



Effect of Environmental Factors on the Distribution of Fungi in Paddy Fields in Najaf

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Abstract

Fungi are the largest organisms found on Earth; hence, it is important to understand the factors influencing their distribution. In the present study, samples were collected in three stages of rice planting: before planting, during cultivation, and after cultivation from paddy fields in Najaf city. These samples were then cultured using potato dextrose agar medium. The BLAST tool (Basic Local Alignment Search Tool) was used for the identification of the fungi, and their counts were determined using a microscope. The results showed that the identified fungi belonged to genera such as *Aspergillus*, *Achroioestachys*, *Actinomucor*, *Cladosporium*, *Curvularia*, *Fusarium*, *Penicillium*, *Proteus*, and *Talaromyces*. The most frequently identified fungi were *Fusarium humuli*, *Aspergillus niger*, *Alternaria alternata*, *Penicillium oxalicum*, *Aspergillus terreus*, and *Penicillium griseofulvum*, respectively. The highest number of colonies and most significant environmental effects were observed before planting and after the agricultural stages. Species distribution was significantly correlated with environmental factors. Additionally, principal component analysis revealed that the greatest impact of environmental parameters on species distribution was explained by the first and second components, which accounted for 46.05% and 30.20% of the variation, respectively.

Keywords: Distribution, Ecology, Environmental factor, Fungi, Paddy field, PCR.

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Introduction

The Middle East represents a very interesting historical and contemporary region, rich in the biodiversity of various species (Albano et al., 2021). Iraq, located within the Middle East, is one of the countries with very rich biodiversity.

However, the study of biodiversity in Iraq has been disrupted due to significant political, social, and wartime changes (Kotsis, 2024). Iraq has vast areas of agricultural land, and the presence of the Tigris and Euphrates rivers has made agriculture dynamic in this region. This dynamic ecosystem suggests that the country harbors a diverse range of fungi, but the study of fungal flora has been largely neglected (Mustafa et al., 2024).

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Fungi represent a unique class of unicellular eukaryotes whose evolutionary origin dates back to the Precambrian era, between 760 million and 1.06 billion years ago. Due to their haploid chromosomes and chitin-based cell walls, fungi have been considered a separate group from plants and classified as monophyletic. The fungal cell membrane contains ergosterol, and their lysine biosynthetic pathway differs from that of other organisms. Moreover, their carbohydrate synthesis pathways, biochemical processes, and enzymatic reactions distinguish fungi from plants and animals (Bhagat et al., 2023).

Fungi play key roles in the environment, which include:

1. Maintaining ecosystem functioning through cooperation with other organisms,
2. Acting as natural decomposers within the global carbon cycle,
3. Playing a dominant role in plant nutrition through symbiotic relationships,
4. Participating in lichen formation,
5. Controlling host population dynamics, and
6. Influencing the life and mortality of plants and animals (Bahram and Netherway, 2022).

Various organisms respond to environmental modifications to adapt to changing conditions. In fungi, responses to environmental changes occur at cellular, morphological, and molecular levels. These responses involve alterations in gene expression, phenotype, physiological flexibility, gene modification, speciation, natural selection, species migration, and behavioral adjustments to enhance adaptation or dominance under environmental changes. Fungi are capable of detecting environmental factors such as temperature, pH, water stress, light intensity, and intracellular or extracellular nutrient concentrations, as well as oxidative stress (Bahram and Netherway, 2022).

The influence of various environmental parameters on organisms and their reactions to these factors has become a priority for international studies, which aim to improve understanding of adaptation mechanisms and the distribution of organisms in the environment

(Bahram and Netherway, 2022). For example, the effects of temperature and air humidity in Basra on the distribution of atmospheric fungi were studied. The results showed a negative relationship between fungal population size and air temperature, whereas a positive relationship was observed with air humidity (Lu et al., 2022). Additionally, the influence of salinity, osmotic stress, and temperature variations on yeast distribution in Iraq has been studied (Alghanem et al., 2023). Similarly, the physicochemical composition of water was found to directly influence fungal population sizes in the spring waters of Kurdistan (SHARMA, 2024).

This study highlights how various ecological parameters of rice paddy fields in Najaf Province, Iraq, influence the distribution of fungi in relation to agricultural activities along the Tigris and Euphrates rivers.

Materials and Methods

Collection of Samples

All experiments were conducted at the Rice Research Station in the Al-Mashkhab area, under the Iraqi Ministry of Agriculture. Samples were collected from locations A (31° 53' 23" N, 44° 30' 03" E), B (31° 53' 22" N, 44° 30' 00" E), and C (31° 53' 19" N, 44° 30' 00" E) (Fig. 1). Sampling was performed three times: before planting, during cultivation, and after cultivation, at depths of 5–10

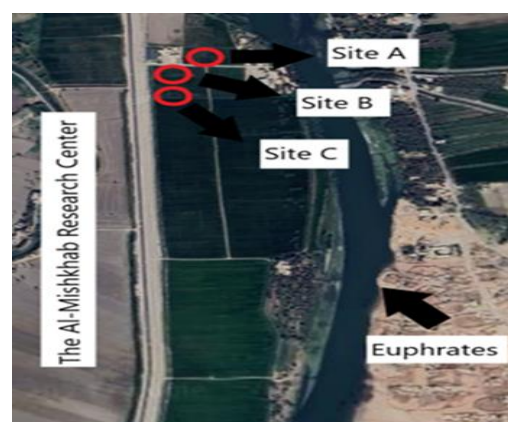


Fig. 1. The Study sites in paddy fields.

cm. All soil samples were sieved through a 2 mm diameter sieve, transferred into polyethylene bags, and stored in a refrigerator for physical and chemical analysis (James and Wells, 1990).

Potato Dextrose Agar (PDA)

Potato dextrose agar (PDA) medium was used to isolate and cultivate fungi from soil samples collected from different regions (Sharma et al., 2011). To culture the soil samples on PDA, the dilution method was used by adding 1 gram of soil sample to 10 ml of distilled water. Then, 1 ml of the suspension was transferred to a sterile tube containing 9 ml of distilled water to achieve a 10^{-4} dilution. Finally, 1 ml of the diluted suspension was added to a petri dish containing 15 ml of PDA medium. The culture medium was incubated at 27 °C for five days to identify the fungal colonies (Tsao, 1960). The number of fungi was counted when colonies became visible, using a microscope (Black, 1965).

Environmental Parameters

Environmental parameters such as electrical conductivity, pH, total nitrogen, phosphorus, potassium, calcium, sodium, magnesium, and organic compounds (e.g., gypsum, calcium carbonate, ammonium, and chlorides), as well as wind speed and humidity, were measured using modern laboratory facilities and methods (Sharma, 2014).

Identification and Extraction of DNA

DNA extraction was performed using a method developed by the U.S. company Zymo Research with their DNA extraction kit (Cat. No. D6005). Maxime PCR PreMix (I-Taq Cat. No. 25026) prepared by iNtRoN was used for the polymerase chain reaction (PCR). Using the official primers (ITS1: TCCGTAGGTGAACCTGCGG) and reverse primer (ITS4: TCCCCTTATTGATATGC), PCR was conducted in a total volume of 20 microliters.

The DNA structure underwent initial denaturation for 5 minutes at 98 °C, followed by 40 seconds of denaturation at 94 °C. Primer annealing was performed for 40 seconds at 55 °C, and initial elongation was carried out for 1 minute at 72 °C.

The PCR concluded with a final elongation step at 72 °C (Mutlag and Hussain).

To identify the isolated fungi, PCR products (ITS1 and ITS4) were sent to the Korean company Macrogen for sequencing of the nitrogen base nucleotides. All nitrogen-based sequences were analyzed using the Basic Local Alignment Search Tool (BLAST) to compare them with data from the National Center for Biotechnology Information (NCBI) for globally recognized fungi.

Results

Identified Species by Nucleotide Sequences

The species identification from nucleotide sequences was performed using advanced BLAST tools to study and identify the sequences obtained from fungi isolated from paddy fields in Najaf Province. The following genera were detected: *Actinomucor*, *Achroiostachys*, *Alternaria*, *Aspergillus*, *Cladosporium*, *Curvularia*, *Fusarium*, *Penicillium*, *Proteus*, and *Talaromyces* (Table 1).

Counting the Number of Identified Fungi

In total, 403 fungi were identified at all stages of agriculture, with the highest number of fungal species recorded before planting, after agriculture, and during agriculture. The most common fungi identified in the paddy fields included *Fusarium humuli*, *Aspergillus niger*, *Alternaria alternata*, *Penicillium oxalicum*, *Aspergillus terreus*, *Penicillium griseofulvum*, *Aspergillus flavus*, *Achroiostachys saccharicola* = *Penicillium dipodomycicola*, *Cladosporium sphaerospermum*, *Curvularia plantarum*, *Penicillium camemberti* = *Talaromyces funiculosus*, *Fusarium chlamydosporum* = *Proteus mirabilis*, and *Actinomucor elegans* (Table 2 and Fig. II).

Measurement of Environmental Parameters

All environmental factors, except for phosphorus and iron content, showed an increase after the stages of agriculture and prior to planting. The

Table 1
The identified fungi in current study.

Identified Fungi	Genome	Sequence ID	Sequence Similarities
<i>Actinomucor elegans</i> (ACEL)	5.8 Ribosomal RNA	MT503293.1	99%
<i>Achroistachys saccharicola</i> (ACSA)	18S Ribosomal RNA	KU845809.1	96%
<i>Alternaria alternata</i> (ALAL)	5.8 Ribosomal RNA	OP984167.1	98%
<i>Aspergillus flavus</i> (ASFL)	5.8 Ribosomal RNA	OR656460.1	98%
<i>Aspergillus terreus</i> (ASTE)	5.8 Ribosomal RNA	OR975589.1	88%
<i>Aspergillus niger</i> (ASNI)	5.8 Ribosomal RNA	MN429196.1	100%
<i>Cladosporium sphaerospermum</i> (CLSP)	5.8 Ribosomal RNA	MH393182.1	96%
<i>Curvularia plantarum</i> (CUPL)	5.8 Ribosomal RNA	OQ555118.1	99%
<i>Fusarium chlamydosporum</i> (FUCH)	5.8 Ribosomal RNA	MT448897.1	99%
<i>Fusarium humuli</i> (FUHU)	5.8 Ribosomal RNA	MW016529.1	98%
<i>Penicillium dipodomyicola</i> (PEDI)	16S Ribosomal RNA	KJ145889.1	97%
<i>Penicillium oxalicum</i> (PEOX)	5.8 Ribosomal RNA	MN795755.1	96%
<i>Penicillium camemberti</i> (PECA)	5.8 Ribosomal RNA	MT529671.1	97%
<i>Proteus mirabilis</i> (PRMI)	NUITM-VP1 DNA	AP026827.1	85%
<i>Penicillium griseofulvum</i> (PEGR)	5.8 Ribosomal RNA	MN133842.1	95%
<i>Talaromyces funiculosus</i> (TAFU)	5.8 Ribosomal RNA	ON063294.1	96%

maximum values of pH, EC, gypsum, organic matter, chlorine, calcium, magnesium, sodium, sulfate, ammonia, phosphorus, and humidity were observed after the agriculture stage. The highest values for bulk density, calcium carbonate, total nitrogen, temperature, and wind speed were recorded prior to planting. Environmental parameters such as potassium and bicarbonate were higher after agriculture and before planting. Organic carbon remained consistently high at all stages (Table 3).

Correlation Between Environmental Parameters

A significant correlation at $p \leq 0.05$ and $p \leq 0.01$ was observed among the environmental parameters. The following correlations were recorded: a positive and significant relationship between chemical elements, inorganic and organic compounds, including phosphorus, potassium, calcium, magnesium, sodium, sulfate, ammonia, chlorine, bicarbonate, and lead. Iron and temperature demonstrated a significant negative correlation with the chemical elements. Other statistical analyses revealed a significant negative correlation between the following pairs:

pH and bulk density, calcium carbonate, and total nitrogen; temperature and wind speed, pH, EC, phosphorus, iron, and gypsum; total nitrogen and temperature; and wind speed and organic matter. On the other hand, calcium carbonate showed the strongest positive relationship with bulk density, EC, and organic carbon. A significant positive relationship was found between pH and gypsum, magnesium, lead, chlorine, ammonia, sulfate, calcium, organic matter, as well as between EC, potassium, calcium, magnesium, sodium, sulfate, ammonia, chlorine, bicarbonate, and lead with gypsum, calcium, sulfate, and chlorine with organic matter. Total nitrogen was positively related to wind speed. It was also observed that none of the environmental parameters showed a significant correlation with organic carbon (Table 4).

Correlation Between Environmental Parameters and Fungal Distribution

A significant relationship between physical parameters and fungal species distribution was recorded at $p \leq 0.05$ and $p \leq 0.01$. The results

Table 2
The identified species of fungi in paddy fields.

Species	Before Planting	During Agriculture	After Agriculture	Num of each species in all stage
<i>Actinomucor elegans</i>	2	0	2	4
<i>Achroiostachys saccharicola</i>	3	0	15	18
<i>Alternaria alternata</i>	6	17	26	49
<i>Aspergillus flavus</i>	4	0	16	20
<i>Aspergillus terreus</i>	8	17	8	33
<i>Aspergillus niger</i>	44	9	2	55
<i>Cladosporium sphaerospermum</i>	0	16	0	16
<i>Curvularia plantarum</i>	2	4	3	9
<i>Fusarium chlamydosporum</i>	0	0	6	6
<i>Fusarium humuli</i>	23	38	21	82
<i>Penicillium dipodomyicola</i>	2	0	16	18
<i>Penicillium oxalicum</i>	41	5	0	46
<i>Penicillium camemberti</i>	5	1	1	7
<i>Proteus mirabilis</i>	0	0	6	6
<i>Penicillium griseofulvum</i>	13	2	12	27
<i>Talaromyces funiculosus</i>	1	1	5	7
All species in each stage	154	110	139	403

Table 3
The results of Environmental parameters of study area.

Parameter	Before Planting	During Agriculture	After Agriculture
Bulk density	1.79 ± 0.018a	1.65 ± 0.001b	1.68 ± 0.06b
pH	7.3 ± 0.02c	7.5 ± 0.04b	7.7 ± 0.03a
EC	2.67 ± 0.005b	2.63 ± 0.068b	3.3 ± 0.2a
CaCO ₃	271 ± 0.83a	233 ± 0.66c	238 ± 0.83b
Gypsum	1.57 ± 0.01b	1.30 ± 0.02c	1.76 ± 0.02a
Organic Matter	10.1 ± 0.25c	10.4 ± 0.05b	11.1 ± 0.01a
Total Nitrogen	0.37 ± 0.0083a	0.02 ± 0.0003c	0.04 ± 0.0003b
Organic Carbon	6.61 ± 0.16a	6.49 ± 0.02a	6.64 ± 0.01a
Phosphorus	15.5 ± 0.183b	16.4 ± 0.130a	15.2 ± 0.005c
Potassium	242 ± 0.16a	218 ± 0.33b	243 ± 1a
Chloride (Cl)	2.62 ± 0.05b	2.44 ± 0.06c	3.17 ± 0.01a
Calcium (Ca)	4.1 ± 0.003b	3.8 ± 0.023c	4.8 ± 0.006a
Magnesium (Mg)	3.55 ± 0.011b	2.76 ± 0.026c	4.09 ± 0.011a
Sodium (Na)	4.73 ± 0.003b	4.42 ± 0.001c	4.79 ± 0.01a
Sulfites (So ₄)	3.38 ± 0.06b	3.23 ± 0.01c	3.70 ± 0.02a
NH ₄	0.015 ± 0.0001b	0.013 ± 0.0001c	0.017 ± 0.0001a
HCO ₃	2.68 ± 0.13a	1.71 ± 0.05b	2.54 ± 0.03a
Lead (Pb)	0.027 ± 0.0006b	0.017 ± 0.0037c	0.033 ± 0.0010a
Iron (Fe)	15.21 ± 0.043b	16.22 ± 0.043a	15.26 ± 0.023b
Temperature	30.3 ± 1.3a	29 ± 1a	23.3 ± 0.16b
Humidity	42 ± 0.02c	45 ± 0.01b	56 ± 0.01a
Wind speed	3.5 ± 0.10a	2.5 ± 0.10b	2.2 ± 0.12c

indicated the following significant positive correlations:

- *Aspergillus flavus* with EC and chlorine
- *Alternaria alternata* with pH, organic matter, temperature, and wind speed
- *Penicillium oxalicum* with bulk density and wind speed
- *Talaromyces funiculosus* with EC
- *Fusarium chlamydosporum* with EC, organic matter, calcium, sulfate, and chlorine
- *Achroiostachys saccharicola* with calcium, phosphorus, organic matter, gypsum, EC,

magnesium, sodium, sulfate, nitrate, chlorine, and lead

- *Cladosporium sphaerospermum* with phosphorus
- *Penicillium camemberti* with wind speed
- *Actinomucor elegans* with organic carbon
- *Proteus mirabilis* with EC
- *Aspergillus niger* with bulk density, calcium carbonate, total nitrogen, wind speed, and temperature (Table 5).

Principal Component Analysis

Environmental factors and fungal species located closer to the axes have a greater influence. Variables near each other show a positive relationship. Positive values for X and Y indicate a positive relationship with the axes. The results showed that the first and second axes explained the greatest changes, with eigenvalues of 17.04 and 11.17, respectively, accounting for 46.05% and 30.20% of the variance. Together, the first and

Table 4
Correlations of Physical parameters in all Planting time.

P value	Bulk density	pH	EC	CaCO ₃	Gypsum	Organic M	Nitrogen	Organic C	P	K	Ca
Bulk density	1										
pH	-0.750*	1									
EC	-0.329	0.755*	1								
CaCO ₃	0.902**	-0.835**	-0.339	1							
Gypsum	0.245	0.34	0.797*	0.206	1						
Organic M	-0.588	0.911**	0.852**	-0.634	0.562	1					
Nitrogen	0.888**	-0.868**	-0.39	0.998**	0.143	-0.679*	1				
Organic C	0.21	0.016	0.375	0.31	0.584	0.419	0.272	1			
P	-0.391	-0.164	-0.691*	-0.375	-0.970**	-0.364	-0.319	-0.484	1		
K	0.566	-0.053	0.523	0.581	0.914**	0.218	0.527	0.633	-0.954**	1	
Ca	-0.098	0.651	0.927**	-0.144	0.933**	0.810**	-0.207	0.517	-0.836**	0.720*	1

* Correlation is significant at the 0.05 level (2-tailed).

** Correlation is significant at the 0.01 level (2-tailed).

Table 4 (Continued)
Correlations of Physical parameters in all Planting time.

P value	Mg	Na	So ₄	NH ₄	CL	HCO ₃	Pb	Fe	TEM	Wind
Mg	1									
Na	0.967**	1								
So ₄	0.937**	0.823**	1							
NH ₄	0.996**	0.949**	0.943**	1						
CL	0.917**	0.794*	0.986**	0.930**	1					
HCO ₃	0.845**	0.945**	0.649	0.805**	0.59	1				
PB	0.957**	0.944**	0.871**	0.950**	0.844**	0.838**	1			
FE	-0.890**	-0.976**	-0.686*	-0.861**	-0.653	-0.974**	-0.886**	1		
TEM	-0.668*	-0.465	-0.873**	-0.694*	-0.898**	-0.225	-0.544	0.276	1	
Wind	-0.034	0.215	-0.331	-0.097	-0.402	0.488	0.022	-0.407	0.699*	1

Table5
Correlation of Environmental parameters and fungi distribution.

P value	Bulk density	pH	EC	CaCO3	Gypsum	Organic M	Nitrogen	Organic C	P	K	Ca
ASFL	0.112	0.406	0.712*	-0.097	0.633	0.51	-0.147	0.308	-0.554	0.482	0.652
ALAL	-0.378	0.784*	0.615	-0.677*	0.332	0.743*	-0.716*	0.036	-0.16	-0.007	0.547
PEDI	0.197	0.335	0.502	-0.131	0.49	0.423	-0.178	0.243	-0.395	0.345	0.503
PEOX	0.682*	-0.61	-0.356	0.655	0.003	-0.718*	0.664	-0.413	-0.223	0.255	-0.259
ASTE	-0.37	0.14	-0.183	-0.267	-0.45	-0.1	-0.249	-0.394	0.458	-0.449	-0.292
TAFU	-0.405	0.481	0.693*	-0.211	0.376	0.49	-0.231	0.188	-0.331	0.245	0.504
CUPL	-0.351	0.155	0.16	-0.276	-0.165	0.08	-0.258	-0.186	0.175	-0.26	-0.078
FUCH	-0.481	0.661	0.718*	-0.31	0.586	0.703*	-0.341	0.265	-0.518	0.37	0.735*
FUHU	-0.322	-0.049	-0.291	-0.315	-0.553	-0.194	-0.274	-0.421	0.548	-0.606	-0.476
ACSA	-0.235	0.636	0.960**	-0.192	0.804**	0.785*	-0.243	0.498	0.708*	0.596	0.898**
CLSP	-0.374	0.106	-0.401	-0.393	-0.639	-0.1	-0.369	-0.354	0.704*	-0.664	-0.459
PECA	0.569	-0.53	-0.333	0.6	0.059	-0.499	0.603	-0.041	-0.2	0.29	-0.161
ACEL	0.095	-0.041	0.32	0.379	0.534	0.252	0.358	0.724*	-0.53	0.615	0.451
PRMI	-0.448	0.538	0.794*	-0.25	0.459	0.564	-0.272	0.201	-0.414	0.289	0.587
PEGR	0.269	-0.094	0.374	0.42	0.543	0.097	0.396	0.432	-0.593	0.625	0.413
ASNI	.812**	-.948**	-0.53	.942**	-0.064	-.763*	.960**	0.225	-0.106	0.328	-0.403

* Correlation is significant at the 0.05 level (2-tailed).

** Correlation is significant at the 0.01 level (2-tailed).

Table5 (continued)
Correlation of Environmental parameters and fungi distribution.

P value	Mg	Na	So ₄	NH ₄	Cl	HCO ₃	Pb	Fe	TEM	Wind
ASFL	0.612	0.536	0.635	0.634	0.667*	0.409	0.532	-0.438	-0.582	-0.25
ALAL	0.297	0.1	0.499	0.356	0.575	-0.124	0.205	0.077	-0.726*	-0.781*
PEDI	0.455	0.389	0.469	0.49	0.529	0.28	0.358	-0.301	-0.468	-0.277
PEOX	0.001	0.164	-0.119	-0.078	-0.198	0.443	-0.002	-0.278	0.341	0.737*
ASTE	-0.39	-0.446	-0.252	-0.388	-0.357	-0.376	-0.44	0.488	0.102	-0.058
TAFU	0.413	0.308	0.516	0.413	0.459	0.184	0.45	-0.212	-0.473	-0.28
CUPL	-0.178	-0.232	-0.08	-0.177	-0.053	-0.287	-0.36	0.251	-0.169	-0.128
FUCH	0.605	0.462	0.740*	0.61	0.712*	0.265	0.592	-0.333	-0.734*	-0.45
FUHU	-0.581	-0.604	-0.485	-0.577	-0.428	-0.6	-0.757*	0.58	0.134	-0.11
ACSA	0.810**	0.685*	0.876**	.829**	0.876**	0.468	0.770*	-0.554	-0.796*	-0.4
CLSP	-0.602	-0.661	-0.475	-0.57	-0.516	-0.625	-0.466	0.696	0.324	-0.26
PECA	0.066	0.213	-0.094	0.017	-0.147	0.399	0.027	-0.319	0.302	0.669*
ACEL	0.568	0.615	0.413	0.56	0.38	0.535	0.628	-0.64	-0.123	0.201
PRMI	0.481	0.364	0.598	0.48	0.563	0.206	0.483	-0.262	-0.589	-0.318
PEGR	0.557	0.614	0.428	0.529	0.395	0.604	0.568	-0.641	-0.162	0.353
ASNI	-0.054	0.2	-0.377	-0.107	-0.412	0.433	0.045	-0.404	0.748*	0.956**

* Correlation is significant at the 0.05 level (2-tailed).

** Correlation is significant at the 0.01 level (2-tailed).

second components explained 76.25% of the changes.

The results indicated that the distribution of *Penicillium griseofulvum* and *Actinomucor elegans* was most influenced by changes in magnesium, nitrate, organic carbon, gypsum, phosphorus, lead, sodium, and bicarbonate. Changes in calcium

carbonate, total nitrogen, bulk density, wind speed, and temperature were most influential on the distribution of *Penicillium camemberti*, *Penicillium oxalicum*, and *Aspergillus niger*. The distribution of *Penicillium dipodomycicola*, *Aspergillus flavus*, *Achroioctachys saccharicola*, *Talaromyces funiculosus*, *Proteus mirabilis*, and *Alternaria alternata* was associated with changes

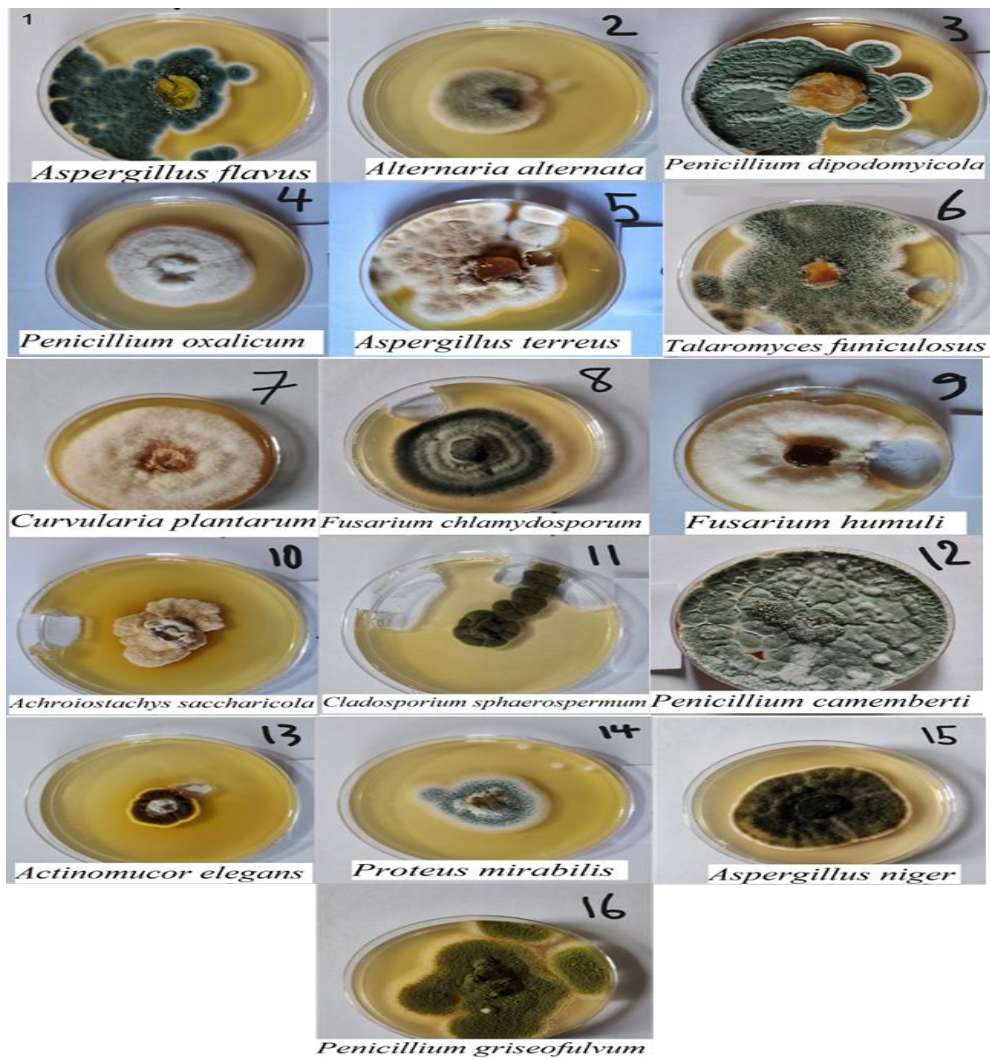


Fig.II. The grown Colonies in PDA medium.

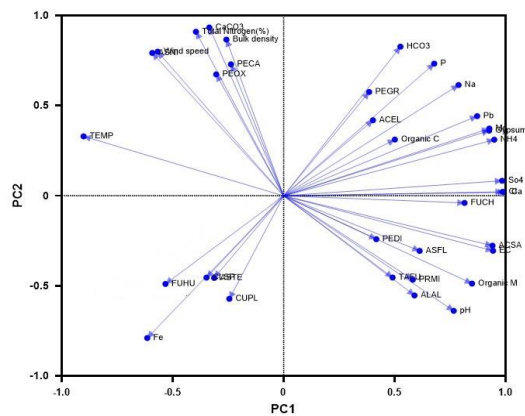


Fig III. Principal component analysis of fungi distribution and environmental parameters

in pH, organic matter, and EC. Additionally, changes in iron concentration had an effect on the distribution of *Fusarium humuli*, *Curvularia plantarum*, *Cladosporium sphaerospermum*, and *Aspergillus terreus* (Fig. III).

Discussion

The study of the distribution of biological species, particularly fungi, in relation to environmental factors is vital for understanding ecological dynamics. Changes in species distribution can be attributed to factors such as global warming and climate change, which are altering ecosystems worldwide (Vitasse et al., 2021). Fungi play a crucial ecological role through their mutualistic relationships with both prokaryotic and eukaryotic organisms. It is estimated that fungi comprise 2 to 3.8 million species, though only 10% have been formally identified (Chethana et al., 2021; Sun et al., 2019).

Ecological research often investigates the influence of environmental factors on species distribution. For instance, genetic research has used techniques such as AFLP polymorphism to differentiate species like *Cryptococcus neoformans* and *Cryptococcus bacillisporus*, highlighting the link between genetic variations and ecological distribution (de Holanda Fonseca et al., 2024). Molecular and morphological methods have also identified distinct fungal species in agricultural environments, including the genera *Fusarium*, *Alternaria*, *Penicillium*, and *Curvularia* in rice fields (Zafrin et al., 2024).

Fungi from the *Fusarium* genus are particularly problematic in paddy fields, causing diseases like root rot and seedling rot (Nikitin et al., 2023). Other genera, such as *Aspergillus* and *Penicillium*, are also prevalent across global rice fields, including those in China and Italy (Conde et al., 2024). In Najaf province, Iraq, several fungi, including *Aspergillus flavus*, *Alternaria alternata*, *Penicillium dipodomyicola*, and *Fusarium humuli*, were isolated from rice paddies, with the highest distributions recorded for *Fusarium humuli*, *Aspergillus niger*, *Alternaria alternata*, *Penicillium oxalicum*, and *Aspergillus terreus*.

Environmental factors such as water, humidity, nutrient availability, and stress conditions are known to influence fungal distribution in rice fields (Khoshru et al., 2023). Temperature and pH have also been found to affect the growth of certain fungi, with increased temperature and pH often inhibiting growth (Yusuf et al., 2023). Studies have shown that soil organic carbon, nitrogen, and pH concentrations, as well as other factors such as soluble silicate, are critical in shaping fungal distribution (Philippot et al., 2024). Furthermore, temperature and rainfall have been shown to influence fungal species distribution in regions such as the Norway (Yu et al., 2023).

Fungi also interact with other soil microorganisms to impact nutrient cycles. They play a role in decomposing organic matter, influencing the soil carbon and nitrogen cycles (Raza et al., 2023). However, environmental factors like excessive concentrations of chemical elements can limit fungal growth due to toxicity (Nieder and Benbi, 2024). Fungal species with higher biodegradation capacities tend to have more diverse populations (Nieder and Benbi, 2024).

The impact of climatic conditions on fungal distribution is not easily predictable, as temperature, water availability, and other factors can influence fungal reproduction, habitat suitability, and competition (Casu et al., 2024). Wind speed, for example, plays a significant role in the dispersal of fungal spores across long distances (Guhl et al.). This study also found that the distribution of the identified fungal species in Najaf province was significantly correlated with environmental parameters, although no correlation was established for some species such as *Penicillium dipodomyicola*, *Aspergillus terreus*, *Curvularia plantarum*, and *Penicillium griseofulvum*.

These findings highlight the importance of understanding environmental factors in fungal distribution, as they are crucial in maintaining ecological balance and managing agricultural health.

Conclusion

The highest distribution of fungal species in the study was observed in the following order:

Fusarium humuli > *Aspergillus niger* > *Alternaria alternata* > *Penicillium oxalicum* > *Aspergillus terreus* > *Penicillium griseofulvum*. Principal component analysis (PCA) revealed that the distribution of *Fusarium humuli* was most strongly associated with changes in iron concentration, showing a positive correlation. The distribution of *Aspergillus niger* and *Penicillium oxalicum* was influenced by several environmental factors, including calcium carbonate, total nitrogen, bulk density, wind speed, and temperature. Specifically, *Aspergillus niger* demonstrated a significant positive correlation with all these

parameters, while *Penicillium oxalicum* was primarily correlated with wind speed and bulk density.

In contrast, *Alternaria alternata* exhibited a closer association with electrical conductivity (EC), pH, and organic matter content, with a significant positive correlation only observed with changes in pH and organic matter. These findings underscore the complex relationships between fungal distribution and environmental factors, highlighting the diverse ecological requirements of different fungal species.

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