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THE SPREADING AND MOLECULAR CHARACTERIZATION OF THE WATER MOULD *Saprolegnia parasitica* IN AL-DIWANIYAH RIVER OF IRAQ

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ABSTRACT: The water mold *Saprolegnia parasitica* is a chief species of oomycetes that affects a wide range of plant, natural ecosystems, fish and the aquaculture industry. The current study aimed to investigate the isolation and identification of some aquatic fungi like *Saprolegnia* spp. and also molecular characterization of *Saprolegnia parasitica* in the water of Al-Diwaniyah river of Iraq using the baiting method for isolation and PCR polymerase chain reaction for molecular diagnosis of fungi. A total of 60 samples were taken from three study sites of river: 25 samples of Al-Shafi'iah city bridge (Najaf road) as first site (S1); 25 samples of Hawly Al-Jamiah road bridge, Umm Al-Khail area, as second site (S2); and 10 samples of Al-Orouba bridge as the third site (S3). Molecular diagnosis was carried out by PCR examination using primers for the rDNA gene and its presence in *Saprolegnia parasitica*, as DNA was obtained at a concentration of 685.4-99.4 µg/µl and a purity of 1.92-1.8. The samples from the first site gave the highest number of 18 isolates (45%), followed by samples from the second site with 14 isolates (35%). The samples from the third site showed a number of 10 isolates (20%). The species *S. parasitica* was more visible during February 2020 with a number of 17 isolates, and less visible in April 2021, with a number of 3 isolates. In conclusion, the variation in the presence of the types of infectious aquatic fungi *Saprolegnia* spp. in the Al-Diwaniyah river of Iraq in different sites and months of 2021 showed a high percentage of isolates for Al-Shafi'iah city bridge (Najaf road) site and the highest number of *Saprolegnia parasitica in* February 2020. There is a need for further examination of oomycetes in different sites of rivers of Iraq in different months.

Keywords: Aquatic fungi, Fish, Fungal disease, Molecular characterization, *Saprolegnia parasitica.*

INTRODUCTION

Water is the necessary factor for the life of living organisms, and it is one of the most important basic elements of the environment on which animal and human life and all his activities are based. Fresh water constitutes the remaining area, and rivers constitute a suitable environment for the growth of several biological communities of micro-organisms such as fungi, bacteria, parasites and viruses (Frenken et al., 2019; Al-Maliki et al., 2021).

Oomycetes is one of those organisms that spread in the aquatic environment, as it plays an important role in the balance of the aquatic environment, especially fresh water, as it analyzes many pollutants and substances through its enzymes that secrete them. They are very sensitive to the efficacy of toxic substances in water (Tsui et al., 2015).

Saprolegnia parasitica is a species that infect a wide range of plant and also cause economically important diseases in animals especially in aquatic animals (Earle and Hintz, 2014). This fungus is characterized by the ability to infect fish and their eggs. So, researchers are recently focused on new methods for controlling this important infection agent (Tedesco et al., 2019; Mostafa et al., 2020; Zhang et al., 2021). Some relative studies have been conducted in Iraq for detection of possible problems in rivers and also its effect on fish health (Touhali and Al-uguali, 2018; Ali et al., 2020; Al-Hassani and Mustafa, 2022).

Mustafa et al. (2019) had reported that presence and concentration of hydrogen peroxide can be useful for controlling *Saprolegnia*sis in fish (common carp). Ali et al. (2020) stated that *Saprolegnia* can have economic hazards due to lethal infection of fish, based on their studies on samples taken from Mosul, Iraq.

With attention to importance of these infectious agents in water health and also aquatic health, the purpose of the study was to survey of aquatic fungi, especially *Saprolegnia parasitica* in the water of Al-Diwaniyah River, and to conduct a phenotypic and molecular diagnosis of the fungus.

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MATERIALS AND METHODS

Samples collection

Water samples were collected monthly for the period from December 2020 to May 2021 at ten samples in the morning from the study sites represented by the Al-Diwaniyah River in three sites represented in the first site (S1) Al-Shafi'iah City Bridge (Najaf Road), and the second site (S2) (Hawly Al-Jamaa Road Bridge, Umm District, Horses), and the third site (S3) (Al-Orouba Bridge). Water samples were taken from all areas of the river from its sides and middle, i.e. square, during the above-mentioned period, i.e. in winter, spring and early summer. Under the surface of the water, at a depth of 20-30 cm, after filling, it was sealed tightly while it was under the surface of the water and was then transferred to the laboratory for laboratory tests.

Fungi isolate

Baitting method

The aquatic fungi were isolated in the current study by the baiting method according to what was mentioned by Al-Rekabi et al. (1996) and Pennisetum spicatum seeds were used as baits for this purpose.

Purification and maintenance of pure cultures

After the emergence of fungal growths, the purification process was carried out by cutting one fungal thread near the top with sterile metal forceps, and transferred under sterile conditions to SDA medium. A piece of culture medium containing the fungal ends was transferred with a 6 mm or 5 cork borer to sterile Petri dishes containing three millet seeds, 15 µl sterile distilled water and one ml of anti-chloramphenicol (Kiziewics, 2006).

Diagnosis of aquatic fungi

The isolated and developing fungi were diagnosed by grafting method based on the phenotypic and agricultural characteristics of the developing colonies and this includes the shape, color and nature of colony growth, as well as using the microscopic phenotypic characteristics of fungal hyphae and sexual and asexual reproductive structures and based on the taxonomic keys mentioned by Watanabe (2002).

Diagnostic method using PCR polymerase chain reaction

A PCR assay was performed to investigate the fungus *Saprolegnia* parasitica, by using the primers of the 18S rDNA gene to diagnose the fungus, according to the manufacturer's instructions which is similar to the methods of Paul et al. (2015) and Al-Maliki et al. (2021).

Fungus DNA Extraction

DNA extraction was carried out from fungi colonies samples using the EZ-10 Spin Column Fungal Genomic DNA Mini-Preps Kit from the Korean company Bioneer. The extraction was carried out according to the company's instructions, which are: 1- About 200 mg of fungal growth colonies were transferred to a sterile ceramic container using liquid nitrogen at a temperature of -169°C (below zero). Then the fungal colonies were crushed and then transferred to sterile 1.5 µl tubes; 2- After that, 180 µl of Universal Digestion Buffer and 20 µl of Proteinase K were added to each sample and then mixed well with the Vortex mixer and then the samples were incubated at 56°C for 30 minutes; 3- Then 100 µl of Universal Buffer PF solution was added and mixed by inverting and then the tubes were incubated at -20°C for 30 minutes; 4- The samples were placed in a centrifuge at a speed of 10000 rpm for 5 minutes. Then transfer the supernatant to a new 1.5 µl tube; 5- Then 100 µl of Universal Buffer BD solution was added and mixed well with a mixture; 6- After that, Absolute ethanol 96% was added to all samples and mixed well with a mixture; 7- The mixture was transferred to special tubes containing a DNA extraction filter equipped with the EZ-10 column kit, which were placed inside university collection tubes with a capacity of 2 µl, and then placed in a centrifuge at 12000 rpm for one minute, and then the precipitate was disposed of; 8- Then 500 µl of Universal PW Solution was added and the tubes were placed in a centrifuge at 12000 rpm for 1 minute and then the precipitate was discarded; 9- Then again 500 µl of Universal Wash Solution was added and then the tubes were placed in a centrifuge at 12000 rpm for 1 minute and then the precipitate was discarded; 10- The DNA-containing EZ-10 column was placed in sterile 1.5 µl tubes and the tubes were then placed in a centrifuge at 12000 rpm for 2 min to dry the EZ-10 column membrane from alcohol and then the precipitate was discarded; 11- After that 50 µl of TE Buffer solution was added to dissolve the DNA inside the EZ-10 filter column and then incubated at room temperature for one minute and then all tubes were placed in a centrifuge at 12000 rpm for one minute to collect the DNA and then transferred to storage At -20°C in the refrigerator until use in the PCR test.

DNA profile assay

The DNA is extracted from the fungal samples through the use of the Nanodrop spectrophotometer (THERMO, USA) to detect and measure the concentration of nucleic acids (DNA and RNA), where the DNA is detected by determining the concentration of DNA (ng\µl DNA). The purity of DNA was measured by reading the absorbance at a wavelength ranging between (260-280 nm) and the device was used as follows: 1) After turning on the Nanodrop device, the DNA measurement program was selected; 2) Sweep the scale substrate twice using blotting paper for the device by placing 1

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µl of ddH2O using a sterile micropipette on the surface of the scale substrate, zeroing and then cleaning the substrate for measuring samples; Press the start button to start the process of measuring the concentration of DNA, using 1 microliter of each sample of the extracted DNA, and then the substrate of the scale device was cleaned again to measure the other samples; The purity of the extracted DNA samples was determined when the absorbance ratio was 1.8, then the extracted DNA is pure.

Analysis of the results of the PCR examination

Agarose gel electrophoresis was carried out using 1.5% agarose gel, reading the PCR product analysis result as follows: 1- Dissolve 1 g of agarose gel in 100 µl of TBE buffer (Tris-borate-EDTA) at 1X concentration using a Microwave device for 5 minutes; 2- The gel was left to cool at 50°C. Then 3 microliters of radioactive DNA dye Ethidium bromide were added and mixed well with the gel; 3- The agarose gel poured into the Tray containing the comb to locate the PCR samples, then the gel were left to solidify at room temperature for 15 minutes and then the comb removed from the gel carefully; 4- The samples that are the result of the PCR product were carried and placed in the gel pits, and then a 100 DNA Marker ladder was added to measure the PCR product in the first hole; 5- After the loading process was completed, the agarose gel was immersed in a buffer TBE Buffer solution at a concentration of 1X, the relay cover was closed, and then the relay was operated using a current of 100 volts and 80 amps for one hour; 6- After the migration process is completed, examine the gel containing the PCR product using a U.V light source to determine the product with the measurement unit.

Statistical analysis

The results were subjected to statistical analysis to find out the significant differences between the studied sites. The significant differences were determined at a probability level of 5% using the least significant difference (LSD) test and the Chi-square test. The percentage of the appearance of fungi in the studied sites was calculated through the following equation:

Percentage = (Number of isolates x 100 / The number of samples examined) x 100%

RESULTS AND DISCUSSION

Isolation and identification

Isolation of the fungus Saprolegnia parasitica

Table 1 shows the numbers of Fungi isolated in this study and the percentage of their appearance using saproide dextrose agar medium and using the grafting method. One species of *Saprolegnia* spp. was isolated and diagnosed. It is the type *S.parasitica* being the most visible in the study areas in terms of its accessibility, where the current study was conducted on the Al-Diwaniyah river in three locations represented in the first site (S1) Al-Shafi'i city bridge (Najaf road), and the second site (S2) (University of Road Bridge, Hawalli, Umm Al-Khail area), and the third site (S3) (Al-Orouba bridge), as it appears from the table below that the total number of samples was 60 samples distributed by 25 samples from the first site, 25 samples from the second site, and 10 samples from the third site. The first site had the highest number of isolates, amounting to 18 isolates, with a percentage of 45%, followed by samples from the second site, with 14 isolates, and with a percentage of 35%. The samples for the third site were 10 isolates and with a percentage of 20%.

This was represented during the study that started from December of the year 2020 until the month of May 2021, and it appeared that there was a significant difference ($P<0.05$) in the numbers of these fungi from one site to another during the study period for the purpose of isolating and diagnosing the aforementioned types of fungi, as rivers represent a suitable environment for the growth of these fungi. Fungi species *Saprolegnia* spp. due to the availability of appropriate ambient conditions such as temperature, acid function, appropriate concentration of dissolved oxygen, lighting, flow velocity and surface tension property, and the variation in the proportions of fungal isolates in the studied sites can be attributed to the difference in the characteristics mentioned, which is consistent with what was reported by Olegovich Bokov et al. (2020) which mentioned that it can be The variation in the fungi that live in Iraqi water in general is attributed to the pollution occurring therein and the difference in physical and chemical properties and other environmental influences that have a direct impact on the presence of these fungi.

Monthly distribution of the fungus *Saprolegnia* parasitica and the effect of its chemical and physical properties

Table 2 shows the monthly distribution and temperature range of *Saprolegnia* parasitica. And the total number of isolates, as the lowest temperature was recorded and was from 12-13 °C at the beginning of the study in December of the year 2020, and the maximum was in May of the year 2021, where the temperature range was from 28-27 °C or less than that by one or two degrees This is consistent with what was mentioned by Watanabe, (2002) and Hafsan et al. (2022), in their study on the types of Fungi *Saprolegnia* in the water of the Shatt al-Arab and their pathogenicity examination, as the temperatures recorded in that region were close to the sites of the current study. The best month in which fungal isolates were obtained in abundance is February 2021, where 17 isolates of *S.parasitica* were isolated, where the temperature range was measured from 14-16°C, and the least number of isolates obtained was in the month of April 2021, as 3 isolates of the aforementioned fungus were isolated, and the temperature range was measured from 20-22°C, and no isolation was obtained in May 2021, which is the last month of the isolation and diagnosis phase, where the temperature

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range was measured from 27-28°C, and the following pictures, the Fungi *Saprolegnia* spp. during the period of isolation and diagnosis, by the appearance of fungal growths on sterilized millet seeds and on SDA culture media in the laboratory (Figures 1 and 2).

Saprolegnia fungi species isolated during the current study

Type I Saprolegnia parasitica Coker

The diameter of the colony of this fungus, after two weeks of growth on millet seeds, ranged 1-1.5 cm. The diameter of the fungal thread at the base reached 25-45 microns. The fungal threads are less branched. The gametes were abundant with different shapes, often regular and sometimes irregular in shape, terminal or inter-site and they exist in the form of chains. The boards are longitudinal or cylindrical, their dimensions range between 35-60 × 250-950 microns, the diameter of the sintered boards is 12 microns, the boards are spherical or pear-shaped, and they are terminal or sideways. With antheridia, cylindrical straight clipboard holders with dimensions 15-30 × 50-200 microns, eccentric oval boards 10-25 microns in diameter, and 2-40 in one capsule filling the cavity (Figures 3-5). Antheridia fungus is characterized by the ability to infect fish and their eggs, which is identical to what Mentioned by Kiziewics (2006) and Hafsan et al. (2022) and its forms are:

Diagnosis of the fungus *Saprolegnia parasitica* PCR technique

The PCR examination was carried out using primers for the rDNA gene to diagnose the fungus *S.parasitica*. The results showed the efficiency of the method in extraction, as DNA was obtained in *S.parasitica* at concentrations of 685.4-99.4 µg/µl and with a purity of 1.80-1.92, noting that the percentage of purity adopted for the reaction is 1.8, as it shows in Table 3.

PCR assay for *Saprolegnia parasitica*

Through what appeared in the electrophoresis on agarose gel at a concentration of 1%, it was observed when examining the gel under ultraviolet rays that light orange bands appeared resulting from the replication process, in reference to the link of the initiator with its complementary sequence in the template DNA. It appeared that the sizes of the pieces of DNA compared with the product size from the measurement of the size of the DNA bundle of *S.parasitica* is 568 bp, all isolates of this species gave a positive result, which numbered 10 pure isolates, and these values were confirmed according to what is found in the NCBI Gene Bank as well as what was confirmed by Sparrow (1960) and through what appeared in the photography after the end of the electrical relay process (Figures 4-6).

Table 1 - The sites of isolates of Fungi, the number of isolates on the Sabouraud agar medium and their percentage.

Sample location	Number of samples examined	Number of isolates	Percentage
S1	25 [a]	18 [a]	45
S2	25 [a]	14 [b]	35
S3	10 [b]	8 [c]	20
Total	60	40	100

*Similar letters: indicate no significant differences. The different letters: indicate the presence of significant differences.

Table 2 - Monthly distribution and water temperature ranges of *Saprolegnia parasitica* and the total number of isolates.

Months	Temperature range (°C)	Number of isolates
December 2020	12-13	5
January 2021	10-11	8
February 2021	14-16	17
March 2021	17-19	7
April 2021	20-22	3
May 2021	28-27	0
Total	40	

Table 3 - Samples of S. parasitica, their concentrations and purity in PCR assay using a Nanodrop spectrophotometer.

Sample	Concentration (ng/ml)	Purity (1.8)
S.parasitica 1	99.4	1.80
S.parasitica 2	238.2	1.82
S.parasitica 3	239.9	1.82
S.parasitica 4	317.6	1.84
S.parasitica 5	320.5	1.84
S.parasitica 6	351.6	1.85
S.parasitica 7	429.3	1.86
S.parasitica 8	493.7	1.88
S.parasitica 9	667.1	1.90
S.parasitica 10	685.4	1.92

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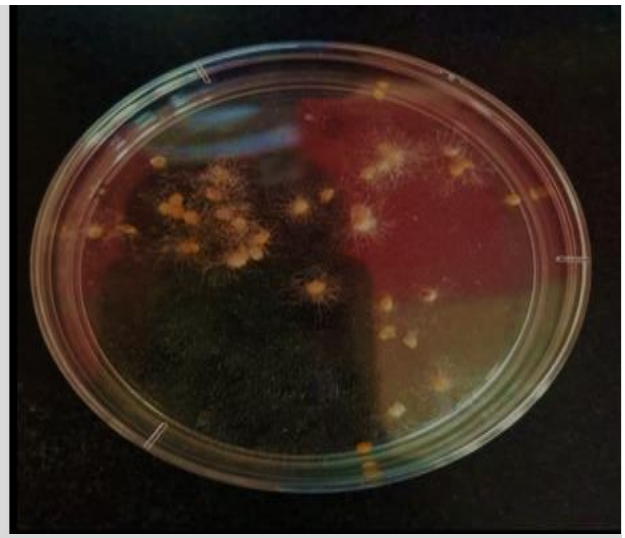

Figure 1- The fungal colony of *Saprolegnia* spp. growing on millet seeds by grafting method and its age, 72 hours.

Figure 2 - The fungal colony of *Saprolegnia* spp. growing on SDA medium and its age is 5 days.

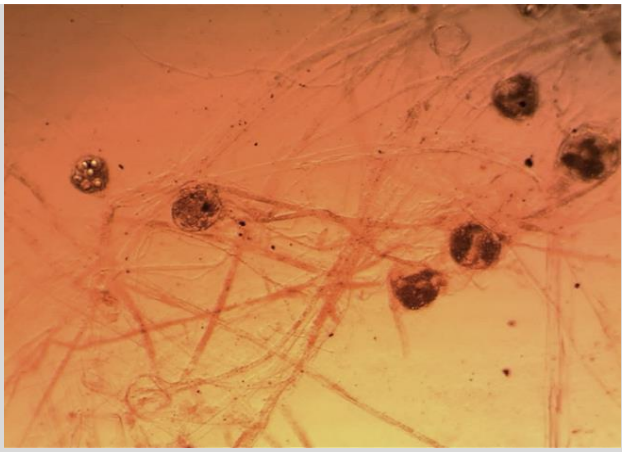

Figure 3 - Detected *Saprolegnia parasitica.*

Figure 4 - *Saprolegnia parasitica* and maturation of sporangia spores in sporangia.

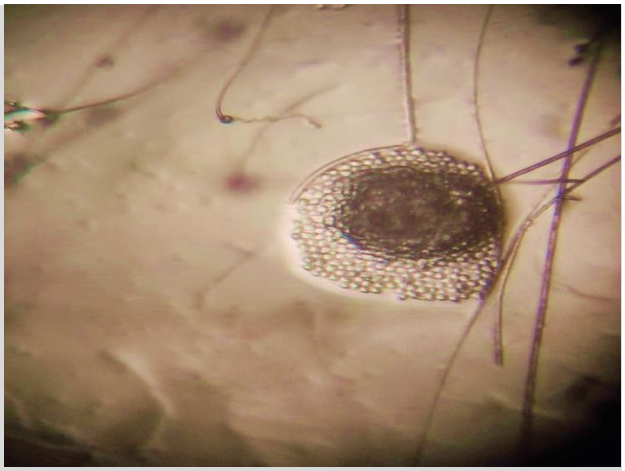

Figure 5 - The sporangia of the fungus *Saprolegnia parasitica* at the moment its wall ruptures and the swimming spores are released.

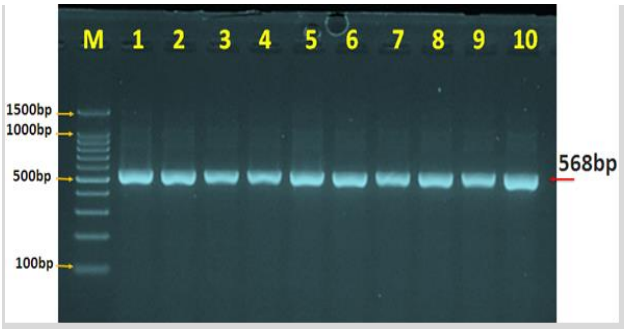

Figure 6 - Agarose gel electrophoresis showing the results of the PCR assay for the 18S rRNA gene ITS1 region for the diagnosis of *S.parasitica*, where the (M) line represents the standard scale (1500-100bp) and the line (10-1) bundles Positive isolates for PCR examination with a yield length of 568bp, knowing that the electrophoresis with agarose gel at a concentration of 1% and an electric current of 100 volts and 80 amps.

CONCLUSION

In conclusion, the variation in the presence of the type of aquatic fungi *Saprolegnia* spp. in the Al-Diwaniyah river of Iraq was evaluated and reported. Also the application of the PCR examination in the molecular diagnosis of the type of fungus is efficient for further studies in different regions.

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DECLARATIONS

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Authors' contribution
Both authors contributed equally to this work.

Conflict of interests
The authors declared that there is no conflict of interest in this study.

REFERENCES

Al-Hassani ST, Mustafa SA (2022). Efficiency of synbiotic as feed additives on growth performance, survival rate and health status in common carp challenged with *Saprolegnia* spp. Iraqi Journal of Agricultural Sciences, 53(2):397-405. DOI: https://doi.org/10.36103/ijas.v53i2.1548

Ali FF, Al-Taee SK, Al-Jumaa ZM (2020). Isolation, molecular identification, and pathological lesions of *Saprolegnia* spp. isolated from common carp, Cyprinus carpio in floating cages in Mosul, Iraq. Veterinary World, 13(12):2759. DOI: https://dx.doi.org/10.14202%2Fvetworld.2020.2759-2764

Al-Maliki LA, Farhan SL, Jasim IA, Al-Mamoori SK, Al-Ansari N (2021). Perceptions about water pollution among university students: A case study from Iraq. Cogent Engineering, 8(1):1895473. DOI: https://doi.org/10.1080/23311916.2021.1895473

Al-Rekabi SA, Naeem RA, and Butty KN (1996). Specificity of Baits in isolation *Saprolegnia*ceae. Al-Mustansiriyah Journal of Science, 7(1):20-22. Google Scholar

Earle G, and Hintz W (2014). New Approaches for Controlling *Saprolegnia* parasitica, the Causal Agent of a Devastating Fish Disease. Tropical Life Sciences Research, 25(2):101-109. http://www.tlsr.usm.my/tlsr25022014/25022014_07.pdf

Frenken T, Agha R, Schmeller DS, van West P, Wolinska J (2019). Biological concepts for the control of aquatic zoosporic diseases. Trends in parasitology, 35(7):571-382. DOI: https://doi.org/10.1016/j.pt.2019.04.003

Hafsan H, Dmitry Bokov,Walid Kamal Abdelbasset,Mustafa M. Kadhim,Wanich Suksatan,Hasan Sh. Majdi, et al. (2022). Dietary Dracocephalum kotschyi essential oil improved growth, haematology, immunity and resistance to Aeromonas hydrophila in rainbow trout (Oncorhynchus mykiss). Aquaculture Research, 53(8): 3164-3175. DOI: https://doi.org/10.1111/are.15829

Kiziewicz B (2006). Water Fungi and Fungus-like Organisms Isolated from Surface Waters Situated in the Białowieża Primeval Forest Using the Liver Fluke Fasciola Hepatica L. of European Bison Bonasus L. as Bait. Polish Journal of Environmental Studies, 15(2):277–281. http://www.pjoes.com/pdf-87868-21727?filename=Water%20Fungi%20and.pdf

Mostafa AA, Al-Askar AA, Yassin MT (2020). Anti-*Saprolegnia* potency of some plant extracts against *Saprolegnia* diclina, the causative agent of saprolengiasis. Saudi Journal of Biological Sciences, 27(6):1482-1487. DOI: https://dx.doi.org/10.1016%2Fj.sjbs.2020.04.008

Mustafa SA, Al-Rudainy AJ, and Al-Faragi JK (2019). Assessment of hydrogen peroxide on histopathology and survival rate in common carp, Cyprinus carpio l. infected with *Saprolegnia*sis. The Iraqi Journal of Agricultural Science, 50(2):697-704. DOI: https://doi.org/10.36103/ijas.v2i50.669

Olegovich Bokov D, Jalil AT, Alsultany FH, Mahmoud MZ, Suksatan W, Chupradit S, Qasim MT, Delir Kheirollahi Nezhad P (2022). Ir-decorated gallium nitride nanotubes as a chemical sensor for recognition of mesalamine drug: a DFT study. Molecular Simulation, 48(5): 438-447. DOI: https://doi.org/10.1080/08927022.2021.2025234

Paul Y, Leung WL, and Hintz WE (2015). Species composition of the genus *Saprolegnia* in fin fish aquaculture environments, as determined by nucleotide sequence analysis of the nuclear rDNA ITS regions. Fungal biology, 119(1):27-43. DOI: https://doi.org/10.1016/j.funbio.2014.10.006

Sparrow FK (1960). Aquatic phycomycetes. 2nd edition, Ann Arbor: The University of Michigan Press, USA. pp. 1187. Google Scholar

Tedesco P, Fioravanti ML, and Galuppi R (2019). In vitro activity of chemicals and commercial products against *Saprolegnia* parasitica and *Saprolegnia* delica strains. Journal of Fish Diseases, 42(2):237-248. DOI: https://doi.org/10.1111/jfd.12923

Touhali IS, and Al-uguali IS (2018). Isolation and identification of *Saprolegnia* parasitica and other fungi from farms fishes in the province of Wasit/Iraq. Journal of Global Pharma Technology, 10(05):135-142. Google Scholar

Tsui CK, Baschien C, and Goh TK (2016). Biology and ecology of freshwater fungi. In Biology of Microfungi Springer, Cham, pp. 285-313. DOI: https://doi.org/10.1007/978-3-319-29137-6_13

Watanabe, T (2002). Pictorial Atlas of Soil and seed fungi morphologies of cultured fungi and key to species, Boca Raton London New York Washington, D.C. DOI: https://doi.org/10.1201/9781420040821

Zhang J, Chen J, Huang Q, MacKinnon B, Nekouei O, Liu H, Jia P, Wang J, Li N, Huang L, Yang Y (2021). Copper/carbon core/shell nanoparticles: a potential material to control the fish pathogen *Saprolegnia* parasitica. Frontiers in Veterinary Science, 8:798. DOI: https://doi.org/10.3389/fvets.2021.689085

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