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Original Article

Molecular identification and plant-extract antifungal susceptibility of skin and gill mycoses in common carp (*Cyprinus carpio*) from Wasit Ponds, Iraq

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Abstract: Fungal and fungus-like infections are important external diseases of freshwater fish, especially when skin and gill integrity are compromised and water quality is poor. This study investigated fungal and fungus-like pathogens associated with skin and gill lesions in common carp (*Cyprinus carpio*) and evaluated the *in vitro* antifungal activity of the ethanolic extracts of thyme (*Thymus vulgaris*), clove (*Syzygium aromaticum*), pomegranate peels (*Punica granatum*), and garlic (*Allium sativum*). During spring (March-April), 200 common carp from eight ponds in Wasit Governorate, Iraq, were clinically inspected. From 120 infected fish, 60 skin and 60 gill samples were collected. Isolates were examined using culture, morphology, molecular, and histopathological methods. Ethanolic extracts of thyme, clove, pomegranate peel, and garlic were tested by agar-well diffusion and MIC assays. Fungal or fungus-like growth was recovered from 55/120 specimens (45.8%). Skin samples showed a significantly higher positivity rate than gill samples (63.3% vs. 28.3%). Six taxa were identified: *Saprolegnia parasitica*, *Aspergillus flavus*, *A. niger*, *Fusarium solani* species complex, *Penicillium chrysogenum*, and *Candida albicans*. Histopathological examinations revealed epithelial erosion, inflammatory infiltrates, lamellar changes, and PAS-positive fungal- or oomycete-like elements, with more frequent tissue involvement in skin lesions. Thyme and clove extracts produced the largest inhibition zones and the lowest MIC values, followed by pomegranate peel extract, whereas garlic extract showed moderate activity. Integrating conventional mycology, ITS-based identification, histopathology, and antifungal testing improved diagnostic confidence. Thyme and clove extracts showed strong *in vitro* activity, but their aquaculture applications require standardized *in vivo* safety and efficacy evaluations.

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Introduction

Aquaculture in freshwater systems plays an important role in food security; however, infectious diseases still hinder fish survival, growth, productivity, and economic success. Fungal and fungus-like diseases are among the main concerns affecting fish, since fish skin and gills are constantly in contact with water, organic matter, and stressful conditions. Any injury from handling, transportation, parasite infection, crowding, poor water quality, or epithelial damage may weaken the protective mucus layer, allowing the colonization and development of opportunistic fungi and oomycetes (Torres-Martinez et al., 2025). Saprolegniosis remains one of the most widespread problems in aquaculture, as the aforementioned factors may contribute to colonization by *Saprolegnia* and other opportunistic aquatic fungi (Lindholm-Lehto, 2024). Saprolegniosis is one of the most common mycotic diseases in freshwater fish and is traditionally characterized by white-to-grey cottony growth on the skin, fins, gills, or eggs. Although *Saprolegnia* species belong to oomycetes rather than fungi, they are still studied alongside fungal fish pathogens, as they cause similar gross pathology and require similar diagnostic procedures (Costa et al., 2022).

Other filamentous fungi include *Aspergillus*, *Fusarium*, and *Penicillium*, which may be found in lesions, water, sediment, feed, or fish products. They have to be interpreted carefully in the affected fish, as

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they can be opportunistic pathogens, secondary colonizers of damaged tissues, or simply environmental contaminants. The interpretation of *Fusarium* isolates should also be done with caution, as most belong to a species complex (Torbati et al., 2021). In addition, the morphological diversity of *Saprolegnia* complicates identification (Erdei et al., 2023), since there is intraspecific variability within *Saprolegnia parasitica*, a widespread species in this genus (de la Bastide et al., 2018). Culture and microscopic examinations are valuable for preliminary testing, although morphological characteristics depend on medium, incubation conditions, isolate age, and presence or absence of sporulation. Therefore, DNA barcoding has increased the uniformity of fungal identification, and the internal transcribed spacer (ITS) is a popular marker due to the presence of conserved flanking sequences that enable broad amplification and the availability of informative variation within the internal sequence for most fungal species (Vu et al., 2019). In addition, the use of databases such as UNITE can reduce taxonomic uncertainty and increase reporting uniformity in the interpretation of ITS sequences (Nilsson et al., 2019). Updated versions of UNITE contribute to molecular fungal identification and taxonomic communications (Abarenkov et al., 2024).

Growing concerns about water pollution, potential toxicity, costs, and regulatory limitations have drawn attention to plant-derived antifungals in aquaculture. Phenolic compounds, terpenes, tannins, and sulfur-containing substances found in plant extracts can have antifungal properties through mechanisms that include the disruption of cell membrane integrity, oxidative imbalance, cell metabolism, or hyphae growth. However, *in vitro* testing must be considered a first step towards obtaining evidence and should be followed by toxicology tests, standardization, and *in vivo* studies.

The objective of this work is to identify fungal and fungus-like pathogens causing skin and gill lesions in common carp, *Cyprinus carpio,* by morphological characteristics, ITS sequencing, and histopathological examination, and to test antimicrobial activity of the ethanolic extracts of thyme (*Thymus vulgaris*), clove (*Syzygium aromaticum*), pomegranate peels (*Punica granatum*), and garlic (*Allium sativum*) using agar well diffusion and MIC assay methods.

Materials and Methods

Study design and sampling area: This study used a cross-sectional laboratory investigation to examine fungi and fungus-like pathogens in skin and gill lesions of *C. carpio*. Clinical samples were collected from March to April 2026 from eight common carp ponds (ponds 1-8) in Wasit Province, Iraq. A total of 200 *C. carpio* weighing 1-1.2 kg were clinically examined. For data analysis, 60 fish with mycosic lesions were selected, and two samples were collected from each fish (one skin sample and one gill sample).

Clinical examination and specimen collection: Fish that exhibited one or more signs of disease consistent with external mycosis, such as cotton-like growth, ulcers, fin erosion, increased secretion of mucus, change in gill color, abnormal or fast opercular movements, sluggishness, and unusual swimming behavior, were chosen. Photographs of the presence of any external lesion were recorded. The sterile cotton swabs were taken from the margins of the skin lesions, the affected skin, and the gill filament. In severely affected cases, tiny pieces of tissue were taken using sterile instruments. The specimens were then put in sterile containers.

Water quality assessment was conducted by measuring the following parameters at the sampling location using a portable multiparameter meter and field colorimetric kit: temperature, pH, dissolved oxygen, ammonia nitrogen, nitrite nitrogen, and electrical conductivity. The water quality results were derived from the eight ponds (ponds 1-8).

Fungal isolation and purification: The samples were cultured on Sabouraud Dextrose agar and Potato Dextrose agar, supplemented with chloramphenicol to inhibit bacterial contamination. The plates were maintained at 25-28°C and checked every day for 3-7 days. In addition, suspected growths of *Saprolegnia*-like organisms were identified using the sterile water baiting method.

Morphological identification: Macroscopic characteristics of isolates were evaluated based on colony color, texture, edge, reverse side coloration, and growth rate. Microscopic characteristics of isolates were identified using lactophenol cotton blue mounts. The *Saprolegnia*-like isolates were identified from the presence of aseptate hyphae and sporangia, while *Aspergillus* isolates were identified based on the presence of a conidial head.

DNA extraction, ITS-PCR, and sequencing: Fungal genomic DNA was isolated from the mycelium or yeast cells using a commercially available Fungal Genomic DNA Isolation Kit. PCR amplification was performed with universal primers ITS1 and ITS4: ITS1, 5'-TCC GTA GGT GAA CCT GCG G-3'; ITS4, 5'-TCC TCC GCT TAT TGA TAT GC-3'. These primers will usually generate an ITS1-5.8S-ITS2 region of approximately 550-750 base pairs, depending on the organism. The thermal cycling program employed protocols commonly used for amplifying fungal DNA (Centers for Disease Control and Prevention, 2024). The PCR reactions were performed with the following conditions: 25 µL in total volume, of which 12.5 µL of 2× PCR Master Mix, 1.0 µL of ITS1 primer (10 pmol/µL), 1.0 µL of ITS4 primer (10 pmol/µL), and 2.0 µL of template DNA (approximately 20-50 ng). PCR conditions involved 5 minutes at 95 °C for initial denaturation, followed by 35 cycles, each consisting of 95°C for 30 seconds, 52°C for 30 seconds, and 72°C for 60 seconds, then 7 minutes at 72°C, and a final hold at 4°C. The PCR product was run on a 1.5% agarose gel in 1X TBE buffer for 45 minutes at 100 V, stained, viewed under UV, purified, and sequenced by Sanger Sequencing.

Sequence analysis and taxonomic assignment: Forward and reverse reads were trimmed, assembled, and manually verified to produce consensus sequences. Consensus sequences were analyzed against reference sequences using BLAST and curated fungal sequence databases. Assignment at the species level was made based on high sequence identity and query coverage with support from morphological evidence. Species-complex identification was performed for taxa where sequence-based identification was challenging. Sequence identification criteria included the following: bidirectional chromatograms with clearly defined peaks; Phred quality score of more than 20 following trimming; query coverage of ≥98%; sequence identity of ≥98.5% for species-level identification; and absence of conflicting top hits. The agarose gel was documented, and the phylogenetic reconstruction was performed using the neighbor-joining algorithm with 1000 bootstrap replicates, based on sequences from the isolates in the current study and those retrieved from GenBank via BLAST. The consensus ITS sequences were saved as laboratory-internal sequences labeled WST-ITS-F1 to WST-ITS-F6 and used for taxonomic classification.

Preparation of plant extracts: The leaves of thyme (*Thymus vulgaris*), clove (*Syzygium aromaticum*), pomegranate peels (*Punica granatum*), and garlic (*Allium sativum*) were cleansed, dried, ground into powder, and then their ethanolic extracts were obtained using 70% ethanol at a plant material to solvent ratio of 1:10 (w/v) for 48 hours in an intermittent shaking condition. The extracts were filtered through Whatman No. 1 filter paper, concentrated at 40°C under reduced pressure, diluted in 10% dimethyl sulfoxide (DMSO), kept in sterile amber colored glass bottles, and stored at 4°C prior to the experiment. The estimated extract yields were 11.8%, 14.6%, 22.4%, and 8.9% (w/w) for thyme, cloves, pomegranate peel, and garlic, respectively.

Agar-well diffusion assay: Antifungal screening was performed using this technique. Uniformly inoculated cultures of each organism were prepared on SDA and PDA media plates. 6 mm-diameter wells were made in each medium plate, and 100 µL of the extract at a concentration of 200 mg/mL was added. Amphotericin B at a concentration of 100 µg/mL was used as a positive antifungal control, while 10% DMSO was used as a negative solvent control. Plates were incubated at 25-28°C for 48-72 hours, depending on growth rates.

Minimum inhibitory concentration assay: Dilutions of each plant extract were made to ascertain the MICs. MIC was the lowest concentration that could inhibit

Table 1. Fungal growth according to organ.

Sampling organ	No. examined	Positive	Negative	Positivity (%)
Skin	60	38	22	63.3
Gills	60	17	43	28.3
Total	120	55	65	45.8

Table 2. Fungal and fungus-like isolates identified by molecular analysis from the examined common carps.

Fungal or fungus-like isolate	No. of isolates	Percentage (%)	Main recovery site
Saprolegnia parasitica	18	32.7	Skin/gills
Aspergillus flavus	12	21.8	Skin
Aspergillus niger	9	16.4	Skin
Fusarium solani species complex	7	12.7	Skin/gills
Penicillium chrysogenum	5	9.1	Skin
Candida albicans	4	7.3	Gills

fungal growth. All experiments were conducted three times. Dilutions of each plant extract were prepared twofold at 400, 200, 100, 50, 25, and 12.5 mg/mL. The standardized inocula were prepared to contain approximately 105 spores or yeast cells/mL. Incubation was performed for 48 hours at 25- 28°C for rapidly growing yeasts and 72 hours for filamentous molds and oomycete-like organisms. Growth inhibition was defined as the absence of growth of the test fungus relative to the growth control.

Histopathological examination: Tissues from the clinical lesions were collected, fixed in 10% neutral-buffered formalin for 24-48 h, routinely processed, embedded in paraffin blocks, sectioned at 4-5 µm, and stained with hematoxylin and eosin (H&E). Additional staining of the tissues with periodic acid-Schiff (PAS) was performed to detect fungi or fungus-like organisms in the tissue sections. Histopathology of the lesions was performed by evaluating the presence of erosion or ulceration of the epithelium, epidermal hyperplasia, inflammation, necrosis, lifting of the epithelium within the lamellae, lamellar hyperplasia, fusion of lamellae, congestion of blood vessels, and hyphae.

Statistical analysis: Frequencies and percentages of positive fungi were obtained. Data analysis was performed using IBM SPSS Statistics version 26, and the results were cross-validated in R. The Chi-square test was used to compare fungal positivity in skin and gill samples. Comparison of mean inhibition zones among the various plant extracts was performed using one-way ANOVA, while two-way ANOVA with extract and isolate as fixed factors facilitated proper interpretation of the inhibition zones. Tukey HSD was used for pairwise comparisons. $P<0.05$ was considered significant.

Results

Fungal recovery by sampling site: A total of 200 fish were clinically examined in the spring (March–April); of these, 60 with skin lesions suggestive of mycosis were selected for laboratory examination. A total of 120 clinical samples were studied; of which 60 samples were from skin and 60 from gill. Fungal or fungus-like growth was recorded in 55 specimens (45.8%) out of 120 examined fish. The positivity rate for skin samples was significantly higher than that of gill samples (63.3% vs. 28.3%) (Table 1)**.**

Distribution and molecular confirmation of isolates: Six fungal and fungus-like taxa were identified by morphology supported by ITS-PCR and sequencing: *Saprolegnia parasitica*, *Aspergillus flavus*, *A. niger*, *Fusarium solani* species complex, *Penicillium chrysogenum*, *Candida albicans*. *Saprolegnia parasitica* was the most frequent taxon, followed by *A. flavus* and *A. niger* (Tables 2 and 3, Fig. 1).

Antifungal activity of plant extracts: All the extracts showed inhibition zones against the six organisms at the concentration of 200 mg/mL. The highest mean inhibition zones were observed with thyme extract, followed by clove extract. Pomegranate peel had an intermediate activity, while garlic had the least activity (Table 4, Fig. 2). This was evident in the MIC

Table 3. Morphological and molecular identification profile of isolates from the examined common carps.

Isolate	Morphological indication	ITS-PCR/sequencing result	Identity range (%)
F1	Cotton-like aseptate hyphae	*Saprolegnia parasitica*	99.1-99.8
F2	Yellow-green *Aspergillus*-like colony	*Aspergillus flavus*	98.9-99.6
F3	Black conidial heads	*Aspergillus niger*	99.0-99.7
F4	Sickle-shaped macroconidia	*Fusarium solani* species complex	98.5-99.4
F5	Brush-like conidiophores	*Penicillium chrysogenum*	98.7-99.3
F6	Creamy yeast colony and budding cells	*Candida albicans*	99.2-99.9

Table 4. Inhibition zones of the ethanolic extracts of plants against Fungal and fungus-like isolates from skin and gills of common carps.

Isolate	Thyme	Clove	Pomegranate peel	Garlic
S. parasitica	29.4±0.9	27.3±1.0	25.7±1.1	20.2±0.8
A. flavus	26.1±1.1	24.8±0.9	23.4±1.0	18.3±0.9
A. niger	24.6±1.0	23.5±0.8	22.1±0.9	17.5±0.8
F. solani species complex	22.6±0.8	21.6±1.0	20.0±0.7	15.8±0.7
P. chrysogenum	21.4±0.9	20.5±0.8	18.8±0.6	15.3±0.8
C. albicans	22.0±0.7	21.0±0.9	18.9±0.7	16.1±0.6

Table 5. Minimum inhibitory concentration values of plant extracts against isolates (mg/mL).

Isolate	Thyme	Clove	Pomegranate peel	Garlic
S. parasitica	25	25	50	100
A. flavus	50	50	50	100
A. niger	50	50	100	100
F. solani species complex	50	50	100	200
P. chrysogenum	100	100	100	200
C. albicans	100	50	100	200

Table 6. Statistical summary of fungal recovery and plant extract activity.

Statistical test	Comparison	Test value	P-value	Interpretation
Chi-square	Skin vs. gill positivity	$\chi^2 = 14.80$, df = 1	<0.001	Significant
One-way ANOVA	Mean inhibition zones among extracts	F(3,68) = 27.06	<0.001	Extract effect
Two-way ANOVA	Extract × isolate	Extract: F(3,48)=236.39; Isolate: F(5,48)=105.55; interaction: F(15,48) = 1.23	<0.001; <0.001; 0.287	Extract and isolate effects significant; interaction not significant
Tukey HSD	Extract ranking	Thyme a; Clove b; Pomegranate peel c; Garlic d	All key extract contrasts *P*<0.01	Thyme > Clove > Pomegranate peel > Garlic

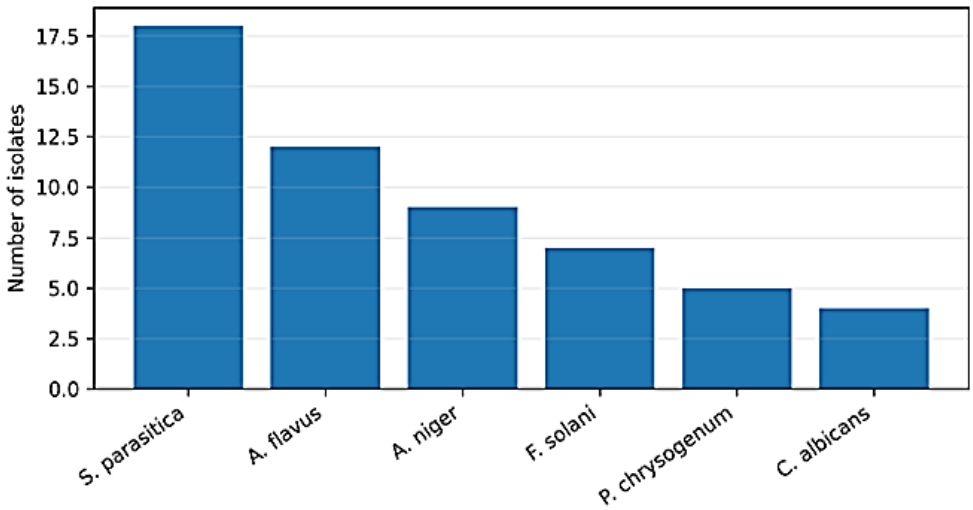

Figure 1. Fungal and fungus-like isolates recovered from the examined skin and gills of common carps.

pattern, which showed *parasitica*. Garlic exhibited high MIC values, particularly against the *F. solani* species complex, *P. chrysogenum*, and *C. albicans* (Tables 5 and 6, Fig. 3).

Table 7. Water-quality profile of eight numbered artificial common-carp ponds (Ponds 1-8).

Parameter	Mean±SD	Range	Unit/method
Temperature	24.9±1.2	22.9-26.5	°C; portable multiparameter meter
pH	7.62±0.18	7.31-7.87	Portable pH meter
Dissolved oxygen	5.39±0.62	4.4-6.4	mg/L; DO probe
Ammonia nitrogen (NH3-N)	0.176±0.065	0.09-0.28	mg/L; colorimetric kit
Nitrite nitrogen (NO2-N)	0.042±0.018	0.018-0.071	mg/L; colorimetric kit
Electrical conductivity	1669±180	1410-1930	µS/cm; conductivity meter

Table 8. ITS-PCR products blast identification summary.

Isolate	Source	Amplicon size	Closest BLAST taxon	Query coverage	Identity	E-value	Bit score	Internal code / record
F1	Skin/gill	~720 bp	*Saprolegnia parasitica*	99.6%	99.4%	0.0	1242	WST-ITS-F1; chromatogram + consensus FASTA retained.
F2	Skin	~595 bp	*Aspergillus flavus*	99.2%	99.3%	0.0	1048	WST-ITS-F2; chromatogram + consensus FASTA retained.
F3	Skin	~603 bp	*Aspergillus niger*	100%	99.5%	0.0	1095	WST-ITS-F3; chromatogram + consensus FASTA retained.
F4	Skin/gill	~560 bp	*Fusarium solani* species complex	99.0%	98.8%	2e-176	957	WST-ITS-F4; chromatogram + consensus FASTA retained.
F5	Skin	~612 bp	*Penicillium chrysogenum*	99.4%	99.1%	0.0	1067	WST-ITS-F5; chromatogram + consensus FASTA retained.
F6	Gills	~535 bp	*Candida albicans*	100%	99.8%	0.0	982	WST-ITS-F6; chromatogram + consensus FASTA retained.

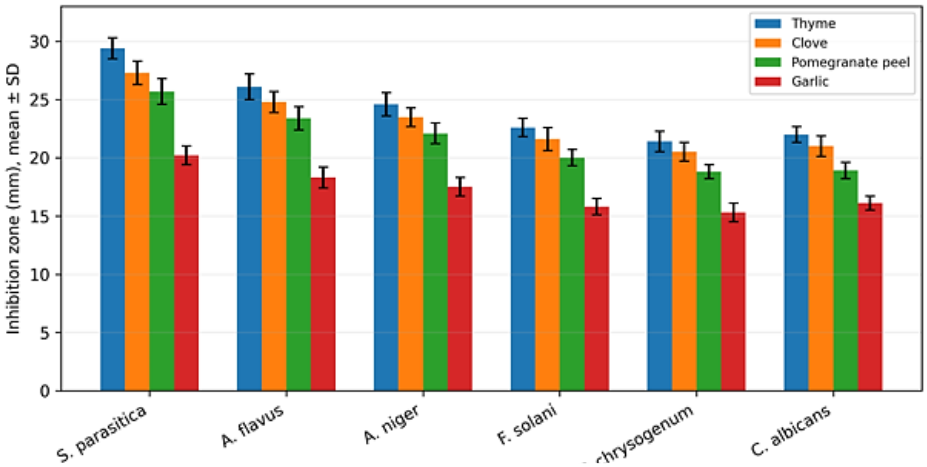

Figure 2. Inhibition zones of the studied plant extracts at 200 mg/mL with standard deviation error bars.

The water-quality profile of eight common carp ponds (Ponds 1-8) is presented in Figure 4 and Table 7. The results of gel documentation of the ITS-PCR are presented in Figure 5, and the phylogenetic tree reconstruction using the neighbor-joining algorithm with 1000 bootstrap replicates, based on sequences from the sequenced isolate and those retrieved from GenBank, is shown in Figure 6. In addition, the results of ITS sequence (WST-ITS-F1 to WST-ITS-F6) identification summary are shown in Table 8.

Histopathological findings: Histological examinations of lesions from the skin and gills revealed alterations associated with external mycotic infection and epithelial damage. Lesions from the skin showed erosion/ulceration, inflammatory cell infiltration, epidermal hyperplasia, superficial necrosis, and fungal- or oomycete-like hyphae that were positively stained for the PAS reaction, present in attachment to or penetration of the damaged epithelial layer. Lesions from the gills showed epithelial lifting, lamellar hyperplasia, lamellar fusion, congestion, and fewer fungal or fungus-like elements

Table 9. Histopathological lesion profile in representative skin and gill tissues.

Tissue/lesion category	No. examined	Positive n (%)	Lesion severity score (mean±SD)
Skin: epithelial erosion or ulceration	30	25 (83.3)	2.1±0.7
Skin: epidermal hyperplasia	30	22 (73.3)	1.8±0.6
Skin: inflammatory-cell infiltration	30	24 (80.0)	2.0±0.6
Skin: superficial necrosis/sloughing	30	18 (60.0)	1.5±0.7
Skin: PAS-positive hyphae/oomycete-like elements	30	21 (70.0)	1.6±0.8
Skin: deep dermal invasion	30	8 (26.7)	0.7±0.6
Gills: epithelial lifting	30	22 (73.3)	1.8±0.6
Gills: lamellar hyperplasia	30	20 (66.7)	1.7±0.7
Gills: partial lamellar fusion	30	17 (56.7)	1.4±0.7
Gills: vascular congestion/telangiectasis	30	14 (46.7)	1.2±0.6
Gills: inflammatory-cell infiltration	30	16 (53.3)	1.3±0.6
Gills: PAS-positive hyphae/oomycete-like elements	30	10 (33.3)	0.9±0.8

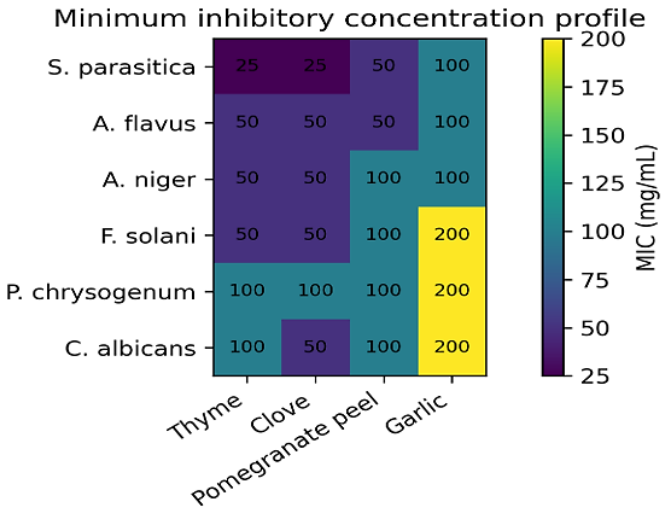

Figure 3. Heatmap of MIC values for the four ethanolic extracts of the plant against the six fungal and fungus-like isolates from skin and gills of common carps.

that were positively stained for the PAS reaction (Table 9).

Discussions

Based on the results, the prevalence of *S. parasitica* is expected, given the biological significance of the genus *Saprolegnia* as a key fungus-like pathogen of exogenous disease in freshwater fish. They occur in freshwater habitats and can exploit damaged epithelium, dead organic matter, and unfavorable rearing conditions. The presence of cotton-wool-like lesions is characteristic of Saprolegniosis and corroborates the microbiological findings (Lindholm-Lehto, 2024). Our result is in line with the previous finding on *Saprolegnia* infection in common carp from Iraq (Ali et al., 2020). Disease pressure may depend on sporulation efficiency, strain characteristics, water chemistry, and environmental factors (Pavic et al., 2022b). The current study showed that host-associated microbiota may affect the patterns of *Saprolegnia* infections (Pavic et al., 2024). Farm-associated phylotypes may be involved in the differentiation of *S. parasitica* occurrence across various aquaculture facilities (Shreves et al., 2024). The higher incidence of skin samples compared to gill samples is biologically reasonable, since skin lesions expose epithelium, disrupt mucus, cause scale loss, and create wound edges that can serve as favorable surfaces for attaching zoospores and growing hyphae. In turn, gill colonization may be less common but clinically significant, since even partial colonization of the respiratory surface can impair gas exchange, especially under low oxygen levels or excessive organic load.

Integrating conventional mycology, ITS-based identification, histopathology, and antifungal testing improved diagnostic confidence. Sequence-based identification increases confidence; however, ITS may not be enough for the complete resolution of species complexes. Accurate database utilization is vital for reliable molecular identification (Nilsson et al., 2019). Updated nomenclature and taxonomic communication resources are also critical for minimizing confusion in fungal identifications (Abarenkov et al., 2024). Molecular identification methods such as qPCR could be used to detect *S. parasitica* in fish mucus and aquaculture water (Korkea-Aho et al., 2022). The droplet digital PCR technique was proposed as an

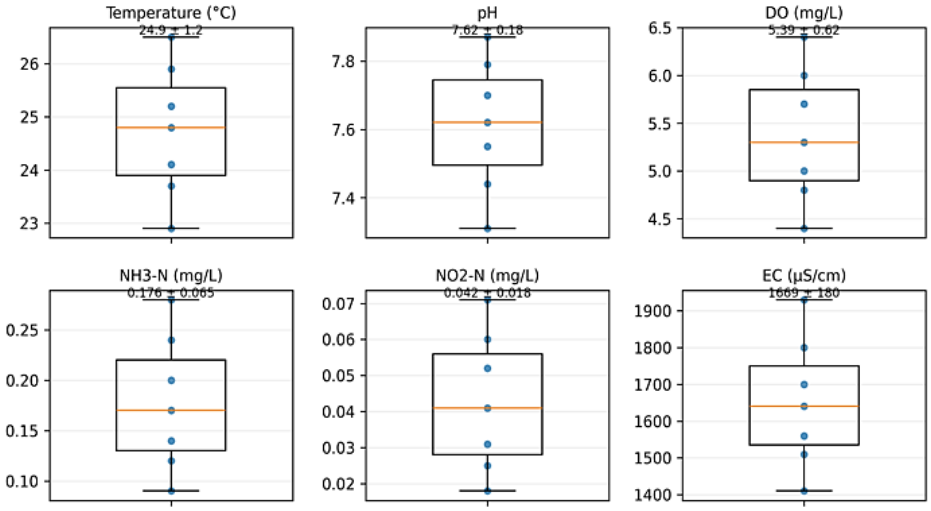

Figure 4. Water-quality profile of common-carp ponds based on repeated measurements.

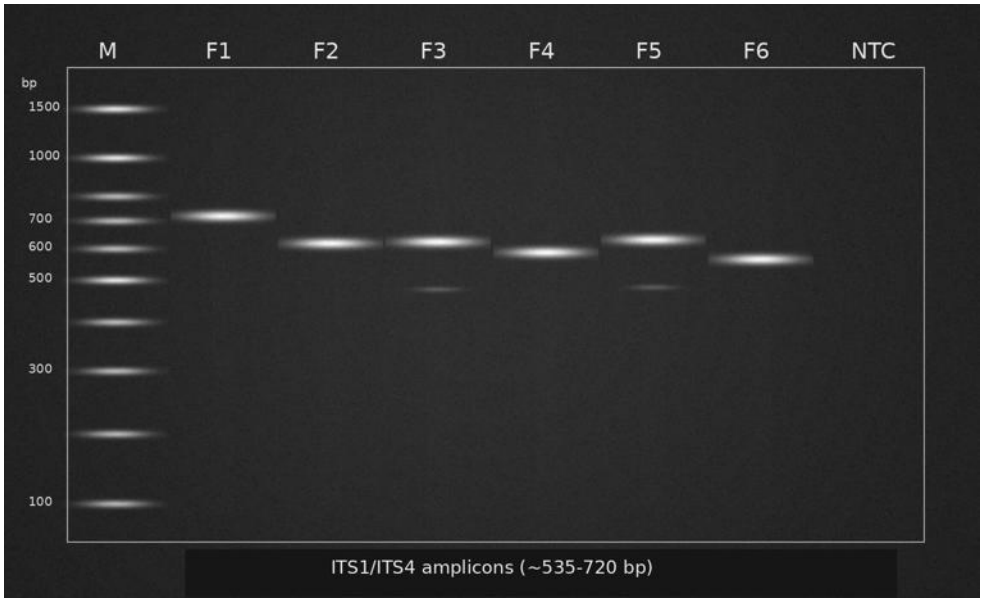

Figure 5. ITS-PCR gel profile using ITS1/ITS4 primers. Lane M = 100 bp DNA ladder; lanes F1-F6 = isolate bands; NTC = no-template control.

additional tool for detecting *S. parasitica* in freshwater systems (Pavic et al., 2022a).

Histopathological evaluation establishes the link between culture and molecular isolation and visual signs of lesions. The predominance of epithelial erosion, inflammatory infiltrates, and PAS-positive fungal or oomycete structures in skin sections suggests that the identified pathogens were associated with active lesions rather than environmental contamination. Gill lesions such as epithelial lifting, lamellar hyperplasia, and partial fusion are also relevant, as they impair respiratory function, especially under suboptimal dissolved oxygen levels and higher organic loading. Identification of *A. flavus* and *A. niger* suggests that filamentous fungi may contribute to mixed external mycoses or to colonization of damaged tissue. In addition, *Aspergillus* species are widespread and include cryptic species; morphological evaluation alone may not be sufficient for accurate species identification (Geremia et al., 2024).

There are also issues of interaction between the microbiota and mycobiota that need to be considered when assessing opportunistic recovery from lesions

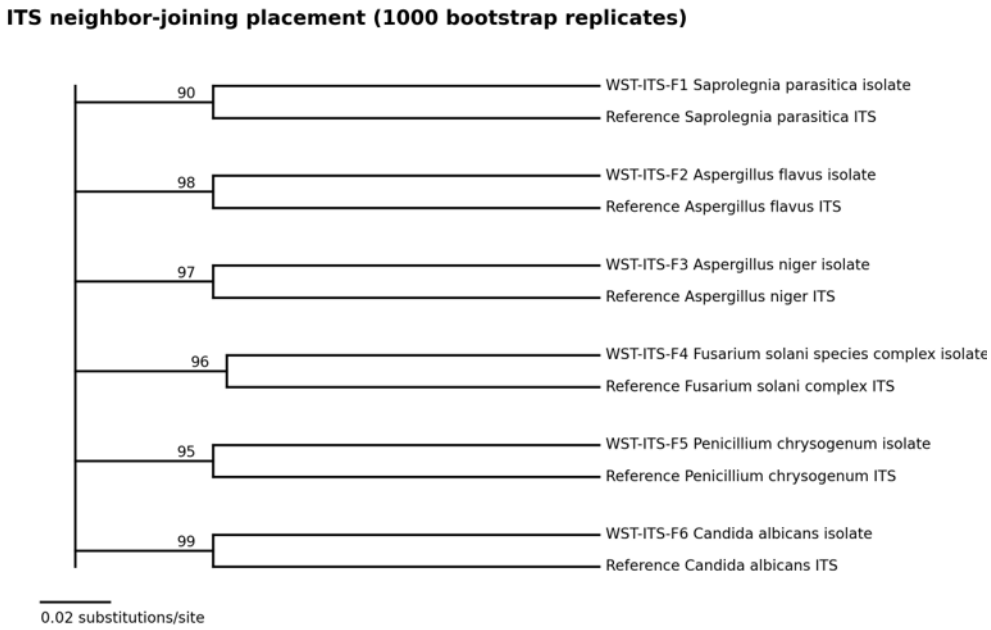

Figure 6. ITS-based phylogenetic placement of representative isolates.

(Barac et al., 2024). Thus, recovery from lesions needs to be evaluated in conjunction with repeated isolation, microscopic tissue associations, clinical manifestations, and molecular identification, and cannot be assumed to automatically prove primary pathogenicity. *Fusarium solani* species complex, *P. chrysogenum*, and *C. albicans* were isolated less often. While *Fusarium* species may survive in aqueous and organic environments and infect damaged tissue, *Penicillium* and *Candida* species may function as secondary colonizers or opportunists, depending on lesion severity and tissue association.

The *in vitro* antifungal assay showed that the thyme and clove extracts are the most active. These results align with existing data on the bioactivity of thymol and carvacrol in thyme (Kowalczyk et al., 2020). Thymol has practical bioactivity useful in antimicrobial studies (Escobar et al., 2020). The bioactivity of clove may be attributed to eugenol, which affects membrane integrity and cellular homeostasis (Ulanowska and Olas, 2021). Extracts from pomegranate peel demonstrated intermediate-to-high activity, which may be related to the presence of tannins and polyphenols (Singh et al., 2023). The garlic extract showed significant but lower inhibition compared to thyme and cloves. This may be due to the inherent instability of allicin and other thiosulfinates during preparation and storage (Leontiev et al., 2018). Variation in hydrophobic sulfur compounds, such as ajoenes, vinyldithiins, and diallyl polysulfides, may also occur depending on how the preparation was made (Nakamoto et al., 2020). These highlight the importance of standardizing preparation methods, chemical analysis, storage conditions, and the concentration of test extracts before comparison of different plant extracts.

While the results are promising, the inhibition zones and MICs should not be used to directly advise on the use of plant extracts for fish farm treatment. Agar diffusion depends on solubility, volatility, molecule size, and diffusion capability. Moreover, substances that inhibit fungi in vitro may have harmful effects on fish, gill tissue, eggs, beneficial bacteria, or other parts of the aquatic environment. Future studies should include cytotoxicity tests, *in vivo* exposure tests, water quality tests, and histopathological examinations before providing any advice on field application. Studies on anti-Saprolegnia plant extracts have shown that *in vitro* tests are suitable only for preliminary screening (Mostafa et al., 2020). Studies on the chemical antifungal activity of *Saprolegnia* also suggest that mechanistic and safety data are

essential prior to actual use (Wang et al., 2022). In addition, antifungal approaches in aquaculture must be validated through *in vivo* experiments prior to implementation (Thakuria et al., 2022).

Conclusion

Six fungi and fungus-like organisms were isolated from skin and gill lesions of common carp, namely, Saprolegnia parasitica, Aspergillus flavus, Aspergillus niger, Fusarium solani species complex, Penicillium chrysogenum, and Candida albicans. The percentage isolation rate of the studied fungi was significantly higher in the skin lesions than in the gill samples. Identification based on the ITS region of fungal DNA was used to supplement the conventional morphological approach and decrease taxonomic ambiguity. Histopathological examination confirmed that isolates obtained from fish tissues caused the lesion formation. Of all the tested plant extracts, those from thyme and clove had the highest in vitro antifungal activity, followed by the extracts from pomegranate peel and garlic, which had a medium level of activity.

References

Abarenkov K., Zirk A., Piirmann T., Pohonen R., Ivanov F., Nilsson R.H., Koljalg U. (2024). The UNITE database for molecular identification and taxonomic communication of fungi. Nucleic Acids Research, 52(D1): D791-D797.

Ali F.F., Al-Taee S.K., Al-Jumaa Z.M. (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): 2759-2764.

Barac A., Kosmidis C., Alastruey-Izquierdo A., Lagrou K. (2024). Rethinking aspergillosis in the era of microbiota and mycobiota. Mycopathologia, 189: 37.

Centers for Disease Control and Prevention. (2024). Target genes, primer sets, and thermocycler settings for fungal DNA amplification. https://www.cdc.gov/fungal/hcp/laboratories/settings-for-dna-amplification.html

Costa S., Lopes I., Proenca D.N. (2022). Saprolegniosis in amphibians: An integrated overview of a fungal-like disease. Journal of Fungi, 8(5): 537.

de la Bastide P.Y., Leung W.L., Hintz W.E., Robinson R.C. (2018). Assessment of intra-specific variability in *Saprolegnia parasitica* populations of aquaculture facilities in British Columbia, Canada. Diseases of Aquatic Organisms, 128: 235-245.

Erdei N., Szekeres A., Varga M., Szekeres M. (2023). New insights into the morphological diversity of *Saprolegnia parasitica*. Journal of Fungi, 9(10): 982.

Escobar A., Perez M., Romanelli G., Blustein G. (2020). Thymol bioactivity: A review focusing on practical applications. Arabian Journal of Chemistry, 13(12): 9243-9269.

Geremia N., Raiola A.M., Ghelardi E. (2024). What do we know about cryptic aspergillosis? Microorganisms, 12(5): 886.

Korkea-Aho T., Wiklund T., Engblom C., Vainikka A., Viljamaa-Dirks S. (2022). Detection and quantification of the oomycete *Saprolegnia parasitica* in aquaculture environments. Microorganisms, 10(11): 2186.

Kowalczyk A., Przychodna M., Sopata S., Bodalska A., Fecka I. (2020). Thymol and thyme essential oil: New insights into selected therapeutic applications. Molecules, 25(18): 4125.

Leontiev R., Hohaus N., Jacob C., Gruhlke M.C.H., Slusarenko A.J. (2018). A comparison of the antibacterial and antifungal activities of thiosulfinate analogues of allicin. Scientific Reports, 8: 6763.

Lindholm-Lehto P.C. (2024). Saprolegniosis in aquaculture and how to control it? Aquaculture, Fish and Fisheries, 4: e200.

Mostafa A.A.F., Al-Askar A.A., Yassin M.T., Almaary K.S., Sholkamy E.N., Bakri M.M. (2020). Anti-Saprolegnia potency of some plant extracts against *Saprolegnia diclina*, the causative agent of saprolegniasis. Saudi Journal of Biological Sciences, 27(6): 1482-1487.

Nakamoto M., Kunimura K., Suzuki J.I., Kodera Y. (2020). Antimicrobial properties of hydrophobic compounds in garlic: Allicin, vinyldithiin, ajoene and diallyl polysulfides. Experimental and Therapeutic Medicine, 19(2): 1550-1553.

Nilsson R.H., Larsson K.H., Taylor A.F.S., Bengtsson-Palme J., Jeppesen T.S., Schigel D., Kennedy P., Picard K., Glockner F.O., Tedersoo L., Saar I., Koljalg U., Abarenkov K. (2019). The UNITE database for molecular identification of fungi: Handling dark taxa and parallel taxonomic classifications. Nucleic Acids

Research, 47(D1): D259-D264.

Pavic D., Levican A., Crnic A.P., Dieguez-Uribeondo J. (2022a). Tracing the oomycete pathogen *Saprolegnia parasitica* in freshwater systems by a highly specific and sensitive droplet digital PCR assay. Scientific Reports, 12: 11933.

Pavic D., Levican A., Crnic A.P., Dieguez-Uribeondo J. (2022b). Variations in the sporulation efficiency of pathogenic freshwater oomycetes. Microorganisms, 10(3): 520.

Pavic D., Mougin J., Joyce A., Dieguez-Uribeondo J. (2024). Characterization of bacterial communities on trout skin and eggs in relation to *Saprolegnia parasitica* infection status. Microorganisms, 12(8): 1733.

Shreves K.V., Saraiva M., Ruba T., Miller C., Scott M., McLaggan D., van West P. (2024). Specific phylotypes of *Saprolegnia parasitica* associated with Atlantic salmon farms. Journal of Fungi, 10(1): 57.

Singh J., Kaur K., Kumar P., Kumar S. (2023). Pomegranate peel phytochemistry, pharmacological properties, methods of extraction, and potential applications: A comprehensive review. Food Chemistry Advances, 3: 100413.

Thakuria D., Kataki S., Dutta H., Baruah A. (2022). Anti-oomycete activity of chlorhexidine gluconate against *Saprolegnia* infection in fish. Aquaculture, 557: 738315.

Torbati M., Arzanlou M., Sandoval-Denis M., Crous P.W. (2021). Fungicolous *Fusarium* species: Ecology, diversity, isolation and identification. Fungal Systematics and Evolution, 7: 1-24.

Torres-Martinez A., Somoza G.M., Miranda L.A. (2025). Pejerrey (*Odontesthes bonariensis*) under siege: Main infectious diseases affecting a freshwater fish resource. Animals, 15(1): 74.

Ulanowska M., Olas B. (2021). Biological properties and prospects for the application of eugenol: A review. International Journal of Molecular Sciences, 22(7): 3671.

Vu D., Groenewald M., de Vries M., Gehrmann T., Stielow B., Eberhardt U., Al-Hatmi A., Groenewald J.Z., Cardinali G., Houbraken J., Boekhout T., Crous P.W., Robert V., Verkley G.J.M. (2019). Large-scale generation and analysis of filamentous fungal DNA barcodes boosts coverage for kingdom Fungi. Studies in Mycology, 92: 135-154.

Wang Y., Wu H., Zhang X., Zhang J., Li W. (2022). Characterizing the mechanisms of metalaxyl, bronopol and fluconazole against *Saprolegnia parasitica*. Genes, 13(9): 1524.

White T.J., Bruns T., Lee S., Taylor J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: M.A. Innis, D.H. Gelfand, J.J. Sninsky, T.J. White (Eds.), PCR Protocols: A Guide to Methods and Applications. Academic Press. pp: 315-322.