Print ISSN: 0375-9237
Online ISSN: 2357-0350

EGYPTIAN JOURNAL OF BOTANY (EJBO)

Biodiversity and seasonality of zoosporic fungi in four water areas of the Nile system (Egypt) and the potentiality of Achlya ambisexualis for polymeric materials degradation

Om Kalthoum H. Khattab, Abdel-Raouf M. Khallil, Yasmin M. Elsaba, Hadeer A. Badawy

PUBLISHED BY
THE EGYPTIAN
BOTANICAL SOCIETY

Biodiversity and seasonality of zoosporic fungi in four water areas of the Nile system (Egypt) and the potentiality of Achlya ambisexualis for polymeric materials degradation

Om Kalthoum H. Khattab[1], Abdel-Raouf M. Khallil[2], Yasmin M. Elsaba[1], Hadeer A. Badawy[1]
[1]Department of Botany and Microbiology, Faculty of Science, Helwan University, Helwan, Egypt
[2]Department of Botany and Microbiology, Faculty of Science, Assiut University, Assiut, Egypt

To unravel the importance of zoosporic fungi in their participation in the cycling of organic matter and humification processes in aquatic ecosystems, this study was conducted to show the biodiversity and seasonality of zoosporic fungi, as well as their potential to degrade several polymeric materials. Using the baiting technique, twenty-nine species related to seven zoosporic fungal genera were collected during four seasons (autumn 2021–summer 2022) from the Nile River (from Helwan and El-Manial regions) and irrigation canals (El-Ismailia and El-Ibrahimia Canals) in Egypt. Some physicochemical parameters of water were evaluated in correlation with zoosporic fungi's occurrence, diversity, and seasonality. Out of isolated fungal taxa, only one species is related to one genus of true zoosporic fungi (i.e., *Allomyces*). In contrast, twenty-eight species are related to six genera of straminipiles fungi (i.e., *Achlya*, *Aphanomyces*, *Dictyuchus*, *Phytophthora*, *Pythium*, and *Saprolegnia*). The Principal Coordinates Analysis (PCoA) was applied to reveal the correlation between the determined abiotic factors and particular species occurrence. The Shannon and Simpson indices were used to estimate species diversity, and fungal dominance and evenness were assessed to illustrate the richness of the recovered fungal taxa. The current study represents the first research concerning the survey, biodiversity, and seasonality of zoosporic fungi in most of the experimented water bodies. The most prevalent isolate, *Achlya ambisexualis*, was selected for molecular identification and tested for its potential to produce cellulase, amylase, protease, lipase, and asparaginase. This study remarkably suggests producing asparaginase (anticancer) utilizing the experimented fungus for the first time.

Keywords: Zoosporic fungi, Seasonal fluctuations, Fungal biodiversity, Principal Coordinates Analysis (PCoA), *Achlya ambisexualis*, Asparaginase

ARTICLE HISTORY
Submitted: May 19, 2024
Accepted: July 20, 2024

CORRESPONDANCE TO
Hadeer A. Badawy,
Department of Botany and Microbiology, Faculty of Science, Helwan University, Helwan, Egypt
Email: Hadeer.ashraf.badawy@gmail.com
ORCID iD: 0009-0003-3376-6678
DOI: 10.21608/ejbo.2024.291008.2850

EDITOR
Prof. Neveen Mahmoud Khalil,
Department of Botany and Microbiology, Faculty of Science, Cairo University Egypt
Email: neveen@sci.cu.edu.eg

INTRODUCTION

Aquatic phycomycetes, investigated by Sparrow (1960), are not a monophyletic group and include taxa whose evolutionary histories are now known to be highly diverse (Baldauf, 2008; Gleason et al., 2017). The term “aquatic phycomycetes” is no longer used, and these organisms are frequently referred to as zoosporic true fungi and zoosporic fungus-like microorganisms (heterotrophic Straminipila) or by the names of the relevant phyla they have been assigned (Gleason et al., 2017). The true zoosporic fungi form a diverse paraphyletic assemblage at the base of the fungal phylogeny and comprise the superphylum Chytridiomyceta (phyla Chytridiomycota, Monoblepharidomycota, and Neocallimastigomycota), the phyla Blastocladiomycota, Olpidiomycota, and Rozellomycota/Cryptomycota (Naranjo-Ortiz & Gabaldón, 2019; Tedersoo et al., 2018). Although Straminipila (Hyphochytriomycota, Oomycota, and Labyrinthulomycota) frequently inhabit environments similar to those of true fungi and share with them many ecological functions, trophic strategies, morphological, and physiological characteristics, they are not considered to be true fungi (Beakes et al., 2014). To simplify the terminology in research that focuses on these groups together, the term "zoosporic fungi" refers to both groups (aquatic oomycetes and true zoosporic fungi) (Czeczuga & Muszyńska, 2004). These organisms are widespread in aquatic environments (Barr, 2001; Sparrow, 1960) and play a pivotal role in microbial ecology (Gleason et al., 2017; Voigt et al., 2013). They are primarily recognized for their role as decomposers of persistent organic matter, such as cellulose, chitin, and sporopollenin (Kiziewicz & Kurzątkowska, 2004; Powell, 1993), as well as their known pathogenicity of *Saprolegnia* and *Aphanomyces* in freshwater animals (Masigol et al., 2023). These organisms exhibit high abundance and diversity on plant debris for decomposition (Nikolcheva & Bärlocher, 2004) and are essential components of food webs (Jephcott et al., 2016). The chemotactic zoospore, the primary means of dispersal and substrate infection (Gleason & Lilje, 2009), is the distinguishing feature of zoosporic fungi. They can use various trophic strategies, including mutualism, parasitism, and saprotrophism (Gleason et al., 2017; Voigt et al., 2013).

Freshwater ecosystems exhibit many variances in terms of their physical and chemical characteristics. Variations in abiotic factors appear to be responsible for changes in the distribution, species composition, and structure of the mycobiota assemblages in terms of ecosystem dynamics (Marano et al., 2011; Shearer

et al., 2007). In addition, one of the key variables impacting the colonization and diversity of freshwater fungi is the sampling season (Cai et al., 2003; Luo et al., 2004; Nikolcheva & Bärlocher, 2005).

Zoosporic fungi are an integral part of any ecosystem biota. However, their role in aquatic ecosystems receives little attention. These fungi in aquatic ecosystems play a key role in the energy transfer from allochthonous and autochthonous particles to other food chain components. Fungal enzymes have gained global recognition for their many applications across multiple industries, such as the food, pharmaceutical, agricultural, chemical, and petroleum sectors (Abdel-Azeem et al., 2021; Ali et al., 2020; Latif et al., 2021; Tammam et al., 2022; El-Fallal et al., 2022). These industries are becoming increasingly popular because of their advantages, which include shorter processing times, lower costs, nontoxicity, higher-quality products, increased efficiency, lower energy input requirements, and environmental friendliness.

Although numerous studies have been conducted on zoosporic fungi in the Nile system in Egypt (Ali, 2007; El-Hissy et al., 1982; Farida et al., 1993; Khallil et al., 1995; Khallil et al., 2020; Moharram et al., 1990), the seasonal fluctuations and biodiversity of zoosporic fungi in the current study areas have received insufficient attention. Therefore, the current study attempts to address this gap and aims to track the seasonality and biodiversity of heterotrophic straminipiles (fungi-like organisms) and true zoosporic fungi in correlation with some physicochemical characteristics of the water in the target study areas. Since the function of zoosporic fungi in aquatic ecosystems (mainly due to their participation in the cycling of organic matter) is receiving increasing demand, the potential of the commonest isolate was tested for some extracellular enzymatic activities.

METHODS

Description of study areas and sampling sites

The study water areas lie in Great Cairo (Cairo and Al-Qalyubia Governorates) and Upper Egypt (Assiut Governorate). Cairo Governorate is located on the banks and islands of the Nile in the north of Egypt, and it is confined between latitudes 22° and 32° north and longitudes 25° and 37° east. In Cairo, the summers are long, hot, humid, arid, and clear, and the winters are cool, dry, and mostly clear. Over the year, the air temperature typically varies from 10 °C to 35.5 °C and is rarely below 7.7 °C or above 38.8 °C. It is also characterized by little rainfall. Al-Qalyubia Governorate is located directly north of Cairo Governorate on the Nile Delta. It is located within 30° north latitude and 31° east longitude, and its height is 19 meters above sea level. It enjoys a semi-tropical desert climate. Over the course of the year, the temperature typically varies from 10.6 °C to 35.6 °C and is rarely below 7.8 °C or above 38.9 °C. The Assiut Governorate (Upper Egypt; 365 km south of Cairo) is situated on the banks of the Nile and is bordered on both sides by a chain of eastern and western mountains. It is confined between latitudes 13° and 27° north and longitudes 14° and 30° east. In Assiut, the summers are long, sweltering, arid, and clear, and the winters are short, cool, dry, and mostly clear. Over the course of the year, the temperature typically varies from 7.7 °C to 38.3 °C and is rarely below 4.4 °C or above 42.2 °C. It is also characterized by little rainfall. El-Ibrahimia irrigation Canal (the biggest irrigation Canal in Egypt) originates from the Nile River at Assiut city, extends about 365 km, and represents the main source for irrigation in middle Egypt Governorates. El-Ismailia Canal originates from the Nile River in Cairo and extends east to El-Ismailia City (Qanal region). The experimented water samples were collected seasonally from El-Ibrahimia Canal (Assiut governorate; Upper Egypt) as well as from three different water bodies (in Great Cairo), either from the Nile River (Helwan and El-Manial regions) or El-Ismailia irrigation Canal (Mostorod region; Qalubyia Governorate), as depicted in Figures 1 and 2.

Collection of samples

Surface water samples were collected seasonally from the four described study areas (Helwan region, El-Manial region, El-Ismailia Canal, and El-Ibrahimia Canal) during the period from autumn 2021 to summer 2022. Water samples were collected in clean and sterile autoclavable glass bottles with a 250-ml capacity. From each of the experimented water areas, five water samples were collected from different points (at about 50-meter intervals) in the bottles containing either sterilized sesame seeds or sterilized halves of maize grains serving as baits. For the determination of some physico-chemical features, water samples (one bottle with a 1 l capacity) were also collected concomitantly from the same water areas and directly brought to the laboratory and

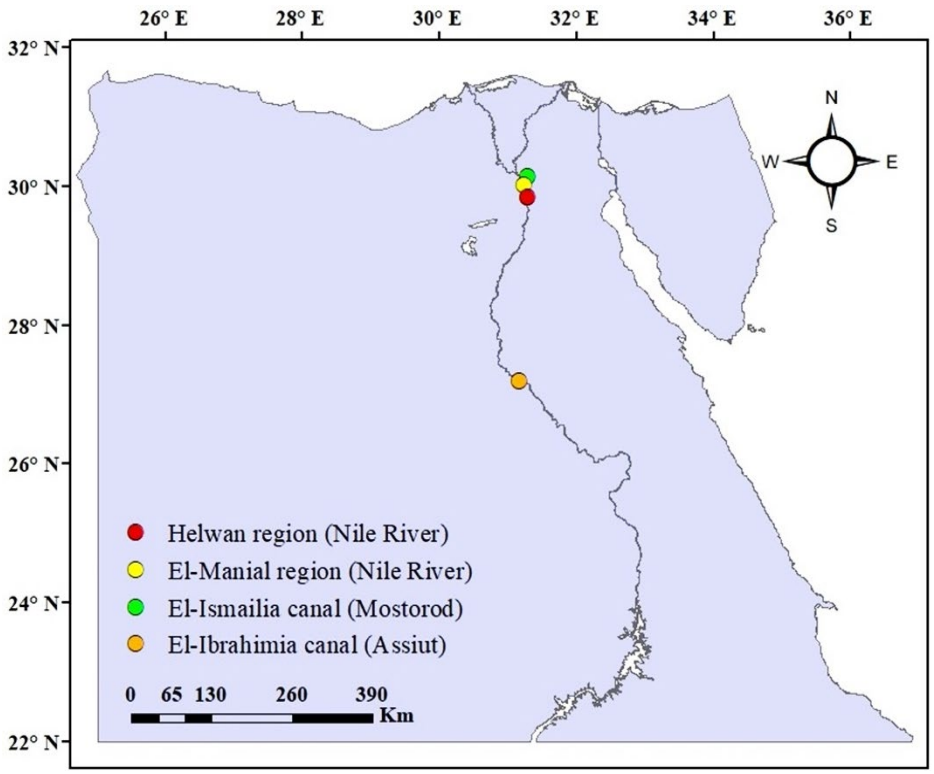

Figure 1. A map showing location the four study areas generated in ArcGIS 10.5.

Figure 2. The four sampling water areas: Nile River: (A) Helwan region and (B) El-Manial region Irrigation canals: (C) El-Ismailia Canal and (D) El-Ibrahimia Canal.

analyzed for selected physico-chemical characteristics, as well as for the recovery of the target heterotrophic straminipiles and true zoosporic fungi.

Limnological study related to physico-chemical characteristics of water

Some physico-chemical characteristics of water samples, such as water temperature, dissolved oxygen, pH, total soluble salts, and organic matter, were determined. Temperature and dissolved oxygen were measured in situ; temperature was measured with a field probe thermometer (liquid mercury in a glass tube), and dissolved oxygen was measured using a dissolved oxygen meter (HANNA Instruments model H1 9145 microprocessor). The pH was measured ex-situ using a pH meter (Jenway Model 3510 pH meter). Total soluble salts were estimated by filtering a known volume of water and evaporating in an oven overnight at 105°C. The dry residue was then weighed, and the amount of total soluble salts per one liter of water was calculated. The organic matter contents were chemically estimated based on Jackson (1973).

Mycological Analysis

Heterotrophic straminipiles and true zoosporic fungi were isolated from the collected water samples using the baiting technique with sterilized sesame seeds and halves of maize grains (Khallil, 1990; El-Hissy and Khallil, 1991). Surface water samples from each site (about 100 ml) were poured under aseptic conditions into sterilized 15-cm Petri dishes (five replicates), some plates containing sterilized sesame seeds (5 seeds for each), and others containing sterilized halves of maize grains (5 halves for each). These dishes were then left overnight at room temperature (El-Hissy and Khallil, 1991). The colonized seeds and grains were then transferred into sterilized 9-cm Petri dishes containing sterile filtered Canal or Nile water with the addition of chloramphenicol (250 mg/l) (Roberts, 1963) to suppress bacterial growth. The dishes were then incubated at 20±2 °C for one month, during which they were examined daily for the growth and identification of zoosporic fungal taxa. After that, the colonized baits were transferred into new Petri dishes with sterilized, filtered Canal or Nile water to refresh their growth. Following their nourishment, these organisms were cultivated on sterile culture media: glucose peptone (GP), as described by Willoughby and Pickering (1977). The recovered taxa were purified and preserved by multiple transfers to new substrates due to difficulties in culturing on agar media. For the determination of the fungal population of zoosporic fungi, the fungal species appearing on one water sample were counted as one colony (CFU). The fungal cultures were maintained on GP media, stored at 8–12 °C, and subcultured every 2-3 months. The occurrence (O), occurrence remarks (OR), and population (P) for each recovered zoosporic taxon were calculated from the total number of collected water samples.

Identification and documentation

The identification of zoosporic genera and species was performed using microscopic examination based on morphological and cultural characteristics (Coker, 1923; Dick and Seymour, 1970; Johnson, 1971, 1977; Karling, 1977; Nikolcheva and Bärlocher, 2005; Scott, 1961; Sparrow, 1960; Waterhouse, 1956, 1968). Photographs were taken using an optical microscope (Olympus CX31PF, Tokyo, Japan) coupled with a camera (XCAM Family 1080P HDMI+USB Output). Some fungal isolates did not form sexual reproductive structures, so they were only identified at the genus level.

Statistical analysis

Principal Coordinate Analysis (PCoA) (Legendre, 1998) was applied to elucidate the correlation between the monitored abiotic factors and the occurrence of particular fungal species. Using PAleontological STatistics (PAST), Version 3.25, USA, the fungal taxa (s) refers to the number of isolated fungal species from collected samples from four water sites; dominance (d) defines the dominance of fungal taxa in assayed water sites in different seasons; the Simpson index illustrates the evenness of the zoosporic fungal communities; and the Shannon index is the diversity index that estimates the number of fungal individuals, taking into consideration the number of fungal taxa. The two-way clustering analysis of the recorded fungal communities within different seasons and the ANOVA (Analysis of Variance) were also estimated using the PAST program. Cluster analysis is used to determine the occurrence of fungal groups in response to various seasons. Moreover, this analysis is used to illustrate the significance of clustering between different fungal communities.

Molecular identification of the most prevalent fungal isolate

The fungal isolate was grown on glucose peptone (GP) (Willoughby and Pickering, 1977) and incubated at 20±2°C for 7 days. The whole procedure, from DNA extraction until the final step of DNA sequencing, was

performed by SolGent Company (Daejeon, South Korea). Fungal DNA was extracted and isolated by Solg™ Genomic DNA Prep Kit according to the manufacturer's protocol. The internal transcribed spacer (ITS) region of the ribosomal DNA was amplified using universal primers: ITS1 (5' - TCC GTA GGT GAA CCT GCG G - 3'), and ITS4 (5' - TCC TCC GCT TAT TGA TAT GC - 3'). Using an ABI 9700 thermal cycler, the polymerase chain reaction (PCR) mixtures were prepared using SolGent EF-Taq according to the manufacturer's protocol. The PCR product was further purified with the SolGent PCR Purification Kit-Ultra (SolGent, Daejeon, South Korea) prior to sequencing. Contigs were created from the sequencing data using the CLCBio Main Workbench program. The obtained sequences were further analyzed using BLAST from the National Center of Biotechnology Information (NCBI) website. Sequences obtained together with those retrieved from the GenBank database (http://www.ncbi.nlm.nih.gov) were subjected to the Clustal W analysis using MegAlign software version 5.05 (DNASTAR Inc., Madison, Wisconsin, USA) for the phylogenetic analysis (Thompson et al., 1994).

Screening of enzymes production

The current study analyzed the enzyme profile of the tested fungus to assess its capacity for producing a range of significant industrial enzymes. All media were inoculated with a 0.5 cm fungal disk from a 7-day-old fungal culture. The plates were incubated at 25°C for 72h. All measurements were made in triplicate.

Cellulase

The ability of cellulose breakdown was assessed by cultivating the fungal isolate on CMC agar medium (g/l), which consisted of carboxymethylcellulose (10.0 g/l), KH_2PO_4 (4.0 g/l), Na_2HPO_4 (4.0 g/l), tryptone (2.0 g/l), $MgSO_4.7H_2O$ (0.2 g/l), $CaCl_2.2H_2O$ (0.001 g/l), $FeSO_4.7H_2O$ (0.004 g/l), and agar (15.0 g/l), with a pH of 7.0 (Behera et al., 2017). Traces of Rose Bengal were added to the media as a cellulose degradation indicator. The presence of a clear zone, which can be enhanced by re-incubating the plates at 50°C for 4–8 hours around the growth, was considered a positive result for cellulase degradation (Makeshkumar, 2011).

Amylase

The extracellular amylolytic activity was assessed by cultivating the fungal isolate on a medium containing (g/l): soluble starch (2.0); yeast extract (15.0); agar (18.0); and one liter of distilled water. The plates were incubated for 3–5 days at 25 °C, then flooded with IKI solution. A clear yellow zone around the colony with a blue background was considered a positive test for starch hydrolysis (Hankin and Anagnostakis, 1975).

Protease

Gelatin agar medium was used for the determination of proteolytic activity, as described by Ammar et al. (1991). The media contain (g/l): 20.0 gelatin and 20.0 agar in 0.2M phosphate buffer, pH = 7. The inoculated plates were incubated at 25 °C for 3–5 days. After the incubation period, the plates were flooded with Frazier's reagent consisting of the following: $HgCl_2$, 5.0 g; 20 ml. conc. HCl; and 100 ml. of distilled water (Bisson and Cabellii, 1979). If the fungus produced protease, a clear zone around the colony against an opaque background could be seen.

Lipase

The extracellular lipolytic activity was assayed according to the method of Sierra (1957). The following media were used to culture the selected fungal isolates: peptone 8.0 g; $CaC1_2.2H_2O$ 0.1 g; Tween 80 (polyoxyethylene sorbitan monooleate) 10 ml.; agar 20.0 g; and one liter of distilled water. Tween 80 was sterilized separately and added to the cooled autoclaved medium. The occurrence of white precipitation or fatty acid crystals around the colony was considered a positive test of lipase activity.

Asparaginase

Asparaginase activity was assayed using a modified Czapek Dox medium (Gulati et al., 1997) that contained (g/l): 6.0 Na_2HPO_4; 2.0 KH_2PO_4; 0.5 NaCl; 0.2 $MgSO_4.7H_2O$; 0.005 $CaCl_2.2H_2O$; and 20.0 L-asparagine in distilled H_2O adjusted to pH 5.5. The medium was supplemented with 2% agar and 0.009% phenol red (Sigma-Aldrich). Production of asparaginase was indicated by a change in the medium's color from yellow to red.

Laccase

The laccase activity was determined according to Coll et al. (1993). The media was composed of, in g/l: 3.0 peptone, 10.0 glucose, 0.6 KH_2PO_4, 0.001 $ZnSO_4$, 0.4 K_2HPO_4, 0.0005 $FeSO_4$, 0.05 $MnSO_4$, 0.5 $MgSO_4$, 20.0 agar (pH 5.0), and 0.02% guaiacol. The inoculated plates were incubated at 25 °C for 3–5 days. Laccase production was indicated when laccase catalyzed the oxidative polymerization of guaiacol, and reddish-brown zones in the medium appeared.

RESULTS

Physico-Chemical Characteristics of Water

The physicochemical characteristics of the collected water samples are summarized in Table 1. Water temperature varied in the sampling seasons (ranging from 14.0 °C to 31.5 °C), recording the highest value (31.5°C) during the summer in El-Ibrahimia Canal, Assiut Governorate, whereas the lowest (14.0°C) in the winter in the Nile River, Helwan, Cairo. The pH values of the water samples were neutral to moderately alkaline (fluctuated between 7.0 and 8.7). Total soluble salts exhibited significant variations and fluctuated between 120 and 470 mg/l, depending on the sampling site and season. The highest values of total soluble salts were recorded in El-Ismailia Canal, Mostorod, Al-Qalyubia Governorate, ranging from 297.0 mg/l during the summer to 470.0 mg/l during the spring, whereas the lowest value (120 mg/l) was recorded in El-Ibrahimia Canal during the winter. The organic matter contents varied between 11.3 mg/l in El-Ibrahimia Canal during the summer and 64.6 mg/l in El-Ismailia Canal during the spring. The dissolved oxygen ranged between 5.3 mg/l and 9.9 mg/l. The highest value (9.9 mg/l) was recorded during the winter in the Nile River, Helwan, but the lowest value (5.3 mg/l) was recorded during the summer in El-Ismailia Canal.

Mycological Analysis (Diversity, occurrence, and seasonality of zoosporic fungi)

Table 2 illustrates the biodiversity, seasonal fluctuations, occurrence, and population of zoosporic fungi in the four study water areas, either the Nile River (Helwan and El-Manial) or irrigation canals (El-Ibrahimia and El-Ismailia), during the four seasons (from autumn, 2021 to summer, 2022). Twenty-nine fungal species related to seven genera of either true zoosporic fungi (*Allomyces*) or straminipiles fungi (*Achlya*, *Aphanomyces*, *Dictyuchus*, *Phytophthora*, *Pythium*, and *Saprolegnia*) emerged using sterilized sesame seeds and halves of maize grains as baits. The results revealed that the most prevalent genera were *Achlya* (7 identified species and one unidentified), *Pythium* (4 identified species and one unidentified), *Dictyuchus* (3 species), and *Phytophthora* (2 identified species and one unidentified) emerging from 100%, 100%, 93.75%, and 68.75% of total samples (high occurrence), respectively; each was represented in all seasons. *Saprolegnia* (7 identified species and one unidentified) was a moderate seasonal occurrence (50% of total samples), represented in autumn and winter but completely missed in both spring and summer. *Allomyces*, appeared in winter and spring, and *Aphanomyces*, in spring and summer, were the less frequent genera (rare occurrences), and each was represented in 12.5% of total samples (one unidentified species for each). *Achlya* and *Saprolegnia* had the broadest species spectra, whereas *Allomyces* and *Aphanomyces* had the narrowest species spectra (only one unidentified species represented each). Regarding particular species, *Achlya ambisexualis* (75% of total samples), *Achlya proliferoides*, and *Dictyuchus sterilis* (68.75% of total samples for each) were the most frequent ones appearing in all seasons; they also emerged from four experimental water areas. The remaining species, as shown in Table 2, had a moderate, low, or rare frequency of occurrence (6.25%–50% of total samples).

Regarding the experimented water areas (Figure 3), the Nile River at Helwan exhibited the highest occurrence of fungal biodiversity with 26 species related to 6 genera, followed by El-Ibrahimia Canal with 20 species related to 6 genera and El-Manial with 17 species related to 5 genera. In contrast, the lowest occurrence of fungal diversity was recorded in El-Ismailia Canal at Mostorod with 15 species related to 5 genera. The most predominant species at Helwan was *Achlya ambisexualis*, which appeared in all seasons. However, at El-Manial, *Dictyuchus magnusii* and *Phytophthora* sp. were the most prevalent species appearing in the sampling seasons except the summer. In El-Ismailia Canal, *Achlya ambisexualis* and *Pythium thalassium* had the highest seasonal occurrences, whereas in El-Ibrahimia Canal, *Achlya proliferoides* had the highest frequency of occurrences; each appeared in all sampling seasons. Furthermore, three fungal species, i.e., *Allomyces* sp., *Achlya conspicua,* and *Saprolegnia turfosa* emerged only from Helwan and disappeared entirely in the other water areas. Conversely, two zoosporic fungal taxa, i.e., *Aphanomyces* sp. and *Phytophthora fragariae* exclusively appeared in El-Ibrahimia Canal. Micrographs of some representative zoosporic fungal species are presented in Figure 4.

Palatability and preference of zoosporic fungi for different baiting substrates

Table 3 shows that the isolated fungal taxa varied in terms of the preference of the baits employed. In this respect, *Achlya americana*, *A. conspicua*, *A. dubia*, *A.*

Table 1. Means of some evaluated physico-chemical features of surface water samples collected from four water areas in Egypt: Helwan region (HE), El-Manial region (MA), El-Ismailia Canal (IS), and El-Ibrahimia Canal (IB) during four seasons (from autumn 2021 to summer 2022).

Seasons	**Autum**				**Winter**			
Water areas	**HE**	**MA**	**IS**	**IB**	**HE**	**MA**	**IS**	**IB**
Temperature (°C)	21.0	20.5	22.0	22.5	14.0	15.5	14.5	19.5
pH	7.4	8.1	7.8	7.9	8.1	7.5	8.3	8.7
Total soluble salts (mg/L)	192.0	240.0	325.0	208.2	188.0	290.3	355.5	120.0
Organic matter content (mg/L)	33.4	15.6	38.6	28.5	39.8	22.5	42.3	26.9
Dissolved oxygen (mg/L)	9.2	8.5	6.7	8.4	9.9	9.0	6.6	9.8
Seasons	**Spring**				**Summer**			
Water areas Parameters	**HE**	**MA**	**IS**	**IB**	**HE**	**MA**	**IS**	**IB**
Temperature (°C)	27.0	25.5	26.0	26.5	28.0	30.0	30.5	31.5
pH	7.8	7.0	8.5	8.2	7.6	7.1	8.0	8.1
Total soluble salts (mg/L)	123.5	244.5	470.0	176.5	236.6	225.0	297.0	231.0
Organic matter content (mg/L)	22.1	18.5	64.6	22.8	26.9	12.1	25.1	11.3
Dissolved oxygen (mg/L)	9.3	7.9	7.1	8.1	7.2	7.1	5.3	6.9

Table 2. Seasonal variations, occurrence (O), and population (P) of zoosporic fungi in surface water samples collected seasonally (autumn 2021 to summer 2022) from five points in each of the four study areas: Helwan region (H), El-Manial region (M), El-Ismailia Canal (S), and El-Ibrahimia Canal (B).

Season	**Autmn**				**Winter**				**Spring**				**Summer**				**Total**				
Water sites	**H**	**M**	**S**	**B**	**H**	**M**	**S**	**B**	**H**	**M**	**S**	**B**	**H**	**M**	**S**	**B**	**O**	**O%**	**OR**	**P**	**P%**
Allomyces sp.	0	0	0	0	3	0	0	0	2	0	0	0	0	0	0	0	2	12.5	R	5	1.58
Achlya	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	16	100	H	132	41.9
A. ambisexualis (Johnson)	4	2	2	2	5	2	3	1	2	0	1	0	2	0	1	0	12	75	H	27	8.57
A. americana (Humphrey)	3	0	0	2	2	1	0	1	3	2	0	0	0	0	0	0	7	43.75	M	14	4.44
A. conspicua (Coker)	0	0	0	0	3	0	0	0	1	0	0	0	0	0	0	0	2	12.5	R	4	1.26
A. dubia (Coker)	0	3	0	0	2	0	2	0	2	0	0	3	0	0	0	0	5	31.25	M	12	3.80
A. flagellate (Coker)	3	0	0	0	3	2	0	3	4	0	0	2	0	0	0	0	6	37.5	M	17	5.39
A. prolifera (Nees)	4	2	0	3	0	0	0	1	0	0	2	0	0	2	1	0	7	43.75	M	15	4.76
A. proliferoides (Coker)	0	0	5	3	2	3	1	2	1	0	3	3	1	0	0	2	11	68.75	H	26	8.25
Achlya sp.	2	0	0	2	4	0	2	0	0	3	0	0	0	2	0	2	7	43.75	M	17	5.39
Aphanomyces sp.	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	3	2	12.5	R	5	1.58
Dictyuchus	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	15	93.75	H	48	15.2
D. magnusii (Lindst)	3	2	0	0	0	2	0	0	0	3	0	0	2	0	0	0	5	31.25	M	12	3.80
D. monosporus (Letig)	0	0	0	3	2	0	0	0	3	0	2	0	2	0	0	0	5	31.25	M	12	3.80
D. sterilis (Coker)	2	0	3	0	3	0	2	2	2	1	1	3	0	3	0	2	11	68.75	H	24	7.61
Phytophthora	1	1	1	0	1	1	1	0	1	1	0	1	0	0	1	1	11	68.75	H	24	7.61
P. inflata (Caros. & Tucker)	0	0	0	0	1	0	0	0	1	0	0	0	0	0	2	0	3	18.75	L	4	1.26
P. fragariae (Hickman)	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	2	2	12.5	R	3	0.95
Phytophthora sp.	2	3	2	0	2	2	1	0	0	3	0	2	0	0	0	0	8	50	M	17	5.39
Pythium	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	16	100	H	36	11.5
P. irregulare (Buisman)	2	1	0	1	0	0	0	0	0	0	0	0	3	2	0	2	6	37.5	M	11	3.49
P. rostratum (E.J. Butler)	0	1	0	0	2	0	0	0	0	0	0	0	0	0	0	0	2	12.5	R	3	0.95
P. thalassium (Atkins)	0	0	2	0	0	1	1	0	0	2	1	0	0	0	1	0	6	37.5	M	8	2.53
P. vexans (de Bary)	0	0	0	0	0	0	0	2	1	0	0	2	0	0	0	0	3	18.75	L	5	1.58
Pythium sp.	3	0	0	0	3	0	0	0	0	0	0	2	0	0	1	0	4	25	L	9	2.85
Saprolegnia	1	0	1	1	1	1	1	1	0	0	0	0	0	0	0	0	8	50	M	65	20.6
S. anisospora (de Bary)	0	0	0	0	1	0	0	2	0	0	0	0	0	0	0	0	2	12.5	R	3	0.95
S. diclina (Humphrey)	2	1	0	0	3	0	2	0	0	0	0	0	0	0	0	0	3	18.75	L	7	2.22
S. ferax ((Gruith.) Kutz)	3	0	2	1	4	2	3	3	0	0	0	0	0	0	0	0	8	50	M	19	6.03
S. furcate (Maurizio)	0	0	0	0	2	1	0	2	0	0	0	0	0	0	0	0	3	18.75	L	5	1.58
S. hypogyna (Seymour)	3	0	2	0	5	3	4	0	0	0	0	0	0	0	0	0	5	31.25	M	17	5.39
S. parasitica (Coker)	0	0	0	1	3	0	0	2	0	0	0	0	0	0	0	0	3	18.75	L	6	1.90
S. turfosa (Minden)	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	6.25	R	1	0.31
Saprolegnia sp.	0	0	0	0	2	2	1	2	0	0	0	0	0	0	0	0	4	25	L	7	2.22
Total																				315	

OR: Occurrence remarks, H: High occurrence (60%–100% of total samples), M: Moderate occurrence (30%–< 60% of total samples), L: Low occurrence (15%–<30% of total samples), and R: Rare occurrence (Less than 15% of total samples).

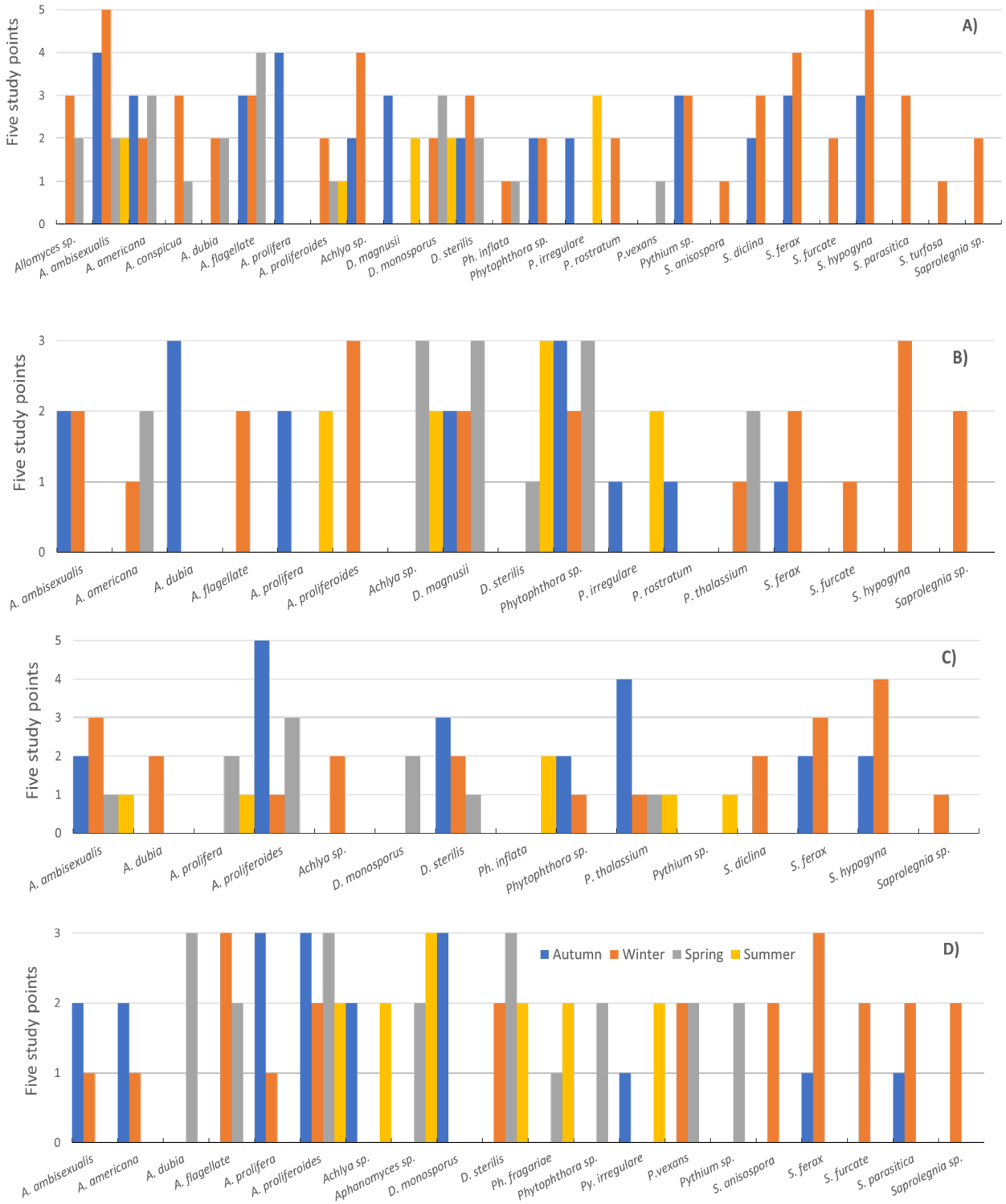

Figure 3. (A) Seasonal fluctuations of zoosporic fungi recovered from Helwan Nile River. (B) Seasonal fluctuations of zoosporic fungi recovered from El-Manial Nile River. (C) Seasonal fluctuations of zoosporic fungi recovered from El-Ismailia canal. (D) Seasonal fluctuations of zoosporic fungi recovered from El-Ibrahimia canal

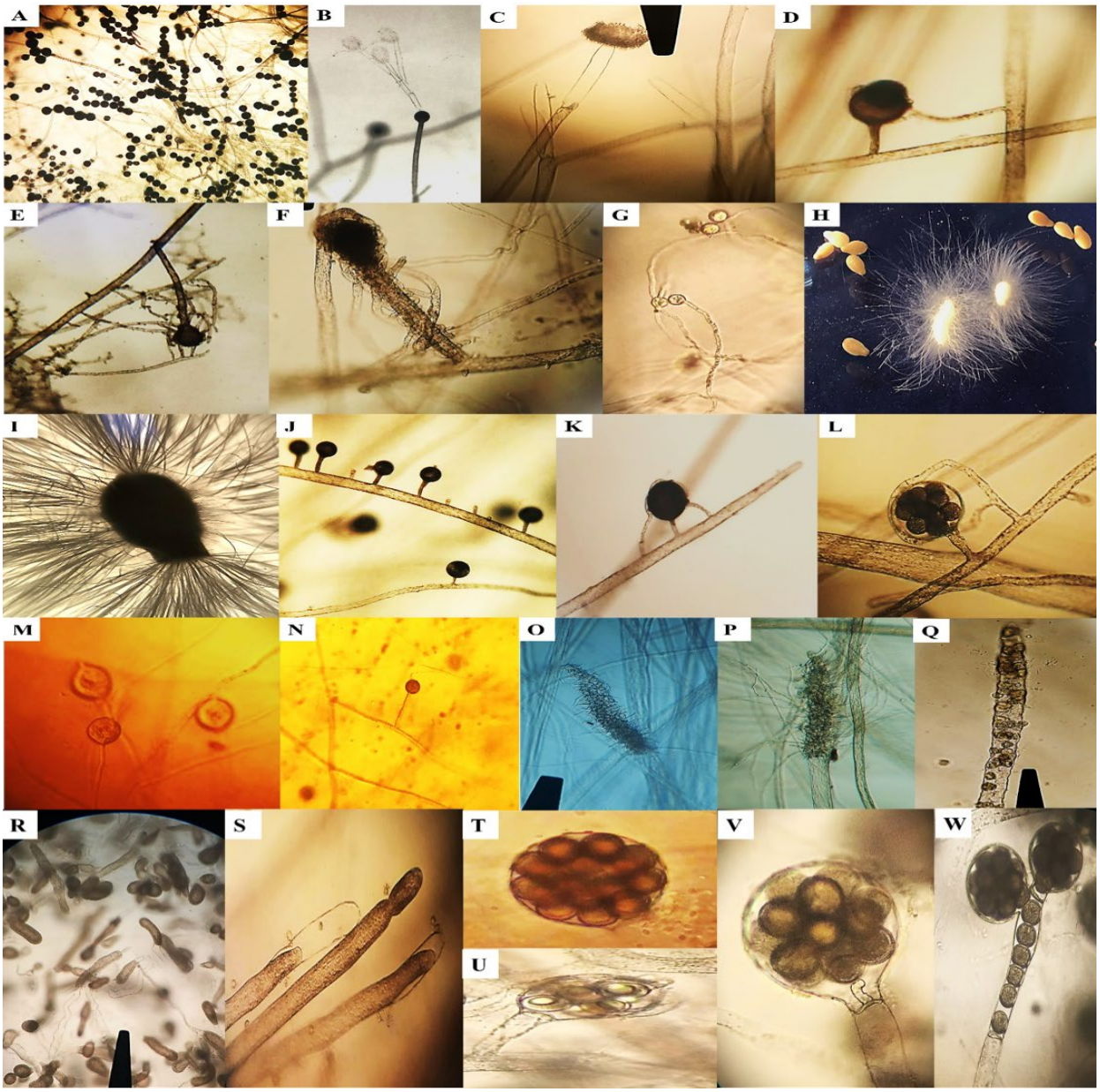

Figure 4. General microscopic characteristics of some representative isolates, A-B: *Achlya* sp. (A: gemmae, B: gemmae germination), C-D: *Achlya ambisexualis* (C: encysment of primary zoospores on sporongial mouth, D: immature oogonia with diclinous antheridia), E: *Achlya flagellate* (immature oogonia with diclinous antheridia), F-G: *Achlya proliferoides* (F: hyphae with antheridial branches entwined around them, G: germination of zoospore cyst by germtube), H-L: *Achlya americana* (H,I: baiting on sesame seed, J: immature oogonia and antheridia, K: immature oogonia with monoclinous antheridia, L: mature oogonia with monoclinous antheridia), M: *Phytphothra* sp., N: *Pythium* sp., O-Q: *Dictyuchus sterilis* (spores emerging from their cysts through sporangium wall leaving a network of cyst walls within), R-T: *Saprolegnia ferax* (R: gemmae, S: proliferation of zoosporagium, T: mature oogonium with centric oospores and pitted wall), U: Saprolegnia anisospora (mature oogonium with eccentric oospores), V: *Saprolegnia hypgyna* (mature oogonium with centric oospores and hypogynous antheridia), W: *Saprolegnia turfosa* (typical short stalked oogonium with centric oospores).

flagellate, *Achlya* sp., *Dictyuchus magnusii*, *D. monosporus*, *Pythium thalassium*, and all species of both *Saprolegnia* and *Allomyces* showed an exclusive preference for sesame seeds as baits since they largely disregarded maize grains. In contrast, *Phytophthora inflata*, *P. fragariae*, *Pythium rostratum*, *P. vexans*, and *Pythium* sp. exclusively colonized maize grains. Both baits were able to recover the remaining fungal taxa. In conclusion, most zoosporic fungi prefer sesame seeds to maize grains as bait.

Table 3. Zoosporic fungi recovered on two baiting substrates (Sesame seeds and maize grains)

Substrates	Sesame seeds	Maize grains
Allomyces sp.	+	-
Achlya	+	+
A. ambisexualis (Johnson)	+	+
A. americana (Humphrey)	+	-
A. conspicua (Coker)	+	-
A. dubia (Coker)	+	-
A. flagellate (Coker)	+	-
A. prolifera (Nees)	+	+
A. proliferoides (Coker)	+	+
Achlya sp.	+	-
Aphanomyces sp.	+	-
Dictyuchus	+	+
D. magnusii (Lindst)	+	-
D. monosporus (Leitg.)	+	-
D. sterilis (Coker)	+	+
Phytophthora	+	+
P. inflata (Caros. & Tucker)	-	+
P. fragariae (Hickman)	-	+
Phytophthora sp.	+	+
Pythium	+	+
P.irregulare (Buisman)	+	+
P. rostratum (E.J. Butler)	-	+
P. thalassium (Atkins)	+	-
P.vexans (de Bary)	-	+
Pythium sp.	-	+
Saprolegnia	+	-
S. anisospora (de Bary)	+	-
S. diclina (Humphrey)	+	-
S. ferax ((Gruith.) Kutz)	+	-
S. furcate (Maurizio)	+	-
S. hypogyna (Seymour)	+	-
S. parasitica (Coker)|	+	-
S. turfosa (Minden)	+	-
Saprolegnia sp.	+	-

Statistical analysis

The diversity of zoosporic fungi

The diversity of zoosporic fungi was calculated, including fungal taxa(s), which represents the number of isolated zoosporic fungal species from collected samples; dominance (d) indicates the fungal taxa dominance in a particular site or season; the Simpson index illustrates the evenness of the zoosporic fungal community; and Shannon index is the diversity index, which estimates the number of fungal individuals while accounting for the number of fungal taxa. Therefore, Table 4 demonstrates that the highest taxa of zoosporic fungi were recorded in the winter compared to the other sampling seasons, irrespective of the sampling water site. The summer was the poorest in zoosporic fungal diversity. Furthermore, the sampling water areas varied in terms of the seasonality of the collected fungi. In this respect, in the winter, the highest fungal taxa were estimated for Helwan with 22 fungal species, followed by El-Ibrahimia Canal with 12 fungal taxa, and both El-Manial and El-Ismailia Canal recording 11 fungal taxa for each. On the other hand, the lowest zoosporic fungal taxa were recorded during the summer in El-Manial with 4 fungal species, both Helwan and El-Ismailia Canal with 5 fungal species for each, and El-Ibrahimia Canal with 6 fungal species.

Consequently, the highest dominance of zoosporic fungi was recorded in the summer compared to the other seasons, irrespective of the experimented water site. The highest Simpson and Shannon diversity indexes for isolated fungal taxa were estimated during the winter, regardless of the experimented water sites. In comparison between the different study water areas in the winter, the highest Simpson index was recorded for Helwan as 0.9465, followed by El-Ibrahimia Canal as 0.9074, El-Manial as 0.898, and El-Ismailia Canal as 0.8884. Moreover, the highest Shannon diversity was recorded for water samples collected from Helwan as 3.003, followed by El-Ibrahimia Canal (2.427), El-Manial (2.335), and El-Ismailia Canal (2.287). Species evenness illustrates the relative abundance of the different species in a specific community. The highest species evenness was recorded in the water samples collected from El-Ibrahimia Canal and El-Manial during the summer, yielding values of 0.9866 and 0.9828, respectively.

Interaction of fungal occurrence with the physicochemical parameters of water samples

The obtained data revealed that most zoosporic fungi prefer low or moderate temperatures, relatively high contents of organic matter, high dissolved oxygen, and relatively low total soluble salts. pH did not display a regular pattern according to the sampling season, water site, or water body; it, thus, did not affect governing the occurrence, biodiversity, and seasonality of the collected zoosporic fungi. The occurrence of collected fungi exhibited a variable preference for determined abiotic factors, depending on the fungal species and the determined parameter. Figure 5 indicates the Principal Coordinate Analysis (PCoA) of the interaction between the isolated zoosporic fungi and the measured physicochemical properties of the water samples collected in different seasons from different water bodies. In winter, the temperature exhibited an interaction with the occurrence of *Achlya prolifera*, *Pythium vexans*, and *Achlya flagellate*, whereas the dissolved oxygen influenced *Achlya americana*, *Achlya proliferoides*, and *Saprolegnia* sp. Zoosporic fungi, namely *Saprolegnia ferax*, *Dictyuchus sterilis*, *Saprolegnia*

Table 4. The diversity of zoosporic fungi isolated from water samples collected from four water areas either Nile River (Helwan, HE and El-Manial, MA regions) or irrigation canals (El-Ismailia canal, IS and El-Ibrahimia canal, IB) during four seasons (from autumn 2021- summer 2022)

	Summer				Autumn			
	HE	**MA**	**IS**	**IB**	**HE**	**MA**	**IS**	**IB**
Taxa (S)	5	4	5	6	13	8	7	9
Individuals	10	9	6	13	36	15	15	18
Dominance (D)	0.22	0.2593	0.2222	0.1716	0.08179	0.1467	0.1667	0.1296
Simpson (1-D)	0.78	0.7407	0.7778	0.8284	0.9182	0.8533	0.8333	0.8704
Shannon (H)	1.557	1.369	1.561	1.778	2.534	1.991	1.875	2.11
Evenness ($e^{H/S}$)	0.949	0.9828	0.9524	0.9866	0.9691	0.9157	0.9317	0.9165
	Winter				**Spring**			
	HE	**MA**	**IS**	**IB**	**HE**	**MA**	**IS**	**IB**
Taxa (S)	22	11	11	12	11	6	6	10
Individuals	58	21	22	23	22	14	10	22
Dominance (D)	0.05351	0.102	0.1116	0.09263	0.1116	0.1837	0.2	0.1074
Simpson (1-D)	0.9465	0.898	0.8884	0.9074	0.8884	0.8163	0.8	0.8926
Shannon (H)	3.003	2.335	2.287	2.427	2.287	1.735	1.696	2.264
Evenness ($e^{H/S}$)	0.9155	0.9386	0.8953	0.9437	0.8953	0.9446	0.9084	0.9617

parasitica, *Saprolegnia ambisexualis*, and *Saprolegnia furcate*, demonstrated an interaction with the pH value of the water samples collected in winter (Figure 5A). Total soluble salts (TSS) influenced the occurrence of *Pythium thalassium* and *Phytophthora* sp., whereas organic matter (OM) was associated with the occurrence of *Achlya dubia*, *Saprolegnia diclina*, *Achlya ambisexualis*, *Saprolegnia hypogyna*, and *Achlya* sp. In spring, the physicochemical parameters of water samples revealed that temperature and dissolved oxygen affected the occurrence of all *Achlya dubia*, *Pythium vexans*, *Dictyuchus sterilis*, *Phytophthora inflata*, *Allomyces* sp., *Achlya conspicua*, and *Achlya flagellate*, while organic matter and total soluble salts influenced the occurrence of *Achlya prolifera* and *Pythium thalassium*, and organic matter affected the prevalence of *Achlya proliferoides* (see Fig. 5B). On the other hand, the pH value of water samples impacted the incidence of *Achlya ambisexualis, Dictyuchus monosporus*, and *Achlya proliferoides.* The physicochemical parameters of the collected water during autumn illustrated that the total soluble salts, organic matter, and temperature affected the frequency of zoosporic fungi, namely *Dictyuchus sterilis*, *Achlya proliferoides*, and *Pythium thalassium*, whereas dissolved oxygen influenced the dominance of *Dictyuchus magnusii*, *Phytophthora inflata*, and *Achlya prolifera* (see Fig. 5C). pH values showed an interaction with *Achlya dubia*, *Pythium rostratum*, *Phytophthora* sp., *Dictyuchus magnusii*, and *Saprolegnia parasitica*. In the summer, the incidence of zoosporic fungi, *Pythium* sp., *Phytophthora inflata*, and *Pythium thalassium* exhibited an interaction with total soluble salts and pH values of water samples, while dissolved oxygen and organic influenced the occurrence of *Achlya ambisexualis*, *Dictyuchus magnusii*, and *Dictyuchus monosporus* (see Fig. 5D). The temperature had an impact on the prevalence of *Aphanomyces* sp. and *Phytophthora fragariae*.

Cluster analysis of zoosporic fungi isolated from the four water sites

The cluster analysis of the collected zoosporic fungi from four water areas during different seasons showed a prominent grouping of the fungal communities (see Fig. 6). The results showed that there is a significant difference between the occurrence of fungal communities in winter compared to the other sampling seasons. In contrast, autumn and summer exhibited a similarity in this respect. The two-way cluster analysis of isolated fungi showcased a noticeable variation between four fungal groups. The first group involved *Saprolegnia anisospora*, *Saprolegnia furcate*, *Saprolegnia turfosa*, *Saprolegnia diclina*, *Saprolegnia parasitica*, *Saprolegnia hypogyna*, *Saprolegnia ferax*, *Saprolegnia* sp., *Achlya ambisexualis*, *Pythium rostratum*, *Achlya americana*, and *Phytophthora* sp. The second group was represented by nine fungal species: namely, *Achlya conspicua*, *Allomyces* sp., *Pythium thalassium*, *Achlya proliferoides*, *Achlya dubia*, *Achlya flagellate*, *Dictyuchus monosporus*, *Dictyuchus sterilis*, and *Pythium vexans*. The third fungal community included five fungal species (namely, *Achlya prolifera*, *Pythium irregulare*, *Dictyuchus magnusii*, *Pythium* sp., and *Achlya* sp.). The fourth fungal group contained three fungal species: namely, *Phytophthora inflata*, *Aphanomyces* sp., and *Phytophthora fragariae*.

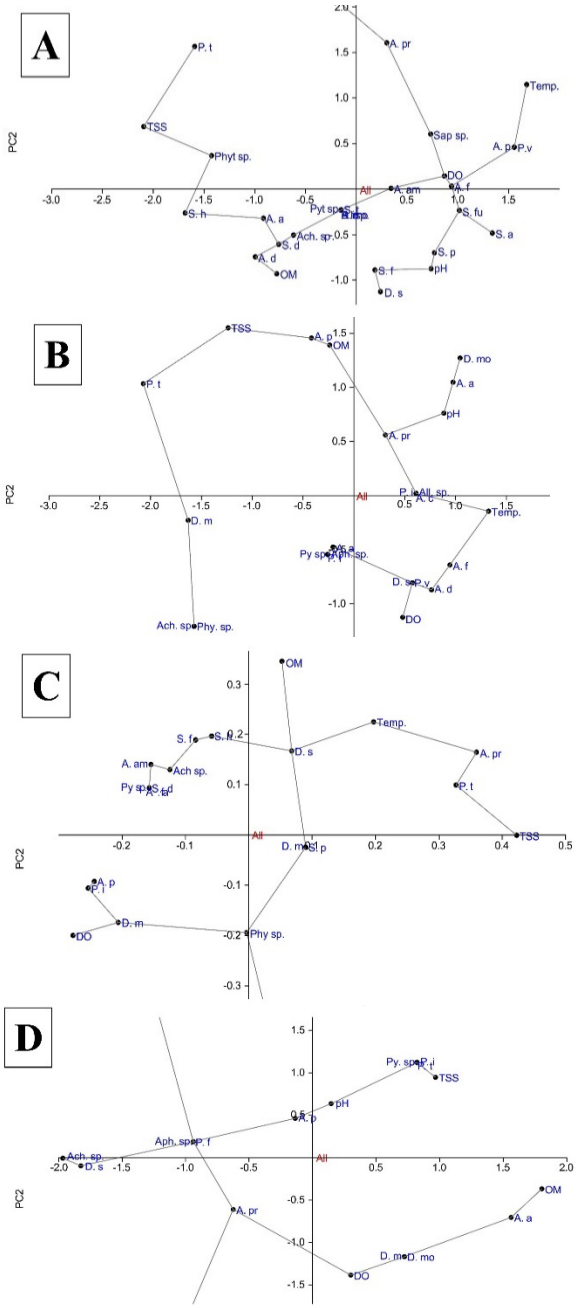

Figure 5. Principal Coordinates Analysis (PCoA) showed the interaction of fungal occurrence with the physicochemical parameters of water samples collected during (A) Winter, (B) Spring, (c) Autumn and (D) Summer. All. sp.= *Allomyces* sp., A. a= *Achlya ambisexualis*, A. am= *A. americana*, A. C= *A. conspicua*, A. d= *A. dubia*, A. f= *A. flagellate*, A. p= *A. prolifera*, A. pr= *A. proliferoides*, Ach sp.= *Achlya sp.,* Aph. sp= *Aphanomyces sp.*, D. m= *Dictyuchus magnusii*, D. mo= *D.* monosporus, D. s= *D. sterilis*, P. i= *Phytophthora inflata*, P. f= P. fragariae, Phy sp.= *Phytophthora sp.*, P. ir= *Pythium irregulare*, P. r= *P. rostratum*, P. t= *P. thalassium*, P. v= *P.vexans*, Py sp.= *Pythium sp.*, S. a= *Saprolegnia* anisospora, S. d= *S. diclina*, S. f= *S. ferax*, S. fu= *S. furcate*, S. h= *S. hypogyna*, S. p= *S. parasitica*, Sap sp.= *Saprolegnia sp.*

ANOVA analysis

The ANOVA (Analysis of Variance) was employed to investigate the statistically significant differences between the recorded zoosporic fungi in the different seasons. Hence, the mean values and variances of the respective fungal groups are compared across different seasons. The ANOVA analysis showed that the occurrence of zoosporic fungi was significantly different throughout different seasons (p=0.000701), as shown in Table 5.

Molecular identification of the most prevalent fungal isolate

nucleotide sequence of fungal DNA was examined using enhanced BLAST (Megablast) searches from the National Center for Biotechnology Information (NCBI) GenBank database, as illustrated in Fig.7. The fungal isolate was identified as *Achlya ambisexualis* and submitted to GenBank as *Achlya ambisexualis* isolate Y6 with the fungus ID PP406471. In addition, the constructed phylogenetic tree of the partial sequence, together with sequences from other fungi in GenBank, revealed that *Achlya ambisexualis* isolate Y6 (PP406471) shares a high similarity of 99% with *Achlya ambisexualis* strain ID FJ545256.

Screening of enzymes production from Achlya ambisexulais

According to Table 6 and Fig. 8, *Achlya ambisexualis* could produce cellulase, amylase, protease, lipase, and asparaginase but not laccase. Protease showed the highest activity zone diameter, whereas amylase demonstrated the lowest activity zone diameter.

DISCUSSION

Water temperature, total soluble salts, organic matter content, and dissolved oxygen were noticeably varied depending on the sampling site and season. The winter was the richest in fungal diversity and occurrence compared to the other sampling seasons. This finding was supported by the two-way cluster analysis and ANOVA statistical analysis. The result showed that the seasonal occurrence of zoosporic fungi was affected by water temperature. It is concluded that most zoosporic fungi prefer relatively low or moderate temperatures. This is consistent with the results of Khallil et al. (1995), Khallil et al. (2020) Klich and Tiffany (1985), and Muhsin (2012), confirming that temperature is a significant factor influencing the diversity, frequency, and seasonal fluctuations of aquatic fungi. Similarly, Marano et al. (2008) found that the maximum frequency of

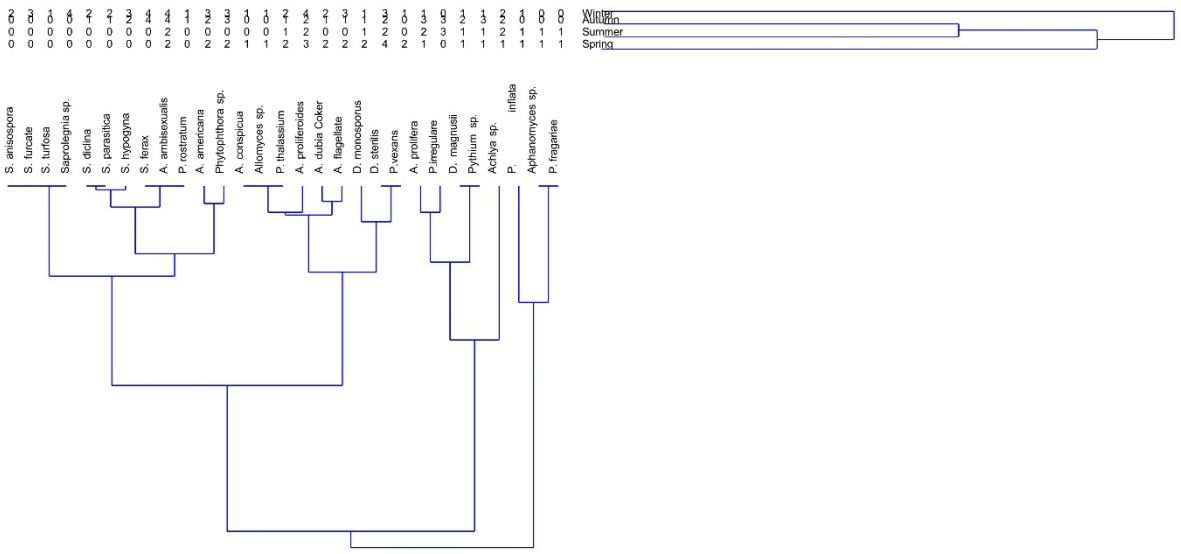

Figure 6. Cluster analysis of zoosporic fungi isolated from four water sites during different seasons showed an obvious grouping of the fungal communities

Table 5. Analysis of Variance (ANOVA) of the seasonality of zoosporic fungi

	Sum of sqrs	Df	Mean square	F	p (same)
Between groups:	23.4052	3	7.80172	6.097	0.000701
Within groups:	143.31	112	1.27956		
Total:	166.716	115	0.0007		

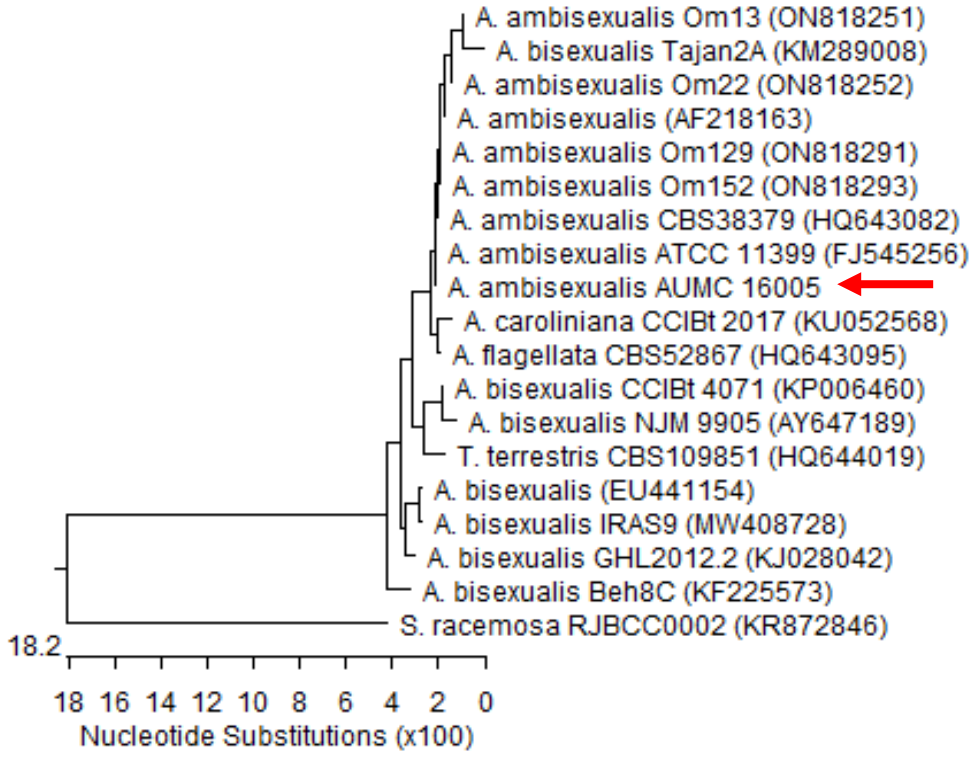

Figure 7. Maximum likelihood phylogenetic tree based on ITS sequencing of rDNA of the fungal strain Achlya ambisexualis AUMC 16005 (arrowed) aligned with closely related sequences of fungal strains accessed from the GenBank. Saprolegnia ramosa is included in the tree as an outgroup strain. (A. =Achlya, S.= Saprolegnia).

Table 6. Qualitative screening for cellulase, amylase, protease, lipase, asparaginase and laccase producers *Achlya ambisexualis* using different tests.

Enzymes	Activity zone Diameter (mm)
Cellulase	34 ±2.3
Amylase	26 ±3.7
Protease	55 ±2.8
Lipase	44 ± 2.6
Asparaginase	51 ±2.0
Laccase	0

*Mean of three replicates and ±standard error mean (SEM)

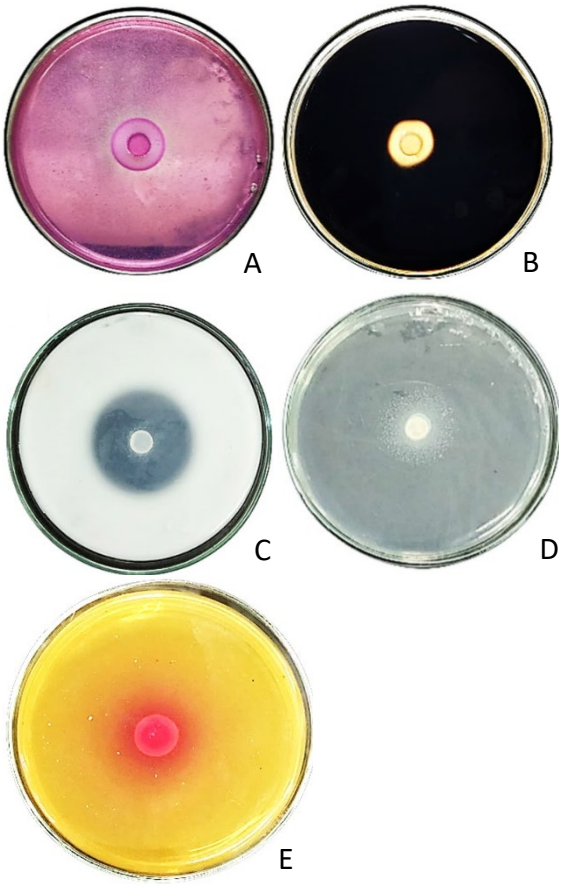

Figure 8. *Achlya ambisexualis* enzymatic activities after incubation for 72 h at 25°C. (A) cellulase, (B) amylase, (C) protease, (D) lipase, and (E) asparaginase.

zoosporic fungi was observed at the lowest temperatures. Voronin (2008) also reported that temperature significantly impacts the development and occurrence of zoosporic fungi and the concentration of dissolved oxygen in the water. Furthermore, Khallil et al. (2020) confirmed that water temperature was one of the main limiting factors governing the seasonal occurrence of zoosporic fungi. Conversely, Paliwal (2009) clarified that the winter period, when the temperature reaches below 12°C, had the lowest number of zoosporic taxa, while the spring and rainy seasons with temperatures between 14.4–16.5 °C had the highest number. According to Nair and Bhat (2016), the monsoon season, followed by the post-monsoon and summer, is the most favorable for recovering water molds. This discrepancy could be explained by the temperature variations among the examined geographic areas. Considering the involvement of zoospores in distinct food webs, according to Gleason et al. (2008), an increase in temperature may lead to an increase in competitors because there are more consumers, such as rotifers, nematodes, and protozoa. Furthermore, the higher water temperature created ideal conditions for bacteria in the summer, which could compete with zoosporic fungi for organic matter. This could account for the low species diversity, abundance, and occurrence of zoosporic fungi observed in these conditions.

The water samples' pH values showed no consistent variations, regardless of the sampling site and season. This aligns with Khallil et al. (2020) who stated that pH did not exhibit any considerable influence on the seasonality and biodiversity of straminipiles and true zoosporic fungi. In contrast, DiLeo et al. (2010) pinpointed that pH was the main factor influencing the abundance of water mold in the field. They also found that zoosporic fungal abundance was significantly decreased when the pH of the naturally acidic water of the Pine Barrens increased, and that improving conditions through a decrease in the pH of the impacted water increased fungal abundance.

Total soluble salts, i.e., the amount of salt dissolved in the water, exhibited significant variations depending on the sampling sites and seasons. The result showed that water sites with higher values of total soluble salts exhibited the lowest occurrence, biodiversity, and population of zoosporic fungi. The water samples collected from El-Ismailia Canal, which recorded the highest total soluble salts, were the poorest in zoosporic fungi, irrespective of the sampling season. Conforming to our results, Khallil et al. (2020) highlighted that high salinity levels had an adverse impact on the diversity, abundance, and seasonal occurrence of zoosporic fungi. In contrast, Gleason et al. (2006) stated that several species of true zoosporic fungus (Chytridiomycota) can withstand varying salinities. Voronin (2008) also indicated that fungi can tolerate higher salt concentrations at lower temperatures.

The current study elucidated that water sites with higher organic matter exhibited the highest

occurrence of zoosporic fungi. In agreement with our findings, Ali (2007), Khallil et al. (2020), Shearer et al. (2007), and Voronin (2008) reported that water samples with high organic matter had the most significant amount of zoosporic fungi. Rarely and uniquely, although the water samples collected from El-Ismailia Canal recorded relatively higher levels of organic matter contents, they were the lowest in fungal occurrence. The elevated salt level may be attributed to this finding. Similarly, Voronin (2008) concluded that levels of dissolved organic matter significantly influence the abundance and distribution of zoosporic fungi. They also found that fungal taxa increase in response to rising organic matter, but only at relatively low salinity and temperature. Voronin and Zhdanova (2023) stated that *Pythium* and *Saprolegnia* (fungi-like oomycetes) can develop in reservoirs with a high concentration of dissolved organic matter and withstand a contaminated environment. The dissolved oxygen shows high variation according to sampling sites and seasons. The current study showed a negative correlation between dissolved oxygen levels and temperature: As water temperature increases, dissolved oxygen levels decrease (i.e., high temperatures reduce oxygen solubility in water). In line with our results, Voronin (2008) showed that the dissolved oxygen amount in water is strongly influenced by temperature. Additionally, there is a positive relationship between dissolved oxygen levels and fungal occurrence, with an increase in dissolved oxygen leading to an increase in fungal occurrence. This aligns with Suzuki (1961), who indicated that the zoospores of aquatic fungi typically concentrate in oxygen-rich water layers. Interestingly, PCoA analysis supported our findings regarding the strong correlation between the determined physico-chemical parameters, the occurrence of collected fungi, and sampling seasons.

Twenty-nine fungal species related to seven genera of either true zoosporic fungi (i.e., *Allomyces*) or straminipiles fungi (i.e., *Achlya*, *Aphanomyces*, *Dictyuchus*, *Phytophthora*, *Pythium*, and *Saprolegnia*) emerged using sterilized sesame seeds and halves of maize grains as baits. All the recovered zoosporic taxa recorded in the current investigation had been reported previously with different frequencies from various water areas in Egypt (Abd-Elaah et al., 2018; Khallil, 1990; Ali, 2007; El-Hissy & Khallil, 1989; Khallil et al., 2020), as well as in other geographical regions including Saudi Arabia (El-Nagdy et al., 2022), Iran (Masigol et al., 2020), Argentina (Marano et al., 2008), and Brazil (Nascimento et al., 2011). Khallil et al. (2020) isolated thirty-four species related to 10 genera (namely, *Allomyces*, *Achlya*, *Aphanomyces, Aqualinderella, Brevilegnia*, *Dictyuchus, Phytophthora*, *Pythiopsis*, *Pythium*, and *Saprolegnia*) from El-Zinnar Irrigation Canal and the El-Ibrahimia Canal, which receive treated sewage water and industrial effluents from a factory for oils and detergents, respectively, during four seasons (from the winter of 2017 to the autumn of 2018). Czeczuga et al. (2007) isolated 88 species (41 true zoosporic fungal species and 47 fungus-like organisms (straminipiles)) on fruit tree petals floating on water. Nascimento et al. (2011) recovered 32 taxa of zoosporic fungi from 48 water samples collected from two areas in Brazil. Our results showed that the most prevalent genera were *Achlya*, *Pythium*, and *Dictyuchus.* In contrast to our results, Yanna, Ho and Hyde (2002) found *Achlya* and *Dictyuchus* to be the predominant genera in Hong Kong. Voronin (2008) also stated that *Achlya* and *Dictyuchus* had the highest seasonal occurrence. On the other hand, El-Hissy et al., (1982) examined the incidence of water molds (straminipiles and true zoosporic fungi) in freshwater habitats of the Nile system and manifested that *Saprolegnia* was one of the most prevalent genera.

In the current study, *Achlya* and *Saprolegnia* exhibited the broadest species spectra. Similarly, Khallil et al. (2020) found that *Achlya* and *Saprolegnia* frequently had the highest species spectra in El-Zinnar and El-Ibrahimia irrigation canals at Assiut, Upper Egypt. Opposed to our results, Ali (2007) showed that *Achlya* did not have the highest species spectrum and clarified that *Pythium* (8 species) had the broadest spectrum of species diversity. However, consistent with our results, Ali pointed out that *Saprolegnia* (7 species) was among the genera with the broadest spectra of species diversity in polluted water drainages across the Nile Delta regions (i.e., Lower Egypt). The most frequent species in the current study were *Achlya ambisexualis*, *Achlya proliferoides*, and *Dictyuchus sterilis.* In agreement with our results, Khallil et al. (2020) observed that *Achlya proliferoides* and *Dictyuchus sterilis* were the most prevalent species, exhibiting the highest frequency of occurrence among the identified species, emerging from El-Zinnar and El-Ibrahimia irrigation canals. On the contrary, Czeczuga et al. (2007) identified *Rhizophydium cornutum*, *R. globosum* (true zoosporic fungi), and *Aphanomyces irregularis*, *A. laevis*, *Saprolegnia litoralis*, *Pythium butleri*, *P. inflatum*, *P. tardicrescens*, and *P. ultimum* (Straminipila) as the

most common species growing on the petals of five fruit tree species floating on water from three types of freshwater habitats (i.e., spring, river, and pond) in north-eastern Poland. Moreover, Kiziewicz (2012) recovered 21 species related to 8 genera of zoosporic fungi from River Springs (Poland), indicating that *Achlya apiculate*, *Aphanomyces laevis*, *Catenophlyctis variabilis*, *Dictyuchus monosporus*, *Pythium debaryanum,* and *Saprolegnia ferax* were the most prevalent taxa.

The preference and colonization of zoosporic fungi varied depending on the baits employed, even though most recovered fungal taxa prefer sesame seeds to maize grains as baits. Some zoosporic fungal taxa were recovered exclusively on sesame seeds, while others, particularly those related to Pythiaceae, only colonized maize grains. Our findings were supported by El-Hissy and Khallil (1989) and El-Hissy et al. (2001), who reported that the highest number of isolated zoosporic fungi appeared on sesame seeds compared to the other employed baits. Afzali et al. (2013) isolated 59 species of zoosporic fungi from Malaysian natural water bodies and fish farms by baiting method using sterilized maize, green peas, fish meat, insect's wings, and hemp seed.

From our results, *Achlya ambisexualis* showed some favourable activity in cellulase, amylase, protease, lipase, and asparaginase. Consequently, this isolate is a promising source of numerous hydrolytic enzymes important to industry and biotechnology. In agreement with our results, Nolan and Bal (1974) noted that cellulase was located at the ultrastructural level and discovered to be present in dictyosomes and vesicles, between the plasmalemma and the cell wall, around the periphery of unknown storage structures, and on the outer surface of the cell wall in the male strain of *Achlya ambisexualis*. Hill and Mullins (1979) showed that within the developing hyphae of *Achlya ambisexualis*, the enzyme cellulase is present in both soluble and insoluble forms. Hill and Mullins (1980) also reported the presence of cellulase in *Achlya ambisexualis* and investigated whether cellulase demonstrated more considerable specific activity in actively growing mycelia than in non-growing mycelia. Hill and Pott (2011) demonstrated that *Achlya ambisexualis* released extracellular proteases during growth on all proteins and protein hydrolysates tested. Khallil and Omar (1993) proved the ability of five tested zoosporic fungi to produce amylase, lipase, protease, and cellulase. Odu et al. (2017) also investigated the ability of some aquatic phycomycetes in Maiduguri, a Semi-Arid Area of Nigeria, to produce lipase. Masigol et al. (2019) confirmed that Oomycota are involved in transforming a big fraction of aquatic Dissolved organic matter (DOM) via their capacity to produce a broad range of extracellular enzymes. The enzymatic repertoire of aquatic fungi makes them one of the most critical players in the microbial loop in various aquatic ecosystems (Sime-Ngando et al., 2010). In our results, *Achlya ambisexualis* did not exhibit laccase activity. Similarly, Masigol et al. (2019) recorded that none of the experimented oomycetes strains exhibited any laccase or pectinase activity. In contrast, Feng and Li (2012) investigated the presence of laccases as a gene family in oomycetes.

CONCLUSION

In total, twenty-nine species related to seven genera of either true zoosporic fungi (*Allomyces*) or six genera of straminipiles (*Achlya*, *Aphanomyces*, *Dictyuchus*, *Phytophthora*, *Pythium*, and *Saprolegnia*) were collected during the current investigation. The diversity and occurrence of the gathered fungal taxa varied depending on some physico-chemical features of the water and the sampling season. It is concluded that there is a clear seasonal dynamic in the occurrence and diversity of zoosporic fungi. The decline in summer is linked to both increased water temperatures and relatively high salinity levels. Continued research on aquatic fungi is vital for increased awareness, understanding, and unveiling of their function, pivotal environmental role in aquatic habitats, their devastating pathogenicity, particularly on fish in natural aquatic habitats, aquaculture, and fish farms, ecosystem stability, particularly in this period of rapid climate change, and aquatic system pollution that may result from industrial production and other human activities. Species identification of zoosporic fungi is mainly based on morphological features, and only one isolate; the most prevalent isolate was selected for molecular identification. Morphological features are sometimes perplexing, vague, and variable. For instance, some determinative features, such as sexual structures, are not always produced in vitro. In conclusion, it is crucial to complement traditional morphology-based identification with molecular-based taxonomy, which includes several markers and will hopefully be applied in our forthcoming studies. *Achlya ambisexualis* showed some promising activity in cellulase, amylase, protease, and lipase. To the best of the researchers' knowledge, this is the first report of *Achlya ambisexualis* to produce asparaginase. Consequently,

the experimental isolate is a promising source of numerous hydrolytic enzymes essential to industrial and medical biotechnology.

ACKNOWLEDGEMENTS

The authors acknowledge with thanks Botany and Microbiology Dept., Helwan and Assiut universities.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

ETHICAL STATEMENTS

This article does not contain any studies with human participants or animals performed by any of the authors.

FUNDING

No funding provided.

REFERENCES

Abd-Elaah, G., Moharram, A. M., & Ghaly, M. W. (2018). Diversity of zoosporic and terrestrial fungi in As-Sant Canal at Assiut, Egypt. *Journal of Basic and Applied Mycology*, 9, 11–32.

Abdel-Azeem, A. M., Yadav, A. N., Yadav, N., & Usmani, Z. (2021). Industrially important fungi for sustainable development. *Springer International Publishing*, 1, 589.

Afzali, S., Hassan, Abdul-Rahim, A., Sharifpour, I., & Sabri, J. (2013). Isolation and identification of Aphanomyces species from natural water bodies and fish farms in Selangor, Malaysia. *Malaysian Applied Biology*, 42(2), 21-31.

Ali, E. H. (2007). Biodiversity of zoosporic fungi in polluted water drainages across Niles' Delta region, Lower Egypt. *Acta Mycologica* 42(1), 99-111. doi:10.5586/am.2007.010.

Ali, E. A. M., Abd Ellatif, S., & Abdel Razik, E. (2020). Production, purification, characterization and immobilization of laccase from Phoma betae and its application in synthetic dyes decolorization. Egyptian Journal of Botany, 60(1), 301-312.

Ammar, M.S., Louboudy, S.S., & Abdul-Raouf, U.M. (1991) Distribution, total viable bacteria and identification of the most potent proteolytic bacterial strains isolated from Aswan City. *Al-Azhar Journal of Microbiology*, 11, 224-238.

Baldauf, S. L. (2008). An overview of the phylogeny and diversity of eukaryotes. *Journal of Systematics and Evolution*, 46(3), 263–273. doi: 10.3724/SP.J.1002.2008.08060.

Barr, D. J. S. (2001). Chytridiomycota. *Systematics and Evolution*, 93–112. doi: 10.1007/978-3-662-10376-0_5.

Beakes, G. W., Honda, D., & Thines, M. (2014). 3 Systematics of the Straminipila: Labyrinthulomycota, Hyphochytriomycota, and Oomycota. *Systematics and Evolution*, 39–97. doi: 10.1007/978-3-642-55318-9_3.

Behera, B. C., Sethi, B. K., Mishra, R. R., Dutta, S. K., & Thatoi, H. N. (2017). Microbial cellulases - Diversity & biotechnology with reference to mangrove environment: A review. *Journal, Genetic Engineering & Biotechnology*, 15(1), 197–210. doi: 10.1016/J.JGEB.2016.12.001.

Bisson, J. W., & Cabelli, V. J. (1979). Membrane filter enumeration method for *Clostridium perfringens*. *Applied and Environmental Microbiology*, 37(1), 55–66. doi: 10.1128/AEM.37.1.55-66.1979.

Cai, L., Zhang, K., McKenzie, E., & Hyde, K. (2003). Freshwater fungi from bamboo and wood submerged in the Liput River in the Philippines. *Fungal Diversity*, 13, 1-12.

Coker, W. C., & Coker, W. C. (1923). The Saprolegniaceae, with notes on other water molds. In *The Saprolegniaceae, with notes on other water molds.* University of North Carolina Press, 1872-1953. doi: 10.5962/bhl.title.6494.

Coll, P. M., Fernandez-Abalos, J. M., Villanueva, J. R., Santamaria, R., & Perez, P. (1993). Purification and characterization of a phenoloxidase (laccase) from the lignin-degrading basidiomycete PM1 (CECT 2971). *Applied and Environmental Microbiology*, 59(8), 2607–2613. doi: 10.1128/aem.59.8.2607-2613.1993.

Czeczuga, B., Mazalska, B., Godlewska, A., Muszyńska, E., & Kuć, K. (2007). Fungi and fungus-like organisms (Straminipila) on fruit tree petals floating in water. *Biological Letters*, 44(1), 41–50.

Czeczuga, B., & Muszyńska, E. (2004). Aquatic zoosporic fungi from baited spores of cryptogams. *Fungal Diversity*, 16, 11-22.

Dick, M. W., & Seymour, R. L. (1970). The genus Saprolegnia. *Nova Hedwigia*, 63(1), 188. doi: 10.2307/3757704.

DiLeo, K., Donat, K., Min-Venditti, A., & Dighton, J. (2010). A correlation between chytrid abundance and ecological integrity in New Jersey pine barrens waters. *Fungal Ecology*, 3(4), 295–301. doi: 10.1016/J.FUNECO.2009.11.004.

El-Fallal, A. A., El-Katony, T. M., Nour El-Dein, M. M., Ibrahim, N. G., & El-Gharabawy, H. M. (2022). Optimizing the Antioxidant Activity of Solid State Fermentation Systems with Pleurotus floridanus and Paecilomyces variotii on Rice Straw. Egyptian Journal of Botany, 62(2), 523-535.

El-Hissy, F. T., & Khallil, A. (1991). Distribution and seasonal occurrence of aquatic Phycomycetes in water and submerged mud in El-Ibrahimia Canal (Upper Egypt). *Mycology*, 4, 311–316.

El-Hissy, F. T., & Khallil, A. M. A. (1989). Studies on aquatic fungi in delta region (Egypt). *Zentralblatt Für Mikrobiologie*, 144(6), 421–432. doi: 10.1016/S0232-4393(89)80059-9.

El-Hissy, F. T., Moubasher, A. H., & El-Nagdy, M. A. (1982). Seasonal fluctuations of freshwater fungi in river Nile (Egypt). *Zeitschrift Fur Allgemeine Mikrobiologie*, 22(8),

521–527. doi: 10.1002/JOBM.3630220803.

El-Hissy, F. T.; Nassar, M. S.; Khallil, A. M. and Abdel-Motaal, F. F. (2001). Aquatic Fungi Recovered from Water and Submerged Mud Polluted With Industrial Effluents. *Journal of Biological Sciences*, 1(9), 854–858. doi: 10.3923/JBS.2001.854.858.

El-Nagdy, M. A., Ali, E. H., Rawaa, A. M., & El-Garni, S. (2022). Comparative study of the diversity of zoosporic fungi (oomycetes and chytrids) in freshwater bodies in Assiut Governorate, Egypt and Jeddah Governorate, Saudi Arabia. *Czech Mycology*, 74(2), 153–179. doi:10.33585/CMY.74204.

Farida, T. E. H., Khallil, A. M., & Abdel-Raheem, A. (1993). Monthly variations of oomycetes (Zoosporic fungi) and aquatic hyphomycetes at sohag. *Acta Societatis Botanicorum Poloniae*, 62(1–2), 67–73. doi: 10.5586/ASBP.1993.012.

Feng, B., & Li, P. (2012). Genome-wide identification of laccase gene family in three *Phytophthora* species. *Genetica*, 140(10–12), 477–484. doi: 10.1007/S10709-012-9696-Z.

Gleason, F. H., Kagami, M., Lefevre, E., & Sime-Ngando, T. (2008). The ecology of chytrids in aquatic ecosystems: roles in food web dynamics. *Fungal Biology Reviews*, 22(1), 17–25. doi: 10.1016/J.FBR.2008.02.001.

Gleason, F. H., & Lilje, O. (2009). Structure and function of fungal zoospores: ecological implications. *Fungal Ecology*, 2(2), 53–59. doi: 10.1016/J.FUNECO.2008.12.002.

Gleason, F. H., Marano, A. V., Lilje, O., & Lange, L. (2017). What has happened to the "aquatic phycomycetes" (sensu Sparrow)? Part I: A brief historical perspective. *Fungal Biology Reviews*, 32(1), 26–33. doi: 10.1016/J.FBR.2017.09.001.

Gleason, F. H., Midgley, D. J., Letcher, P. M., & McGee, P. A. (2006). Can soil Chytridiomycota survive and grow in different osmotic potentials? *Mycological Research*, 110(7), 869–875. doi: 10.1016/J.MYCRES.2006.04.002.

Gleason, F. H., Scholz, B., Jephcott, T. G., van Ogtrop, F. F., Henderson, L., Lilje, O., Kittelmann, S., & Macarthur, D. J. (2017). Key ecological roles for zoosporic true fungi in aquatic habitats. *Microbiology Spectrum*, 5(2). doi: 10.1128/MICROBIOLSPEC.FUNK-0038-2016.

Gulati, R., Saxena, R. K., & Gupta, R. (1997). A rapid plate assay for screening L-asparaginase producing micro-organisms. *Letters in Applied Microbiology*, 24(1), 23–26. doi: 10.1046/J.1472-765X.1997.00331.X.

Hankin, L., & Anagnostakis, S. L. (1975). The use of solid media for detection of enzyme production by fungi. *Mycologia*, 67(3), 597. doi: 10.2307/3758395.

Hill, T. W., & Mullins, J. T. (1979). Association of latent cellulase activity with a membrane fraction from vegetative hyphae of *Achlya ambisexualis*. *Mycologia*, 71(6), 1227. doi: 10.2307/3759109.

Hill, T. W., & Mullins, J. T. (1980). Hyphal tip growth in Achlya. II. Subcellular localization of cellulase and associated enzymes. *Canadian Journal of Microbiology*, 26(9), 1141–1146. doi: 10.1139/M80-188.

Hill, T. W., & Pott, M. P. (2011). Regulation of extracellular proteases in *Achlya ambisexualis*. *Canadian Journal of Botany*, 75(3), 440-444. doi: 10.1139/B97-046.

Jackson, M. L. (1973). Soil chemical analysis, Prentice Hall of India Pvt. Ltd., New Delhi, India, 498, 151–154.

Jephcott, T. G., Sime-Ngando, T., Gleason, F. H., & Macarthur, D. J. (2016). Host–parasite interactions in food webs: Diversity, stability, and coevolution. *Food Webs*, 6, 1–8. doi: 10.1016/J.FOOWEB.2015.12.001.

Johnson, T. W. (1971). Aquatic fungi of Iceland: *Pythium*. *Mycologia*, 63(3), 517. doi: 10.2307/3757549.

Johnson, T. W. (1977). Aquatic fungi of Scandinavia: Species of *Brevilegnia*. *Mycologia*, 69(2), 287. doi: 10.2307/3758654.

Karling, J. S. (John S. (1977). Chytridiomycetarum iconographia : an illustrated and brief descriptive guide to the chytridiomycetous genera with a supplement of the hyphochytriomycetes. 414.

Khallil, A. M. A., & Omar, S. A. (1993). Influence of the insecticide Dimethoate on some metabolic activities of five zoosporic fungi. *Journal of Basic Microbiology*, 33(6), 405–411. doi: 10.1002/JOBM.3620330607.

Khallil, M. A. (1990). Mycoflora associated with some freshwater plants collected from Delta region (Egypt). *Journal of Basic Microbiology*, 30(9), 663–674. doi: 10.1002/JOBM.3620300909.

Khallil, A. M., El-Hissy, F. T., & Ali, E. H. (1995). Seasonal fluctuations of aquatic fungi recovered from Egyptian soil (Delta Region). *Journal of Basic Microbiology*, 35(2), 93–102. doi: 10.1002/JOBM.3620350206.

Khallil, A. R. M., Ali, E. H., Hassan, E. A., & Ibrahim, S. S. (2020). Biodiversity, spatial distribution and seasonality of heterotrophic straminipiles and true zoosporic fungi in two water bodies exposed to different effluents at Assiut (Upper Egypt). *Czech Mycology*, 72(1), 43–70. doi: 10.33585/CMY.72104.

Kiziewicz, B. (2012). Frequency and distribution of zoosporic true fungi and heterotrophic straminipiles from river springs. *Polish Journal of Environmental Studies*, 21(4), 923–927.

Kiziewicz, B., & Kurzątkowska, A. (2004). Aquatic fungi and fungus-like organisms isolated from surface waters situated near Białystok in Podlasie Province of Poland using the insect Notonecta glauca as bait. *Mycologia Balcanica*, 1, 117–123.

Klich, M. A., & Tiffany, L. H. (1985). Distribution and Seasonal occurrence of aquatic saprolegniaceae in northwest Iowa. *Mycologia*, 77(3), 373–380. doi: 10.1080/00275514.1985.12025114.

Latif, S., Mohamed, A. G., Sueyoshi, K., Mohamed, H. E., & Saber, N. E. S. (2021). Effect of Bacillus subtilis on some physiological and biochemical processes in barley (Hordeum vulgare L.) plant grown under salt stress. Egyptian Journal of Botany, 61(1), 141-153.

Legendre, P., & Legendre, L. (1998). Numerical Ecology, Volume 20, Second Edition (Developments in Environmental Modelling). BOOK, 1006.

Luo, J., Yin, J., Cai, L., Zhang, K., & Hyde, K. (2004).

Freshwater fungi in Lake Dianchi, a heavily polluted lake in Yunnan, China. *Fungal Diversity*, 16, 93–112.

Makeshkumar, V. (2011). Isolation and characterization of rapid cellulose degrading fungal pathogens from compost of agro wastes. *International Journal of Pharmaceutical & Biological Archive*, 2(6):1695–1698.

Marano, A. V., Barrera, M. D., Steciow, M. M., Donadelli, J. L., & Saparrat, M. C. N. (2008). Frequency, abundance and distribution of zoosporic organisms from Las Cañas stream (Buenos Aires, Argentina). *Mycologia*, 100(5), 691–700. doi: 10.3852/07-198.

Marano, A. V., Barrera, M. D., Steciow, M. M., Gleason, F. H., Pires-Zottarelli, C. L. A., & Donadelli, J. L. (2011). Diversity of zoosporic true fungi and heterotrophic straminipiles in Las Cañas stream (Buenos Aires, Argentina): Assemblages colonizing baits. *Fundamental and Applied Limnology*, 178(3), 203–218. doi: 10.1127/1863-9135/2011/0178-0203.

Masigol, H., Khodaparast, S. A., Mostowfizadeh-Ghalamfarsa, R., Rojas-Jimenez, K., Woodhouse, J. N., Neubauer, D., & Grossart, H. P. (2020). Taxonomical and functional diversity of Saprolegniales in Anzali lagoon, Iran. *Aquatic Ecology*, 54(1), 323–336. doi: 10.1007/S10452-019-09745-W.

Masigol, H., Khodaparast, S. A., Woodhouse, J. N., Rojas-Jimenez, K., Fonvielle, J., Rezakhani, F., Mostowfizadeh-Ghalamfarsa, R., Neubauer, D., Goldhammer, T., & Grossart, H. P. (2019). The contrasting roles of aquatic fungi and oomycetes in the degradation and transformation of polymeric organic matter. *Limnology and Oceanography*, 64(6), 2662–2678. doi:10.1002/LNO.11242.

Masigol, H., van West, P., Taheri, S. R., Fregeneda-Grandes, J. M., Pârvulescu, L., McLaggan, D., Bliss, T. T., Mostowfizadeh-Ghalamfarsa, R., Pourmoghaddam, M. J., & Grossart, H. P. (2023). Advancements, deficiencies, and future necessities of studying Saprolegniales: A semi-quantitative review of 1073 published papers. *Fungal Biology Reviews*, 46, 100319. doi: 10.1016/J.FBR.2023.100319.

Moharram, A. M., El-Hissy, F. T., & El-Zayat, S. A. (1990). Studies on the mycoflora of Aswan High Dam Lake, Egypt: Vertical fluctuations. *Journal of Basic Microbiology*, 30(3), 197–208. doi: 10.1002/JOBM.3620300311.

Muhsin, T. M. (2012). Aquatic fungi of Iraq: A review. *Marsh Bulletin*, 7(1), 39–47.

Nair, S. K., & Bhat, D. (2016). Diversity and seasonal appearance of aquatic fungi in three streams of western ghat forests of Goa, India. *Biodiversity Journal*, 7 (2), 215–222.

Naranjo-Ortiz, M. A., & Gabaldón, T. (2019). Fungal evolution: diversity, taxonomy and phylogeny of the Fungi. *Biological Reviews of the Cambridge Philosophical Society*, 94(6), 2101–2137. doi: 10.1111/BRV.12550.

Nascimento, C. D. A., Gomes, E. P. C., & Pires-zottarelli, C. L. A. (2011). Occurrence and distribution of zoosporic organisms in water bodies from Brazilian Cerrado. *Mycologia*, 103(2), 261–272. doi: 10.3852/10-139.

Nikolcheva, L. G., & Bärlocher, F. (2004). Taxon-specific fungal primers reveal unexpectedly high diversity during leaf decomposition in a stream. *Mycological Progress*, 3(1), 41–49. doi: 10.1007/S11557-006-0075-Y.

Nikolcheva, L. G., & Bärlocher, F. (2005). Seasonal and substrate preferences of fungi colonizing leaves in streams: traditional versus molecular evidence. *Environmental Microbiology*, 7(2), 270–280. doi: 10.1111/J.1462-2920.2004.00709.X.

Nolan, R. A., & Bal, A. K. (1974). Cellulase localization in hyphae of *Achlya ambisexualis*. *Journal of Bacteriology*, 117(2), 840. doi: 10.1128/JB.117.2.840-843.1974.

Odu, C. E, Ogbonna, C.I.C, Chukwu, O.O.C, Onyimba, I.A., & Ndirmbula, J.B. (2017). Lipase production by some aquatic phycomycetes in Maiduguri a semi-arid area of Nigeria. *European Scientific Journal*, 13(27), 286–286. doi: 10.19044/esj.2017.v13n27p286.

Paliwal, P. (2009). Distribution of Aquatic Fungi in Relation to Physicochemical Factors of Kosi River in Kumaun Himalaya. *Nature and Science*, 7(3),70–74.

Powell, M. J. (1993). Looking at mycology with a janus face: A glimpse at chytridiomycetes active in the environment. *Mycologia*, 85(1), 1–20. doi: 10.1080/00275514.1993.12026239.

Roberts, R. E. (1963). A study of the distribution of certain members of the saprolegniales. *Transactions of the British Mycological Society*, 46(2), 213–224. doi: 10.1016/S0007-1536(63)80077-7.

Scott, W. W. (1961). A monograph of the genus Aphanomyces. *Technical Bulletin. Virginia Agricultural Experiment Station*, 151.

Shearer, C. A., Descals, E., Kohlmeyer, B., Kohlmeyer, J., Marvanová, L., Padgett, D., Porter, D., Raja, H. A., Schmit, J. P., Thorton, H.A., & Voglymayr, H. (2007). Fungal biodiversity in aquatic habitats. *Biodivers Conserv*, 16, 49–67. doi: 10.1007/s10531-006-9120-z.

Sierra, G. (1957). A simple method for the detection of lipolytic activity of micro-organisms and some observations on the influence of the contact between cells and fatty substrates. *Antonie van Leeuwenhoek*, 23(1), 15–22. doi: 10.1007/BF02545855.

Sime-Ngando, T., Lefèvre, E., & Gleason, F. H. (2010). Hidden diversity among aquatic heterotrophic flagellates: ecological potentials of zoosporic fungi. *Hydrobiologia*, 659(1), 5–22. doi: 10.1007/S10750-010-0230-Y.

SPARROW F.K. JR. (1960): Aquatic Phycomycetes. – xxv + 1187 pp., University of Michigan Press, Ann Arbor. 132(3436), 1308. doi: 10.1126/science.132.3436.1308.

Suzuki, S. (1961). The diurnal migration of zoospores of aquatic fungi in a shallow lake. *Shokubutsugaku Zasshi*, 74(873), 138–141. doi: 10.15281/JPLANTRES1887.74.138.

Tammam, A., El-Aggan, W., Badry, H., Abou-Shanab, R., & El-Sawy, S. (2022). Influence of local isolates of

arbuscular mycorrhizal fungi on growth and physiological performance in Zea mays grown in phosphorus-deficient calcareous soil. Egyptian Journal of Botany, 62(1), 131-148.

Tedersoo, L., Sánchez-Ramírez, S., Kõljalg, U., Bahram, M., Döring, M., Schigel, D., May, T., Ryberg, M., & Abarenkov, K. (2018). High-level classification of the Fungi and a tool for evolutionary ecological analyses. *Fungal Diversity*, 90(1), 135–159. doi:10.1007/S13225-018-0401-0.

Thompson, J. D., Higgins, D. G., & Gibson, T. J. (1994). CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research*, 22(22), 4673–4680. doi: 1 0.1093/nar/22.22.4673.

Voigt, K., Marano, A. V., & Gleason, F. H. (2013). Ecological and economical importance of parasitic zoosporic true fungi. *Agricultural Applications,* 2nd Edition, 11, 243–270. doi: 10.1007/978-3-642-36821-9_9.

Voronin, L., & Zhdanova, S. (2023). Distribution and role of fungi and fungus-like organisms in zooplankton of freshwater ecosystems (review). *Transactions of Papanin Institute for Biology of Inland Waters RAS*, 7–16. doi: 10.47021/0320-3557-2023-7-16.

Voronin, L. V. (2008). Zoosporic fungi in freshwater ecosystems. *Inland Water Biology*, 1(4), 341–346. doi: 10.1134/S1995082908040056.

Waterhouse, G. M. (1956). The genus *Phytophthora*. Diagnoses (or descriptions) and figures from the original papers. *Miscellaneous Publications. Commonwealth Mycological Institute*, 12.

Waterhouse, G. M. (1968). The Genus *Pythium Pringsheim*. Diagnoses (or descriptions) and figures from the original papers. *Mycological papers*, *Commonwealth Mycological Institute*, 110, 71.

Willoughby, L. G., & Pickering, A. D. (1977). Viable saprolegniaceae spores on the epidermis of the Salmonid fish Salmo trutta and Salvelinus alpinus. *Transactions of the British Mycological Society*, 68(1), 91–95. doi: 10.1016/S0007-1536(77)80157-5.

Yanna, Ho, W., & Hyde, K. (2002). Fungal succession on fronds of Phoenix hanceana in Hong Kong. *Fungal Diversity*, 10,185-211.