	3.28
22	3	No growth	3	4.91
23	No growth	1	1	1.63
24	No growth	No growth	-	-
25	2	1	3	4.91
26	2	No growth	2	3.28
27	5	2	7	11.47
28	No growth	1	1	1.63
29	No growth	No growth	-	-
30	2	1	3	4.91
Total	41	20	61	39.31
	67.2%	32.8%		

Table 3: The number and Percentage of fungi isolated from Siganus rivulatus

Isolates	Exo (skin)	Endo (gills)	Total (No.)	Percentage (%)	
Aspergillus niger	32	16	45	0.71	0.35
Penicillium sp	9	4	13	0.69	0.31

Table 4: Macroscopic characteristics of fungal strains isolated from Siganus rivulatus

Genera	characteristics	Colony morphology
Penicillium	Usually green, the colony is fluffy and powdery. The backside is dark or colorless.	
Aspergillus niger	Colonies are initially white, then yellow, and finally have a grainy, black front. The colonies are colorless to pale yellow on the back.	

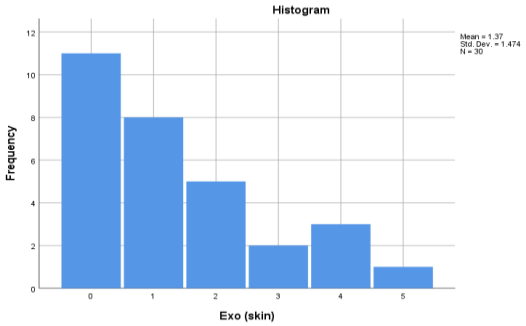


Figure 2: The number of fungi isolated from skin

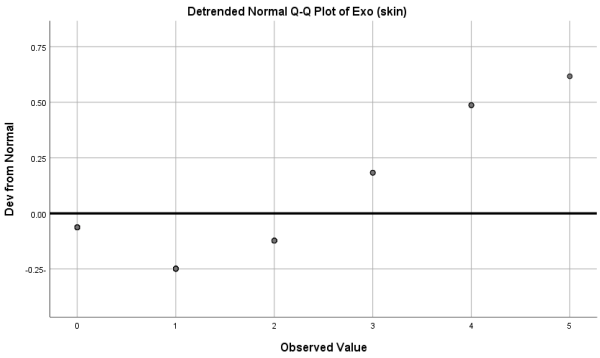


Figure 3: The observed fungi from skin.

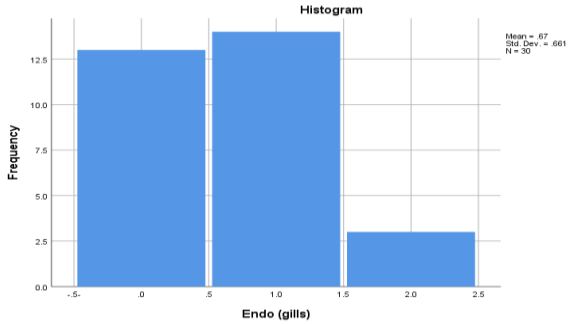


Figure 4: The number of fungi isolated from gills.

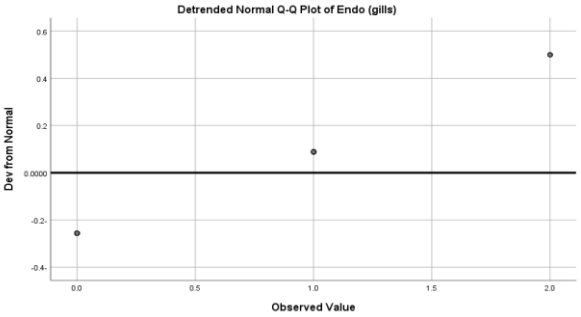


Figure 5: The observed fungi from gills.

Conclusion:

This study confirms the occurrence of Aspergillus niger and Penicillium spp. in the skin and gills of Siganus rivulatus from the Qaminis region. Although no visible disease symptoms were observed, the presence of mycotoxin-producing fungi poses potential health risks to fish and consumers. Regular monitoring and hygienic handling practices are recommended to mitigate fungal contamination in marine environments. Further studies should investigate seasonal variability and antifungal susceptibility to support aquaculture biosecurity programs.

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