

Detection of Fungi in Irrigation Water Sources using Different Isolation Methods

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Abstract

This study was conducted over a 6-month period (2018-2019) to study the inherent fungal contaminants of irrigation water in Al-Kharj farms. Water samples were collected from three sites of the wells (the north and south sides and the plastic housing) at the farms. Sewage samples were also collected. One hundred and sixty-seven fungal isolates were obtained from the wells and the sewage. These isolates belonged to 16 genera, of which 13 were deuteromycetes, one was a zygomycetes and one was an oomycete. The results showed that *Pythium*, *Aspergillus*, *Rhizoctonia*, *Rhizopus*, and *Fusarium* genera were found throughout most months of the year. The results for each month were similar in terms of the frequency of fungi detected (99.96–100%) and the fungi isolation frequency (average, 6.247–6.25%). *Pythium*, *Aspergillus*, *Rhizoctonia*, *Penicillium*, and *Diplococcium* were identified in the water samples taken from the wells and the plastic housing. *Alternaria* and *Fusarium* were identified in water samples taken from the wells, while *Candida* and *Macrophoma* were also found in water samples taken from the well, while *Cladosporium* was found in the water samples and the sewage samples. *Rhizopus* was found in water samples taken from all of the wells near the plastic housing. *Ulocladium* was found in water samples taken from the wells and the plastic housing, while *Thielaviopsis* was found in water samples taken from the wells near the housing and the sterile fungus was found in water samples taken from the wells and the plastic housing. Moreover, we found that the seed trap method was the best method for fungi isolation (isolation frequency, 11.17%) and was better than the direct method of isolation (isolation frequency, 8.53%).

Keywords: Well-Farm, Deuteromycete, zygomycetes, oomycete, seed traps

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INTRODUCTION

Water is a vital resource for all known living organisms, as there is no life without it.¹ The Earth's surface is 71% water and fresh water comprises 3% of the total amount of water on the Earth's surface.² This small percentage of fresh water plays an essential role in human life, as it is the main source of water for human consumption and it is suited to human needs.³ It is estimated that 80% of water use in the Middle East is for irrigation purposes.⁴ There are many sources of water pollution, but the most notable source is microbial contamination. Sewage or untreated wastewater releases a large amount of dissolved organic material, suspended materials, and harmful microbiological organisms.⁵

Fungi are one of the most important biological sources of water contamination. These fungi may originate from the soil. Several studies have been conducted to detect the presence of fungi in water. Muhsin et al.⁶ isolated several fungi from well water, including *Alternaria alternate*, *Aspergillus* sp., *Trichoderma viride*, *Penicillium* sp., *Fusarium oxysporum*, and *Cladosporium* sp. Moreover, Rajanaika et al.⁷ isolated *Pythium*, *Allomyces*, *Rhizopus*, *Achyla*, *Alternaria*, *Aspergillus*, *Saprolegnia*, *Chaetominm*, *Cladosporium*, *Fusarium*, *Trichoderma*, and *Penicillium* from the Tunga River in India. Kraume and Awad⁸ found 60 species of fungi belonging to 27 genera in wastewater samples, including 8.8% *Geotrichum*, 75% *Penicillium*, 65.7% yeasts, and 55.5% *Trichoderma*.

The aim of the present study was to identify pathogenic and other contaminating fungi in water samples collected from wells and sewage, which is used for irrigation at some Al-Kharj farms due to the lack of irrigation water. The fungal genera present in irrigation water samples and the physical and chemical properties of the water samples were analyzed.

MATERIALS AND METHODS

Sample Collection

Water samples were collected from three sites of wells (the north and south sides and the plastic housing) at Al-Kharj farms and from the sewage. Samples were collected in clean, sterile 600-mL plastic bottles that were opened under water and filled with the water sample. The water

temperature was also measured directly using a thermometer, and an additional clean, sterile 600-mL plastic bottle was filled to measure mineral element concentrations.

Fungi Isolation

1-Direct Isolation

Khaimi⁹ used the plate-pouring method, in which 1 mL of each water sample was placed on a sterile Petri dish with a 9-cm diameter. Five replicates were plated per sample. Potato dextrose agar (PDA) was added and sterilized using steam autoclave sterilization. Chloramphenicol was added to the medium at a concentration of 250 mg/L. The plates were rotated to achieve homogenization between the sample and culture media and were then incubated at 25°C for 7-21 days. The number of growing fungal colonies was determined and the fungi were purified by transferring a small part of the outer fungal growth of the colony to new plates containing PDA. These plates were used for identification using taxonomic keys. The frequency of isolated fungal species was calculated using the following equation:

frequency (%) = number of fungal isolates/total number of isolates × 100. Moreover, the prevalence was calculated using the following equation: prevalence (%) = number of samples containing fungi/total number of samples × 100.

2- Seed Traps

The seed trap method described by Hussain et al.¹⁰ was used to isolate fungi from water samples. Fifty chard seeds were sterilized using an autoclave and placed in 15 mL of each water sample (three replicates for each source) for 24 hours. The seeds were dried with sterile blotting paper and then transferred to a sterile 9-cm-diameter Petri dish containing PDA, with 10 seeds per plate. Chloramphenicol was added to the medium at a concentration of 250 mg/L and the plates were incubated at 25°C for 7-21 days. The fungi were purified and the number of the growing fungal colonies, the frequency of isolated fungal species, and the appearance of the fungi were determined as described for the direct isolation method.

Chemical Analyses

The temperature of the water samples was measured directly using a thermometer. pH was also measured using a pH meter, as described by Jackson.¹¹ Electrical conductivity and the

Table 1. The percentage of the fungi appearance at different water sources in six months

Isolated Fungi	December	January	February	March	April	May
<i>Aspergillus</i>	0	17.39	7.5	2.85	29.62	32.25
<i>Alternaria</i>	18.18	0	0	28.57	0	0
<i>Candida</i>	6.81	0	0	0	0	0
<i>Cephalosporium</i>	0	0	27.5	0	0	0
<i>Cladosporium</i>	0	0	12.5	0	1.85	9.67
<i>Diplococcium</i>	29.54	0	0	0	0	0
<i>Fusarium</i>	6.81	0	0	5.71	5.55	3.22
<i>Macrophoma</i>	0	0	0	0	1.85	0
<i>Mycelia sterilia</i>	0	0	0	0	0	4.83
<i>Penicillium</i>	0	10.86	0	0	1.85	0
<i>Pythium</i>	0	4.34	50	45.71	35.18	27.41
<i>Rhizoctonia</i>	6.81	23.91	0	8.57	11.11	22.58
<i>Rhizopus</i>	13.63	32.6	2.5	0	12.96	0
<i>Thielaviopsis</i>	0	0	0	8.57	0	0
<i>Trichoderma</i>	0	10.86	0	0	0	0
<i>Ulocladium</i>	18.18	0	0	0	0	0
Total	99.96	99.96	100	99.98	99.97	99.96
Rate	6.247	6.247	6.25	6.248	6.248	6.247

concentrations of dissolved positive and negative ions were measured using the method described by Richard.¹²

RESULTS

Fungi Isolation

One hundred and sixty-seven fungal isolates were obtained from well water and sewage water samples collected from Al-Kharj. These fungi belonged to 16 genera and included 13 deuteromycetes, one zygomycete, and one oomycete. The frequency of fungi isolation was equal between different samples (99.96–100%), with an isolation rate of 6.247–6.25%. *Pythium*, *Rhizopus*, *Rhizoctonia*, *Aspergillus*, and *Fusarium* were the most frequently isolated genera, as shown in Table 1.

Direct Isolation Method

As shown in Table 2, the prevalence of fungi in the samples was 8.53%. *Pythium*, *Aspergillus*, *Cladosporium*, *Rhizopus*, *Rhizoctonia*, *Candida*, *Penicillium*, *Alternaria*, *Fusarium*, *Macrophoma*, *Diplococcium*, *Trichoderma*, and *Mycelia sterilia* were isolated, but *Ulocladium*, *Thielaviopsis*, and *Cephalosporium* were not detected.

Well water samples had the highest percentage of fungal isolates, at 11.80%, and the

plastic housing samples were the least polluted with fungi, at 5.90%.

Seed Trap Method

Fungal isolation using the seed trap method resulted in an overall fungal prevalence of 11.17% (Table 3). *Pythium*, *Rhizoctonia*, *Aspergillus*, *Rhizopus*, *Cephalosporium*, *Ulocladium*, *Diplococcium*, *Trichoderma*, *Alternaria*, *Fusarium*, *Mycelia sterilia*, *Penicillium*, *Cladosporium*, and *Thielaviopsis* were isolated, but *Candida* and *Macrophoma* were not detected using this method.

The frequent appearance of *Pythium*, *Rhizoctonia*, *Aspergillus*, and *Rhizopus* was clear using both the direct isolation and seed trap methods.

Chemical Analysis of Water Samples

As shown in Table 4, the temperature range was from 11.9–35.3°C during the study period. A high temperature had adverse effects on *Alternaria*, *Candida*, *Ulocladium*, *Trichoderma*, and *Cephalosporium*, while temperatures above 25°C had a direct effect on *Macrophoma* and *Thielaviopsis*. The effect of temperature on the rest of the fungi ranged from a direct effect to the opposite effect.

The pH values ranged from 5.6–7.8. Acidic pH had an adverse effect on *Candida* at and acidic

Table 2. The percentage of the appearance of fungi, isolated by direct isolation- per site

Isolated Fungi	The North well	The South well	The Plastic houses well	Sewage Water	Appearance %
<i>Aspergillus</i>	27.77	27.77	27.77	11.11	22.216
<i>Alternaria</i>	5.55	16.66	0	0	6.664
<i>Candida</i>	0	16.6	0	0	3.332
<i>Cephalosporium</i>	0	0	0	0	0
<i>Cladosporium</i>	16.66	16.66	0	22.22	11.108
<i>Diplococcium</i>	0	0	0	0	1.11
<i>Fusarium</i>	16.66	0	0	0	8.886
<i>Macrophoma</i>	22.22	0	0	0	4.444
<i>Mycelia sterilia</i>	0	0	5.55	0	1.11
<i>Penicillium</i>	5.55	16.66	0	0	5.552
<i>Pythium</i>	33.33	38.88	38.88	33.33	35.55
<i>Rhizoctonia</i>	44.44	16.66	16.66	11.11	21.106
<i>Rhizopus</i>	16.66	16.66	0	16.66	13.328
<i>Thielaviopsis</i>	0	0	0	0	0
<i>Trichoderma</i>	0	0	0	0	2.222
<i>Ulocladium</i>	0	0	0	0	0
Total	188.84	166.61	88.86	94.43	136.6
Rate	11.80	10.41	5.55	5.90	8.5375

and alkali pH had adverse effects on *Thielaviopsis*. Direct effects on *Diplococcium*, *Ulocladium*, *Cephalosporium*, and *Alternaria* were observed at acidic and alkali pH. *Trichoderma* and *Fusarium* were affected by alkali pH. The effect of pH on the other fungi varied.

The sodium concentration ranged from 4.56–801.4 mL/L, the potassium concentration from 0.06–81.66 mL/L, the calcium concentration from 0.3–9.5 ml/l, the chlorine concentration from 1–22 mL/L, the magnesium concentration from 1–22.4 mL/L, and the bicarbonate concentration from 1–13.5 mL/L. These elements had adverse effects on *Trichoderma* for the duration of the study, while potassium and bicarbonate had adverse effects on *Penicillium*. Moreover, sodium, calcium, chlorine, magnesium, and bicarbonate had positive effects on *Cephalosporium*, while potassium had negative effects on *Cephalosporium* and *Alternaria*. Sodium, potassium, and bicarbonate had direct effects on *Alternaria*. Sodium, calcium, potassium, and chlorine had adverse effects on *Fusarium*, while sodium, potassium, chlorine, and magnesium had adverse effects on *Thielaviopsis*, and calcium had a direct effect on *Thielaviopsis*. Sodium and calcium also had positive effects on *Penicillium*. Bicarbonate, chlorine, and magnesium had positive effects on *Penicillium*, while sodium

and potassium had adverse effects on *Mycelia sterilia*. Moreover, magnesium and bicarbonate had a direct effect on *Mycelia sterilia* throughout the study period.

DISCUSSION

Fungi Isolation

As shown in Table 1, the reason for the presence of *Rhizoctonia*, *Pythium*, and *Fusarium* during the study period may be attributed to the fact that these fungi are soil fungi that may be transmitted during irrigation processes. This is supported by several studies showing the presence of soil fungi in water sources.^{13,14} The reason for the abundant presence of *Aspergillus* may be attributed to the fact that this fungus is able to produce large numbers of asexual breeding units, it is able to survive in different environments, and it secretes enzymes that enable it to benefit from different food sources.¹⁵ *Rhizopus* is a widespread fungus that may exist in water. *Candida*, which is a genus of pathogenic fungi, was also isolated, indicating the serious public health impact of water from these wells if it was used for drinking or domestic purposes.

The fungi isolated in this study were similar to those isolated from other water sources, such as the Nile River,¹⁶ drinking water,¹⁷

Table 3. The percentage of the appearance of fungi, isolated by seeds traps per site

Isolated Fungi	The North well	The South well	The Plastic houses well	Sewage Water	Appearance %
<i>Aspergillus</i>	11.11	22.22	27.77	38.88	31.107
<i>Alternaria</i>	16.66	33.33	0	0	13.33
<i>Candida</i>	0	0	0	0	0
<i>Cephalosporium</i>	16.66	16.66	16.66	11.11	12.218
<i>Cladosporium</i>	0	0	0	11.11	2.222
<i>Diplococcium</i>	16.66	16.66	16.66	0	13.328
<i>Fusarium</i>	0	5.55	0	0	2.22
<i>Macrophoma</i>	0	0	0	0	0
<i>Mycelia sterilia</i>	11.11	0	0	0	2.22
<i>Penicillium</i>	0	0	5.55	0	1.11
<i>Pythium</i>	8.883	838.8	33.33	72.22	46.662
<i>Rhizoctonia</i>	27.7	27.77	33.33	5.55	19.994
<i>Rhizopus</i>	16.66	22.22	16.66	16.66	18.884
<i>Thielaviopsis</i>	11.11	0	0	0	3.332
<i>Trichoderma</i>	0	0	0	0	3.332
<i>Ulocladium</i>	16.66	0	11.11	0	8.886
Total	183.28	183.29	161.07	155.53	178.847
Rate	11.455	11.455	10.66	9.720	11.177

polluted water from the Shatt Al-Arab River and its ramifications¹⁸ and well water¹⁹, but at different frequencies.

Direct Isolation Method

The results presented in Table 2 show that well water had the highest incidence of fungi, at 11.80%. The plastic housing well water was the least polluted, as the fungi incidence was 5.90%. The lack of fungi in the plastic housing may be attributed to the frequent use of chemical pesticides near the water of the plastic housing.

Seed Trap Method

The results presented in Tables 2 and 3 show that the seed trap method was the most efficient at isolating pathogenic and non-pathogenic fungi, such as *Ulocladium*, *Thielaviopsis*, and *Cephalosporium*, which were not isolated using the direct isolation method.

The results showed that the lowest incidence of fungi was in sewage water (9.72%), and this may be due to the lack of oxygen in sewage water and the high percentage of pollutants, resulting in an inappropriate environment for these fungi.

Chemical Analysis of Water Samples

The difference in the effect of temperature on the fungi confirmed that each fungus has a

certain temperature range at which it can grow. Borut and Johnson²⁰ found that there is a lack of response of fungi to physical and chemical factors. Moreover, Nakagiri et al.²¹ reported that temperature changes affect the geographical distribution of fungi.

The difference observed in the effect of pH values on fungi confirmed that each fungus has a certain pH range within which it can grow. Suzuki and Nimura²² reported that a pH range of 3-10 was appropriate for the majority of fungi to grow in aquatic environments. However, Mehrotra and Gupta²³ found that pH had a minor effect on the presence of fungi. Khaimi⁹ isolated *Aspergillus niger*, *A. flavus*, *A. terms*, *Penicillium sp.*, *Cladosporium herbarum*, *Aspergillus sp.*, *Cladosporium sp.*, *Ulocladium botrytis*, *A. foetidus*, *Phomopsis*, *Phoma exigua*, *Pythium*, and yeasts and showed that they were able to grow at pH 6.5–8. However, salt concentrations from 0.63–6.81 had adverse effects on *Trichoderma*, *Candida*, and *Thielaviopsis* and direct effects on *Ulocladium*, *Cephalosporium*, *Macrophoma*, and *Alrernaria*. The effects of salt concentration on the rest of the fungi were varied.

The difference in the effect of salts on different fungi confirmed that each fungus has a

Table 4. Temperature, pH, EC and mineral elements of the water of the sites under study during a period of six months

Location	Factors	December	January	February	March	April	May
The North well	° C	18	12.8	17.5	32.3	27.3	30
	Ph	6.65	6.81	7.2	7.6	7.6	7.2
	EC	3.66	4.6	3.22	2.14	1.87	1.59
	Na		24.5	11.84	22.8	17.52	307.2
	K		4.9	5.83	0.09	0.48	3.62
	Ca		2.2	2.8	2.5	9.5	4.6
	CL		22.5	10.8	13	8.5	5
	Mg		21.8	22.4	9	8.5	16.5
	HCO ₃		4.4	5.2	6	1	4
The South well	° C	14.6	13.4	14.5	25.6	30.9	31.2
	Ph	6.66	6.8	7.3	6.9	7.4	7
	EC	2.49	3.11	2.1	2.66	2.1	1.97
	Na		16.5	10	28.6	35.4	801.4
	K		3.20	4.96	0.09	0.32	5.44
	Ca		1.3	1.6	2	8	6.5
	CL		14.5	8.9	16.5	13.5	8.5
	Mg		14	13.4	20	14.5	12
	HCO ₃		3.2	2.8	3.5	1.5	9
The Plastic houses well	° C	15.3	18.2	22	35.3	25.6	26.3
	Ph	6.82	6.67	7.4	7.5	7.6	7.2
	EC	1.12	3.22	2.77	2.97	1.66	1.96
	Na		12.75	9.78	26.3	12.8	400.71
	K		3.10	4.45	0.06	0.32	3.62
	Ca		2.4	2.3	2.5	7.5	6.5
	CL		10.30	8.6	22	9.5	11
	Mg		15.8	20.4	18.5	10	10.5
	HCO ₃		4.4	4	2.5	2	6
Sewage Water	° C	14	13.2	19	28.1	25.3	25.6
	Ph	5.98	6.58	6.8	6.7	7.1	6.1
	EC	2.34	3.08	1.89	1.46	1.58	2.35
	Na		11.85	8.36	17.16	13.78	398.48
	K		25	8.55	0.97	3.54	81.66
	Ca		0.5	0.6	1	5.4	6
	CL		9.8	7.51	16	16.5	21
	Mg		14.4	10.6	11	11.6	8
	HCO ₃		10.8	9.3	2.3	1	13.5

sustainable range of salt concentrations at which it can grow. Abdel-Fattah et al.²⁴ found that the total number of fungi is significantly affected by the total concentration of dissolved salts, especially sodium and calcium. We found that the effects of metallic elements on fungi were varied, resulting in the stabilization of some fungi, as shown in Table 4.

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DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

ETHIC STATEMENTS

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

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