Mycobiota of Sparids *Diplodus sargus*, (Linnceus, 1758) from Northwestern Libya

[1]Al-Masri, T.A, [1]Sarah A. Benzeglam, and [1]Abdulmaula Hamza
[1]Biology Department, Faculty of Education-Tripoli, University of Tripoli, Tripoli, Libya
Corresponding author: ta.elmasri@uot.edu.ly

Article history
Received: Dec 12, 2023 Accepted: Dec 15, 2023

المستخلص:

تمت دراسة الفطريات المرتبطة بسمكة الجراجوز (الدنيس الابيض) *Diplodus sargus* الغير مصابة ظاهرياً والمجمعة من سوق الحوت وسوق ميناء طرابلس البحري بميناء طرابلس البحري. تم فحص 54 سمكة وبعد عملية العزل والفحص المجهري تم تعريف الفطريات باستخدام المفاتيح الخاصة بتعريف الفطريات. تم عزل 12جنسا فطري منها *Aspergillus* sp و *Saccharomyces* spp لكونها أكثر الفطريات المعزولة وعزلت من جميع العينات . يليها فطر *Penicillium* sp أظهر معدل انتشار أقل 96.3% في حين أن فطر *Fusarium* sp و *Leptosphaeria* sp. تم تحديدها بشكل أقل. نظراً لعدم وجود أوساط غذائية خاصة تم تعريف الفطريات على مستوى الجنس فقط. يوفر هذا البحث رؤى قيمة حول الفطريات المرتبطة بسمكة الدنيس الأبيض ويسلط الضوء على ضرورة إجراء المزيد من الدراسات لتحديد الفطريات على مستوى النوع وفهم الآثار البيئية المحتملة.

الكلمات المفتاحية: الفطريات، الدنيس الابيض، ليبيا.

A B S T R A C T:

The mycobiota associated with apparently non-infected White Seabream *Diplodus sargus* from Tripoli waters was investigated. A total of 54 fish were examined macroscopically and microscopically using standard fungal identification keys. Analysis revealed the presence of 12 fungal genera, with *Aspergillus* and *Saccharomyces* spp. being the most commonly isolated, and detected in all specimens. *Penicillium* sp. exhibited a prevalence rate of 96.3%, while *Fusarium* sp. and *Leptosphaeria* sp. were identified to a lesser extent. Due to the absence of specific standard media for species identification, the collected fungi were grouped as "genre Spp." This research provides valuable insights into the mycobiota associated with White Seabream and highlights the need for further studies to enhance species-level identification and understand the potential ecological implications.

Keywords: Fungi, *Diplodus sargus*, Libya.

Introduction:

The vast expanse of the aquatic realm, encompassing a myriad of marine, brackish, and freshwater ecosystems, harbors a diverse array of life, including a remarkable assemblage of fungal species. While freshwater fungi have been extensively studied [1], their marine counterparts remain largely unknown, their diversity and ecological roles are still unexplored [2]. In particular, the fungal mycobiota associated with marine fishes with limited information available, despite the potential impact of these fungi on fish health and marine aquaculture activities [3].

Fungi, the abundant inhabitants of both terrestrial and aquatic ecosystems, play a pivotal role in the decomposition of organic matter, nutrient cycling, and the maintenance of ecological balance [4]. Recent studies have begun to unveil the hidden world of marine fungi, demonstrating their remarkable diversity and ecological importance [3]. Marine fungi exhibit a wide range of adaptations to the unique challenges of the marine environment, including salinity tolerance, the ability to adhere to underwater surfaces, and the production of specialized enzymes to degrade marine organic matter [2].

The interactions between marine fungi and fish are complex and multifaceted. While some fungi are known to act as pathogens, causing diseases in fish [1], others play beneficial roles, such as aiding in digestion or providing protection against other pathogens [5]. The vast majority of marine fungi, however, are saprophytes, deriving their nourishment from decaying organic matter [5]. Studies on marine fungi in Libya are limited [6, 7], the present study aims to uncover the fungal taxa associated with the White Seabream *Diplodus sargus*, a common marine fish found in Libyan waters. We identified a diverse group of fungal species inhabiting the skin and gills of this marine fish, providing valuable insights into the fungal mycobiota of Libyan marine ecosystems.

Materials and Methods:

Area of study and the studied taxon

The White Seabream *Diplodus sargus* (L .1758) is distributed across the Mediterranean Rocky shores, and from the eastern Atlantic Ocean to the Black Sea, and encompasses the coasts of Mauritania to Senegal [8]. This adaptable marine species occupies a prominent position in commercial and recreational coastal fisheries in Libya and the Mediterranean [9]. It exhibits a streamlined, laterally compressed body, perfectly suited for navigating the marine environment. Its silvery scales provide camouflage against predators, while its strong caudal fin propels it swiftly through the water. The white seabream's mouth is small and terminal, with well-developed lips and numerous teeth adapted for grazing on algae and invertebrates [10].

Figure 1: White Seabream *Diplodus sargus*

Fish sampling : Fifty-four specimens of White Seabream were obtained from Bab Al Bahr Fish Market in Tripoli, during the January to April, 2023. Fish specimens were kept in cold containers and sent to the Biology Laboratory at Faculty of Education Tripoli, for external examination and dissection. (Figure 1).

Figure 2: Bab Al Bahr Fish Market of Tripoli

Fungal isolation and identification: Fish specimens were initially rinsed with tap water to remove debris and then immersed in 70% ethanol for five minutes to disinfect the surface. Following disinfection, the samples were washed with sterile distilled water to remove residual ethanol. Subsequently, a smear of the outer surface including the head was prepared and inoculated onto Sabouraud Dextros Agar (SDA) supplemented with gentamicin (50 mg/ml) to selectively inhibit bacterial growth. For the remaining fish, they were dissected into 3 cm sections and subjected to the same disinfection and washing procedures. These sections were then plated onto Petri dishes containing the nutrient medium, with three sections placed in each dish. All inoculated plates and smears were incubated at room temperature for 3-4 weeks to facilitate fungal growth. After incubation, fungal colonies were observed macroscopically and microscopically, and their development was documented through photographs [11].

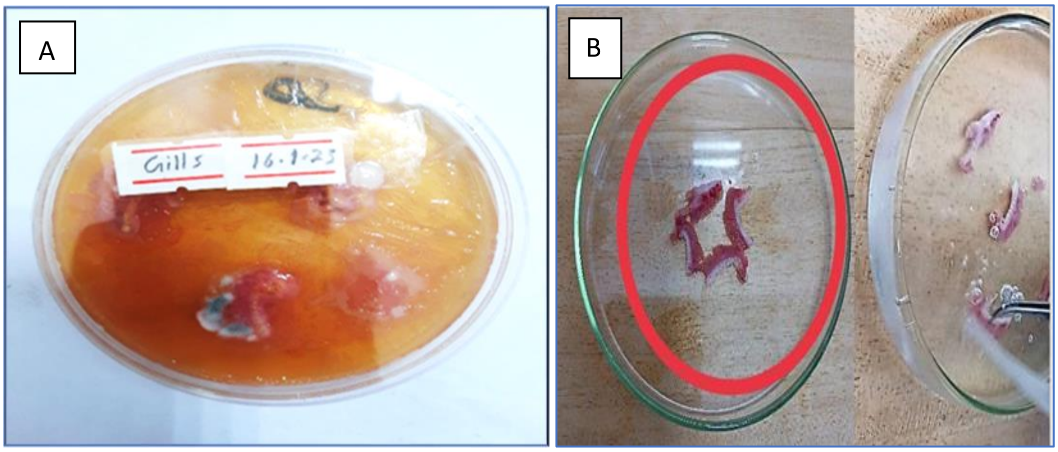

Figure 3. skin and gill tissues on Sabouraud Dextros Agar (SDA) culture medium

Statistical analysis:

Frequency of occurrence for the fungal species in skin and gills, the *Chi* square and pairwise correlation coefficient was calculated using Julus.ai platform, an artificial intelligence statistical program.

Results and discussion

In our study, we investigated the presence of fungi on the skin and gills of Seabream, a common marine fish. The frequency distribution of fungal presence revealed a higher occurrence on the gills (10 out of 12) compared to the skin (7 out of 12).

Table 1 : Fungal genera from Sparids *Diplodus sargus* from western coast of Libya

Fungus genera		Skin	Gills
Zygomycetes	*Circinella sp*	-	+
	Rhizopus sp	+	-
Ascomycetes	*Chaetomium sp*	-	+
	Leptosphaeria sp	+	-
	Saccharomyces sp	+	+
Mitotosporic fungi	*Alternaria sp*	+	+
	Aspergillus sp	+	+
	Cladosporium sp	-	+
	Epicoccum sp	-	+
	Exophiala sp	-	+
	Fusarium sp	+	+
	Penicillium sp	+	+

This suggests a slightly higher prevalence of fungi on the gills compared to the skin, which could be due to the gills providing a more favorable environment for fungal colonization due to factors such as higher moisture content and nutrient availability [11]. In general skin and fin infection are considered less serious as compared to gills. Opportunistic fungi impact fishes primarily when they are stressed or immunocompromised due to adverse environmental conditions. They may also target fishes susceptible to bacterial or viral infections. Furthermore, fungi exploit fishes that have lost their protective mucus layer due to trauma or excessive handling. This highlights the selective nature of fungal attacks on fishes [11].

The Chi-Square test indicated no statistically significant association between fungal infection on the skin and gills. This outcome was unexpected, considering the hypothesized correlation due to their anatomical proximity. Further analysis revealed a weak inverse correlation between the presence of fungal species in the two organs, with a correlation coefficient of approximately -0.098. This result aligns with the Chi-Square test, further reinforcing the conclusion that fungal colonization on the skin and gills operates independently.

The absence of a significant correlation could be attributed to several factors. The skin and gills likely possess distinct microenvironments and immune responses, which might differentially influence fungal colonization and growth, which is in line with findings of Cabillon and Lazado [12]. This underscores the need for further research to elucidate the specific ecological and biological factors that govern fungal presence in these distinct anatomical locations.

To compare the results of our study with published articles of similar studies, we can draw on existing literature that has examined the presence of fungi on marine organisms [3,4,5,10,11,12]. For instance, studies have shown that marine fungi can exhibit specific colonization patterns depending on the host's body part, environmental conditions, and the host's immune responses [5].

The ’orrelation coefficient of approximately -0.098 indicates a very weak inverse relationship between the presence of fungi on the skin and gills. This weak correlation aligns with findings from, who observed that the presence of fungi on various marine host surfaces is often independent, with each microenvironment selecting for different fungal communities.

As shown in the table 1 and Figure (4) fungal genera were identified that belong to the class, Ascomycetes (3 spp.) , Zygomycetes (2 spp.) and mitotosporic fungi (7spp).

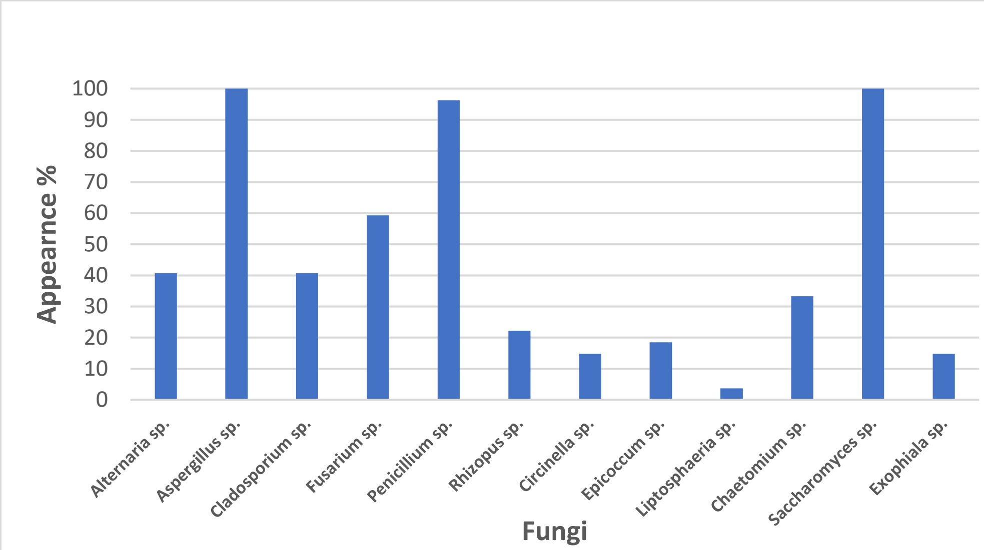

Figure 4. Frequency of appearance of isolated taxa from *Diplodus sargus*.

A characteristic population of mycobiota associated with aquatic animals was given by [1] and [4]. Of these fungi *Aspergillus*, *Cladosporium*, *Fusarium*, and *Penicillium* species were also found in the present study. In present work the Ascomycetes, *Chaetomium* and the Hyphomycetes, *Cladosporium* genera are typically infect the surface of the fish skin and are rarely internal tissue invader. These brown to black pigmented genera are ubiquitous in the soil. They occur as plant pathogen or as saprophytes on paper products, wood, plant debris and in air [14]. No obligate Marine species of Epiccocium has been described. Representatives of this genus has been occur on washed up Algae and other detritus on beaches environment [15]. The less common isolated fungus was the Ascomycetes *Leptosphaeria* sp. (٪3.7). The genus *Leptosphaeria* is saprobic or pathogen on stem or leaves of herbaceous or woody plants in terrestrial or aquatic habitats [5], Recently [7] reported *Leptosphaeria albopunctata* and *Leptosphaeria oreamaris* from submerged and driftwood from western coast of Libya.

Cirrenalia sp. (Fig. 5A), a mucoraceous species, one of the smallest genera of the Family mucoraceae, closely related to Mucor but different in forming sporangia with persistent sporangial wall. Previously reported from a sand beaches environment in Mexico [16], and from the tidal zone in Iraq [17]. *Rhizopus* sp. Has been characterized as an opportunist pathogen, effecting fish health as they have virulence factors which grant them to become primary causative infection [11]. Species of *Exophiala* (known as blacky yeasts), are opportunistic fungal pathogens that may infect a broad range of warm- and cold-blooded animals including Salamonds and Atlantic Cod [15].

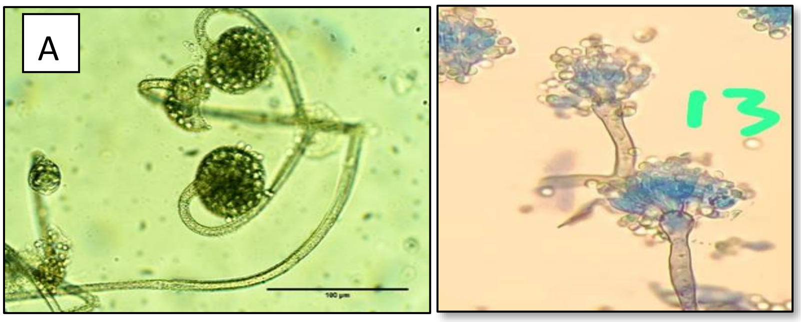

Figure 5. A: *Circinella* sp B: *Aspergillus* sp

Ichthyphonus sp. Is an endemic in both fresh and marine water fishes [18], has a resting double walled multinucleated spores. It is an internal fungus causing chronic, systemic granulomatosis disease. In the absence of visual lesions, only macroscopic examination confirmed by histology determine if the organism is *Ichthyphonus*(excluded). [19].

In summary, our results are in agreement with the existing literature, which suggests that fungal colonization on marine organisms is a complex process [2, 4], influenced by a multitude of factors, and that the presence of fungi on different anatomical locations can occur independently of each other. Further research is needed to elucidate the specific ecological and biological mechanisms driving these patterns.

Conclusion:

Based on the study, fungi Isolated obtained from 54 apparently non infected White seabream *Diplodus sargus* are: *Alternaria* sp *Aspergillus* sp *Cladosporium* sp *Fusarium* sp *Penicillium* sp *Rhizopus* sp *Circinella* sp *Epicoccum* sp *Leptosphaeria* sp *Chaetomium* sp *Saccharomyces* sp *Exophiala* sp. The most common isolated fungal taxa were *Aspergillus*, and Saccharomyces species and the less common was *Leptosphaeria* sp. Future investigations should focus on characterizing the microenvironment differences between the skin and gills. Additionally, exploring the role of specific immune responses in each location could provide valuable insights into the observed independent patterns of fungal colonization.

Acknowledgements: the authors would like to express their gratitude and thanks to Dr. M.S. Ghenghish for her assistance in confirmation of fungi taxonomy and comments on this manuscript.

References:

1. Grossart, H. P., Van den Wyngaert, S., Kagami, M., Wurzbacher, C., Cunliffe, M., & Rojas-Jimenez, K. (2019). Fungi in aquatic ecosystems. *Nature Reviews Microbiology*, *17*(6), 339-354.
2. Sen, K., Sen, B., & Wang, G. (2022). Diversity, abundance, and ecological roles of planktonic fungi in marine environments. Journal of Fungi, 8(5), 491.
3. Naya-Català, F., Piazzon, M. C., Calduch-Giner, J. A., Sitjà-Bobadilla, A., & Pérez-Sánchez, J. (2022). Diet and host genetics drive the bacterial and fungal intestinal metatranscriptome of gilthead sea bream. *Frontiers in Microbiology*, *13*, 883738.
4. Danger, M., Gessner, M. O., & Bärlocher, F. (2016). Ecological stoichiometry of aquatic fungi: current knowledge and perspectives. *Fungal Ecology*, *19*, 100-111.
5. Jones, E. G., Suetrong, S., Sakayaroj, J., Bahkali, A. H., Abdel-Wahab, M. A., Boekhout, T., & Pang, K. L. (2015). Classification of marine ascomycota, basidiomycota, blastocladiomycota and chytridiomycota. *Fungal Diversity*, *73*, 1-72.
6. Ghenghish M.S. (2017). New taxa for Marine fungi from western coast of Libya. American J. Biology and life science. vol. 5(6)51-54.
7. Kafu R, Almasri T. and Ghenghish M. 2022. Lignicolous Marine Fungi from Libya. Global Journal of Science Frontier Research. 22, H1 (Feb. 2022), 65–68.
8. Hanel, R., & Tsigenopoulos, C. S. (2011). Phylogeny, evolution and taxonomy of sparids with some notes on their ecology and biology. *Sparidae*, 51-73.
9. Elbaraasi, H., Elabar, B., Elaabidi, S., Bashir, A., Elsilini, O., Shakman, E., & Azzurro, E. (2019). Updated checklist of bony fishes along the Libyan coasts (southern Mediterranean Sea). *Mediterranean marine science*, *20*(1), 90-105.

10. Mouine, N., Francour, P., Ktari, M. H., & Chakroun-Marzouk, N. (2007). The reproductive biology of *Diplodus sargus sargus* in the Gulf of Tunis (central Mediterranean). *Scientia marina*, *71*(3), 461-469.

11. Iqbal, Z., & Saleemi, S. (2013). Isolation of pathogenic fungi from a freshwater commercial fish, Catla catla (Hamliton). *Sci Int (Lahore)*, *25*(4), 851-855.

12. Cabillon, N. A. R., & Lazado, C. C. (2019). Mucosal barrier functions of fish under changing environmental conditions. *Fishes*, *4*(1), 2.

13. Refai MK, Laila K, Amany M, Shimaa ES. The assessment of Mycotic settlement of freshwater fishes in Egypt. J Am Sci, 2010; 6(11): 823—31

14. Meyer, F. P. (1991). Aquaculture disease and health management. *Journal of animal science*, *69*(10), 4201-4208.

15. Kohlmyer j, Volkmann-Kohlmyer B (1991). Illustrated Keys of Higher filamentous Marine fungi. *Botanica Marina* vol .34 .1-61.

16. Gonzalez M.C, Mureta-Figuroa N, Madina-Ortiz C, Hanlin R.T.(2010) . New record of Circinella muscle from hydrocarbon polluted sand beach of Tabasco, Mexico. Mycotaxon .VoI. 113.(1):111-117.

17. Abdullah S.K, Al-Saadoon A.H, Al-Salihy, M.H. (2007). Fungi from the tidal zone of Khawr Al-Zubair canal Southern Iraq. Marsh Bulletin. 2:18 - 31.

18. Robert RJ., 2012. Fish Pathology, 4th edtion. Widely Blackwell USA.

19. Huntsberger, C. J., Hamlin, J. R., Smolowitz, R. J., & Smolowitz, R. M. (2017). Prevalence and description of Ichthyophonus sp. in yellowtail flounder (Limanda ferruginea) from a seasonal survey on Georges Bank. *Fisheries Research*, *194*, 60-67.