Regulatory Mechanisms in Biosystems

ISSN 2519-8521 (Print)
ISSN 2520-2588 (Online)
Regul. Mech. Biosyst.

Inhibitory effect of leaf extracts of *Citrus sinensis* and *C. aurantium* on fungi isolated from the adjacent soil

M. A. Al-Rejaboo, A. A. Mahmood, Z. S. Almola, S. W. Al-Ameen

University of Mosul, Mosul, Iraq

Article info

Received 08.01.2025
Received in revised form 03.02.2025
Accepted 28.02.2025

Department of Biology, College of Science, University of Mosul, Mosul, Iraq. Tel.: 00-964-770-300-10-83. E-mail: mahaalrejaboo2@uomosul.edu.iq, abeerahmed@uomosul.edu.iq, zaksbio67@uomosul.edu.iq

Al-Rejaboo, M. A., Mahmood, A. A., Almola, Z. S., & Al-Ameen, S. W. (2025). Inhibitory effect of leaf extracts of Citrus sinensis and C. aurantium on fungi isolated from the adjacent soil. Regulatory Mechanisms in Biosystems, 16(1), e25039. doi:10.15421/0225039

A study was conducted on the fungal content of some soils adjacent to *Citrus sinensis* (sweet orange) trees and *C. aurantium* (bitter orange) trees as well as uncultivated soil from scattered gardens belonging to the University of Mosul in the city of Mosul, Iraq. The most common fungi in these soils were *Aspergillus niger* then *Aspergillus fumigatus* and *Aspergillus flavus*, and with less frequency *Penicillium melanoconidium* and *Cladosporium cladosporioides*. *Saccharomyces cerevisiae* and *Pichia fermentans* were identified by PCR amplification and DNA sequencing of molecular gene markers and *Pichia fermentans* was recorded for the first time in the gene bank of the National Center for Biotechnology Information (NCBI): *Saccharomyces cerevisiae* MAZSS2 with the gene bank sequence LC768856.1and MAZSS1 with the sequence LC767347. In our research, it was found that the soils most contaminated with fungi were those next to orange trees while fungal contamination was light and almost non-existent in uncultivated soils, Testing the impact of sweet orange and bitter orange aqueous leaf extract on the growth of various soil-isolated fungi revealed that *Penicillium melanoconidium* was more sensitive to the two extracts compared to the fungi *Cladosporium cladosporioides* and *Aspergillus niger*. The two extracts had a gradual inhibitory effect that increased with increasing concentration.

Keywords: genetic sequence; *Pichia fermentans*; *Aspergillus niger*; *Aspergillus fumigatus*; *Aspergillus flavus*; *Saccharomyces cerevisiae; Citrus sinensis*.

Introduction

Soil microorganisms are essential for soil content and components and have many roles, such as use in assessing soil conditions, encouraging plant growth. They have a role in soil texture. soil and affect the soil's physical, chemical, and biological properties, acting as a complementary mediator of biological processes and life support in the soil environment (Maw, 2020). Fungi constitute a large part of the soil microbes, greater than the natural presence of bacteria, depending on the soil depth and nutritional conditions (Koorem et al., 2014; Su et al., 2022) stated that fungi play an important role in the functioning of the ecosystem, especially in forests and agricultural soils. They play an important role in many basic processes, such as the decomposition of organic matter, the liberation of elements through the mineralization process, and the regulation of the soil's biological activity. Fungi thrive in soil due to their extraordinary adaptability and ability to take on a variety of shapes in response to difficult and unfavorable conditions. They can break down different types of organic molecules due to their ability to produce a wide range of extracellular enzymes (Olorunfemi et al., 2018). These fungal genera have interactions with citrus trees growing in these soils, which sometimes lead to beneficial effects but often to disastrous consequences. Among these fungal genera are *Aspergillus* sp., *Penicillium* sp., *Alternaria* sp., *Mucor* sp., and *Cladosporium* sp., which researchers consider to be the main causes of diseases of citrus trees (Timmer et al., 2013; Ratana-Kumar et al., 2015; Wang et al., 2023). The fungus *Aspergillus niger* causes black mold disease on citrus trees and infects mechanically damaged fruits. The species belonging to this fungus genus such as *A. terrus*, *A. fumigatus A. flavus* are toxic and produce aflatoxins (Mailafia et al., 2016; Amenu et al., 2024). The high amount of vitamin C in citrus fruits, along with sugar, organic acids, minerals, and amino acids, makes them highly favored by consumers. Citrus trees have effects on plant species, and it was found that herbaceous plants such as *Cynodon dactylon* L., *Chenopodium album* L., and oats (*Avena sativa* L.) do not grow under orange trees, and it was noted that water extracts and decaying leaves of these trees and volatile compounds from their green, aged leaves inhibited the germination of seeds and seedling growth of these plant species (El Sawi et al., 2019). Fungi are a dominant and effective microbial mass in the soil, particularly in the soil of forests and trees (Zhang et al., 2019). They are one of the main pathogens that play a major role in causing damage to crops, and many types of fungi are pathogenic to plants and humans. They are particularly insidious as they exist in plants far from human sight, and their disease symptoms usually appear on the vegetative system of the plant after they have completely destroyed its root group, which increases its danger if many of them are resistant to unfavorable environmental conditions and can survive in the soil and infected plant debris for a long time (Prusky & Wilson, 2018; Juan-García, 2020). The various compounds produced by plants, including phenolic compounds, alcohols, polysaccharides, and other compounds, have a major and influential role on the growth of fungi. Many of them inhibit the growth of fungi. Thus, plant residues, whether their remains or what is shed during washing, have a significant impact on the types of natural microbial organisms in the soil (Al-Rejaboo & Jalaluldeen, 2019; Al-Sumaidaiea & Al-Rejaboo, 2023; Al-Rejaboo et al., 2024).

The most popular techniques for identifying species are those that use DNA molecular marker technology. Numerous techniques for using DNA have been created, including RFLP, rolling circle amplification, sequencing technologies and PCR (Wei et al., 2022). The latter approach, which is based on genotypic characters, produces findings quickly, effectively, and possibly more accurately than the previous method, which relies on phenotypic characters. It is not necessary to cultivate an organism in order to isolate it, unlike the with basic procedures (Aslam et al., 2017).The food and other industries may find use for citrus peels, which are a promising bioresource of antifungal drugs. HPLC-DAD was used to identify twelve polyphenols, of which narirutin and hesperidin were the most prevalent. There were two phenolic acids and ten flavonoids. *Rhizoctonia solani*, *Macrophomina phaseolina*, *Fusarium solani*, *Alternaria solani*, and *Trichothecium roseum* are among the fungi that are inhibited in growth by extracts from citrus peels. Extracts from the leaves of *C. sinensis* showed a notable antifungal effect against *Aspergillus fumigatus* and *A. niger*. A remarkable antifungal activity was demonstrated by the ethanol extract against *Aspergillus fumigatus* (ATCC 16404) and *Aspergillus niger* (ATCC 16404) (Mohammed et al., 2019). In this study, as for molds, they were diagnosed based on the phenotypic and microscopic traits and following the taxonomic keys; as for yeasts, they were diagnosed based on the polymerase chain reaction (PCR amplification) and DNA sequencing of molecular gene markers were applied to the yeast isolates obtained from this investigation so the fungi present in the soil near citrus in the gardens of the University of Mosul were isolated and identified In addition, we measured bioactivity of the aqueous extract of *Citrus sinensis* and *C. aurantium* at four concentrations on *Aspergillus niger*, *Cladosporium cladosporioides* and *Penicillium melanoconidium*.

Materials and methods

Soil sample collection. Samples were collected from the soils adjacent to the plants under study in three replicates. Samples were collected from three soils under the influence of orange trees, which are from a children's nursery garden, the garden of the Deanship of the College of Science, and the garden of the Department of Mechanical Engineering, while the samples adjacent to the orange trees were collected from the College of Education for Pure Science garden, the garden of the Civil Engineering Department, the garden of the Department of Debt Affairs, and the Agricultural Division. A control sample was taken from uncultivated soils, far from plant residues, in front of the Biology Department.

Isolation of fungi by dilution method. The indirect method (dilution method) was used, where 1g of each sample was weighed and placed in 9 mL of sterile distilled water, and shaken well. 1 mL was withdrawn and added to 9 mL of sterile distilled water to obtain a 1–10 dilution, and a series of dilutions was made until a 5–10 dilution was obtained, after which 1 mL of concentration 10^{-5} was grown on a Petri dish containing PDA medium and repeated with three dishes for each concentration. The dishes were incubated at 25 °C for seven days, with the results being observed and recorded, and the work was done under appropriate sterilization conditions (Raja et al., 2017).

Preparation of agarose gel and electrophoresis process of extracted DNA. To migrate and detect DNA, a 2% agarose gel was prepared. The migration device was operated by passing an electric current with a voltage difference of 5 volts/cm, and the process took 1.5–2.0 hours. The gel was then photographed under ultraviolet light using a gel documentation device to make it possible to see the DNA bands and also the product of the PCR reaction (Ibrahim & Faisal, 2024).

Detecting the presence of the highly conserved ITS region in yeast using PCR technology. The DNA concentration in all study samples was adjusted by dilution with TE Buffer solution to obtain the concentration required to perform PCR reactions (Table 1, 2). Take 50 ng/µL for each sample. The master reaction mixture was prepared for each PCR reaction by mixing the DNA sample and the primer for each gene with the components of the master mix inside a 0.2 mL Eppendorf tube prepared by the New England Biolaps. The reaction volume was fixed to 20 µL with distilled water, and the mixture was discarded. It was placed in the Microfuge device for a period of between 3–5 s to ensure that the reaction components were mixed, then the reaction tubes were inserted into the Thermocycler device to perform the multiplex reaction using the special program for each (Khaleel et al., 2023).

Extracting DNA from the gel. The bands resulting from the PCR reaction were extracted from the gel in order to purify them and send them for nucleotide sequence testing, based on the analysis kit provided by the company (Geneaid).

Preparation of the aqueous extract. The the water extract of the leaves of bitter orange and sweet orange trees was prepared by mixing 500 g of the fresh leaves after washing them and cutting them into small pieces with 500 mL of distilled water using an electric mixer for 5 minutes and leaving the resulting suspension in a dark place at a temperature of 10 °C for 24 hours for soaking Then the mixture was filtered using several layers of gauze, then the filtrate was exposed to a Remi centrifuge at a speed of 3000 d/min for 10 minutes, and a filtration process was performed again with a Buchner funnel using Whatman No. 1, and the extract was sterilized by pasteurization at a temperature of 62 °C for 15 minutes, and the sterilized extract received in sealed sterile bottles and placed in the refrigerator so that the filtrate was sterile and ready for addition.

Table 1
Elected ITS regions

Primer	Sequence
Forward (ITS1)	TCCGTAGGTGAACCTGCGG
Reverse (ITS4)	TCCTCCGCTTATTGATATGC

Table 2
Elected ITS regions

Stage	Temperature	Time	Cycle Number
Initial denaturation	95	6 min.	1
Denaturation	95	45 sec.	
Annealing	58	1 min.	35
Extension	72	1 min.	
Final extension	72	5 min.	1

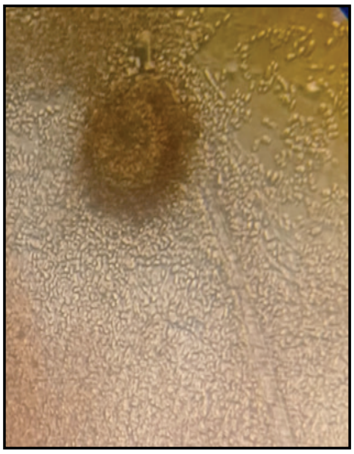
Testing the inhibitory effectiveness of the plant extracts against the growth of fungi. The plant extracte were mixed with the previously prepared sterile food medium (PDA) and cooled to 50 °C to prepare concentrations 10–20–30–40 mg/mL at a rate of three replicates for each concentration. These dishes were cultivated by taking a tablet of the edge of the fungal colony at the age of one week using a cork borer with a diameter of 5 cm and placed in the center of the plate under sterile conditions, and then the dishes were incubated at a temperature of 28 °C for a week (Mahmood & Almola, 2024).

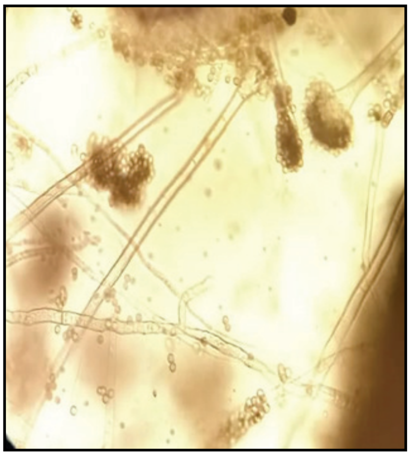
Results

Three areas were chosen for isolation for each plant (all gardens are affiliated with the University of Mosul in the city of Mosul in Northern Iraq). It was found that the most common fungal species in the selected soils was *Aspergillus niger* and followed by other fungi such as *Penicillium* sp, *Fusarium* sp, *Cladosporium* sp. Also, varying numbers of yeasts were observed, including *Saccharomyces cerevisiae* and *Pichia fermentans*. From Table 3, we note that the fungal content of the uncultivated soils was very low, and the fungus *Aspergillus niger* also appeared in it. Thus, it became clear that the cultivated soils are richer in fungal content than the uncultivated soils, according to the results we obtained, and that the fungus *Aspergillus niger* is the dominant fungus in these soils belonging to the gardens of the University of Mosul.

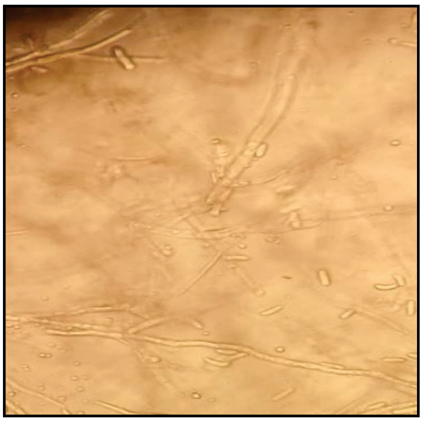
The soil of orange trees was more contaminated with fungi than other soils, as 13, 9, 6, 5 colonies of *Aspergillus niger* were isolated from Deanship of the College of Science, Department of Mechanical Engineering, College of Education for Pure Science, children's nursery. Then 4 colonies of *Aspergillus fumigatus*, *Fusarium* sp. In addition to *Penicillium*, *Cladosporium*, *Curvularia*, *Rhizoctonia*, *Rhizopus*, and *Helminthosporium* (Fig. 1) appeared and were isolated at lower frequencies.

Saccharomyces cerevisiae exists in all gardens soils of Mosul University, two samples of isolated yeasts were randomly selected to detect their genetic structures and diagnose them genetically. One of them was a yeast sample isolated from the soil adjacent to *Citrus aurantium* trees, and the other was isolated from the soil adjacent to *Citrus sinensis* trees. As shown in Figure 2, is the genome extracted from the yeast sample. The chromosomal sequence of the fungus *Saccharomyces cerevisiae* isolated from the soil was identified Figure 3. The yeast has been registered as a new isolate at the National Center for Information and Technology NCBI, Gen Bank: *Saccharomyces cerevisiae* MAZSS2, with a GenBank LC768856.1, as shown in Figure 3a. A second yeast is registered in the International GenBank under the name *Pichia fermentans* MAZSS1, with the GenBank number LC767347.1 and with a genetic sequence as shown in Figure 3b. A genetic affinity tree was designed between the different strains isolated from different countries of the world (Fig. 4), we notice a closeness between the Iraqi strain *Pichia fermentans* MAZSS1 with the Italian strains and then the Denmark strain.

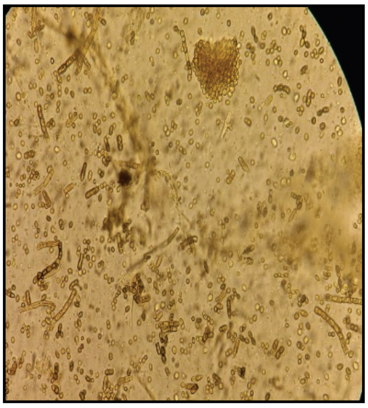


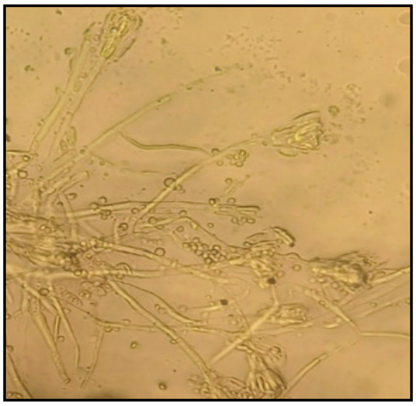


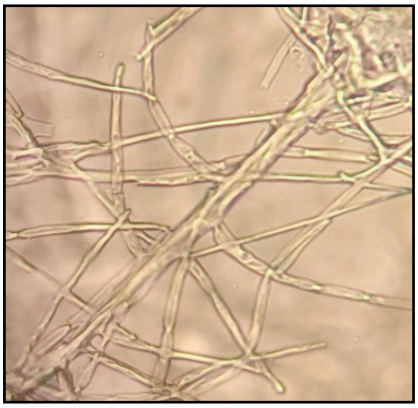
a – Aspergillus niger *b – Aspergillus fumigatus* *c – Aspergillus flavus*



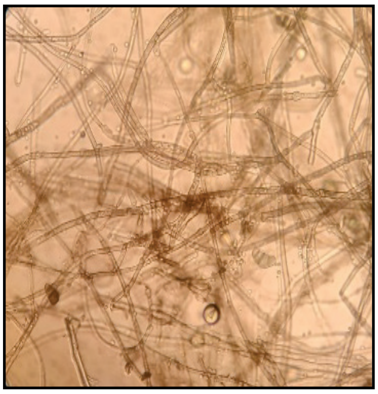
d – Cladosporium cladosporioides *e – Penicillium melanoconidium* *f – Rhizoctonia solani*

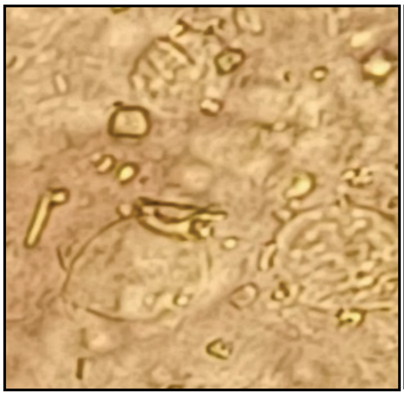


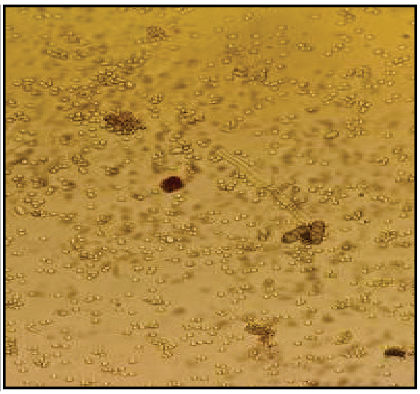




g – Phytophthora sp. *h – Rhizopus* sp. *i – Fusarium* sp.







j – Curvularia sp. *k – Geotrichum candidum* *l – Helminthosporium* sp.

Fig. 1. Microscopic photo at 40^X for some molds isolated from the soil of university garden

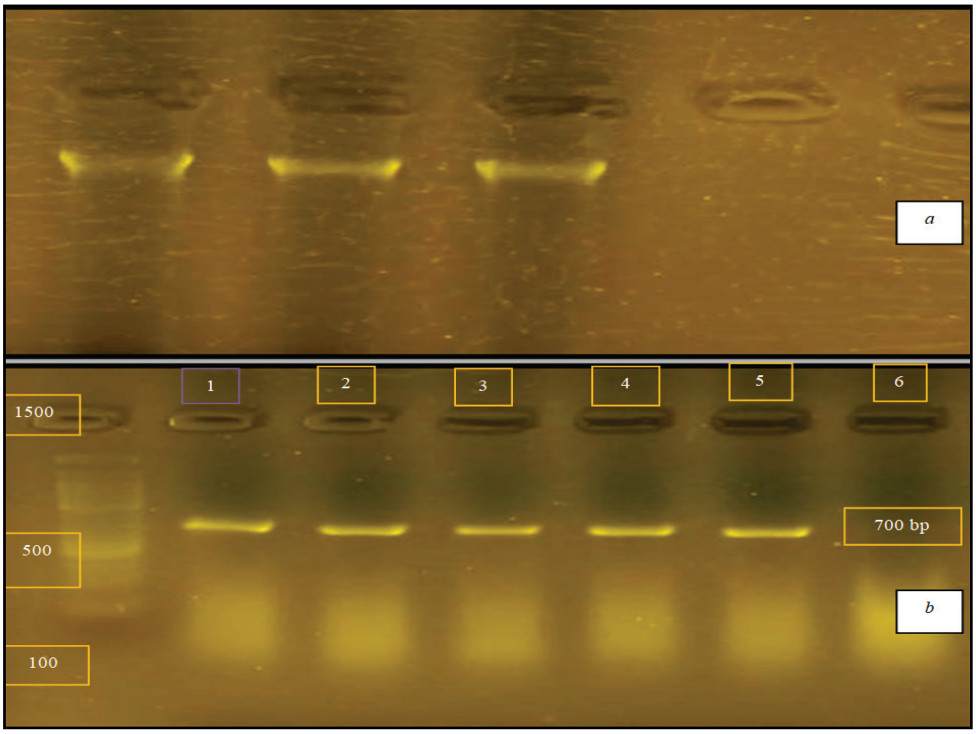


Fig. 2. The genome extracted from the yeast samples (*a*), and the product of the PCR reaction for the ITS region is the product of the 700 bp reaction of the yeast (*b*)

a – Saccharomyces cerevisiae MAZSS2
CTCCAAGCCCTATTGACTCTTACCCGGAGTTTCAGCTAAAAGCTATACTTACTACCTTTATTTTATGTTTACTTTTTATAGACT
GTCTTTTCATCCTACTCCTTCGCACTTGTCTCTCGCTACTGCCGTGCAACAAACACTAAATCAAAACAGTAAAATACTACATC
AAAACGCATATTCCCTAGAAAAAAATTTTCTTACAATATACTATACTACACAATACATAATCAGTGACTTTCGTAACAACAA
TTTCCTTCACTCTCCAACTTCTCTGCTCGAATCCCTACATAATAATATATCAAATCTACCGTCTGGAACATCATCGCTATCCAG
CTCTTTGTGAACCGCTACCATCAGCATGTACAGTGGTACCCTCGTGTCATCTGCAGCGAGAACTTCAACGTTTGCCAAATCAA
GCCAATGTGGTAACAACCACACCTCCGAAATCTGCTCCAAAAGATACTCCAGTTTCTGCCGAAATGTTTTATTGTAGAACAG
CCCTATCAGCATCGAGAGGAATGCCGTCCAATCCGGCACTTTAGATGGGGTAACTCCCAGCG

b – Pichia fermentans MAZSS1
TTTTGAGTACCTTCGTTTCCTCGGCGGGTTCGCCCGCCGATTGGACAATTTAAACCATTTGCAGTTGCAATCAGCGTCTGAAA
AAACATAATAGTTACAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAGTG
TGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCCTTGGTATTCCATGGGGCATGCCTGTTCGAGC
GTCATTTGTACCTTCAAGCTCTGCTTGGTGTTGGGTGTTTGTCTCGCCTCTGCGCGTAGACTCGCCTCAAAACAATTGGCAGC
CGGCGTATTGATTTCGGAGCGCAGTACATCTCGCGCTTTGCATTCAGAACGACGACGTCCAAAAGTACATTTTTACACTCTTG
ACCTCGGATCAGGTAGGGATACCCGCTGAACTTAAGCATATCAATAAGCGGAGGA

Fig. 3. Gene sequence of the yeast *Saccharomyces cerevisiae* (*a*), and gene sequence of the yeast *Pichia fermentans* (*b*)

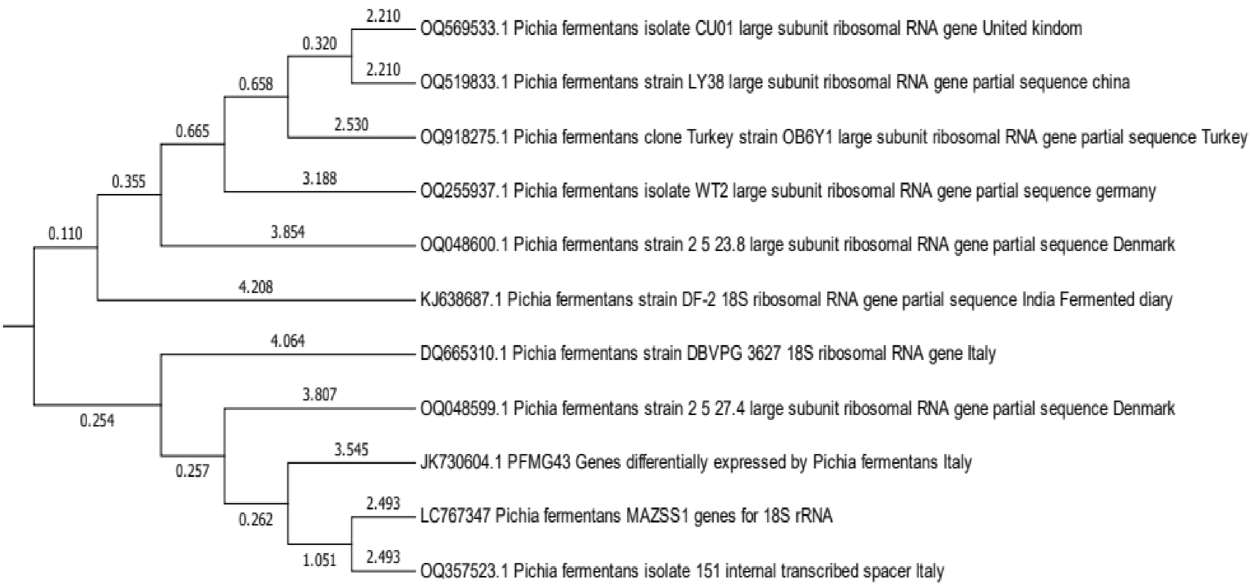


Fig. 4. Genetic affinity tree between different strains isolated from different countries of the world and *Pichia fermentans* MAZSS1

Table 3
Fungi isolated from the soil of different gardens of the Mosul University

No.	Trees	Gardens	Isolated fungi	Frequency, %
1	*Citrus sinensis*	Children's nursery	*Aspergillus niger* *Saccharomyces cerevisiae*	5 2
2	*Citrus sinensis*	Deanship of the College of Science	*Aspergillus niger* *Fusarium* sp. *Penicillium* sp.	13 3 2
3	*Citrus sinensis*	Department of Mechanical Engineering	*Aspergillus niger* *Penicillium* sp.	9 2
4	*Citrus sinensis*	Mosque university	*Aspergillus flavus* *Cladosporium cladosporioides* *Helminthosporium* sp. *Phytophthora* sp. *Rhizopus* sp. *Fusarium* sp.	2 1 1 1 1 1
5	*Citrus aurantium*	College of Education for Pure Science	*Aspergillus niger* *Saccharomyces cerevisiae* *Aspergillus fumigatus* Yeast *Fusarium* sp. *Curvularia* sp. *Penicillium* sp.	6 1 4 2 4 1 1
6	*Citrus aurantium*	Civil Engineering Department	*Aspergillus niger* *Saccharomyces cerevisiae* Yeast *Penicillium* sp. *Cladosporium* sp. *Aspergillus* sp.	2 2 1 1 1 1
7	*Citrus aurantium*	Department of Debt Affairs, Agricultural Division	*Aspergillus niger* *Pichia fermentans*	1 2
8	*Citrus aurantium*	Mosque university	*Geotrichum candidum* *Rhizoctonia solani* *Penicillium melanoconidium* *Helminthosporium* sp. *Aspergillus* sp. Yeasts	1 1 1 1 1 1
9	Uncultivated soils	Biology Department	*Aspergillus niger*	3

From Figure 5 and 6, it was observed that the mold *Penicillium melanoconidium* was more sensitive to the two extracts compared to the other two fungi, while there was no significant effect of the two extracts on the fungus *Cladosporium cladosporioides*. As for the fungus *Aspergillus niger*, the two extracts had a gradual inhibitory effect that increased with increasing concentration. In general, it was found that the aqueous extract of the leaves of *Citrus aurantium* inhibited the growth of the studied fungi more than the *Citrus sinensis* extract.

Balamurugan (2014) evaluating aqueous extracts of bitter orange leaves against the fungus *Macrophomina phaseolina* isolated from dry root rot samples of sesame plants, found that the extracts were more effective in inhibiting this fungus. The results of Ahmed et al. (2010) study showed the antimicrobial activity of oil extracted from dry and fresh orange peels against bacteria and fungi such as *Aspergillus niger*, *Rhizopus* sp. and *Penicillium melanoconidium.* Three concentrations (1000, 2000, and 4000 ppm) of orange peel oil were used and showed. This extract showed inhibition against fungi. The fungus *Rhizopus* spp. was the most affected fungus, as the percentage of inhibition at a concentration of 4000 was 41%. The results of the study of Mohammed et al. (2019) showed that the Estonian coriander plant extract, ethanolic and aqueous, has high inhibitory effectiveness against the plant pathogenic fungi *Fusarium oxysporum*, *Aspergillus niger*, and *Penicillium melanoconidium.* All extracts showed a high inhibitory effect against these fungi, though the inhibitory effectiveness varied according to the solvent and the concentration of the extract. It ranged from 63.1% to 78.1%, and the concentration of 20 µL/mL for the three extracts achieved a high inhibitory effect on the growth of these fungi. The results of the Al-Zaidbaqy & Hmawndi (2019) study in testing the antagonistic effectiveness of leaf extracts of some types of forest trees *Olea europea* and *Thuja oreintalis* against two pathogenic fungi of many plants *Rhizopus stolonifer* and *Aspergillus niger* showed that there was an inhibitory activity of the extracts against the two fungi studied. The lowest rates of fungal growth when using a concentration of 0.5 µg/mL for green olive leaf extract, green cypress, and thuja, respectively, were 2.3, 2.1, 2.2 cm, with an inhibition rate of fungal growth amounting to 69.3%, 73.4%, 72.8% against *Rhizopus stolonifer* and 1.9, 0.9, 1.6 cm with a fungal growth inhibition rate of 80.2%, 89.0%, 76.7% against *Aspergillus niger*. *Citrus aurantifolia* peels had the highest concentrations of total phenolics and flavonoids, with 96 mg GAE/g and 54 mg QE/g, respectively (Benredjem, 2023).

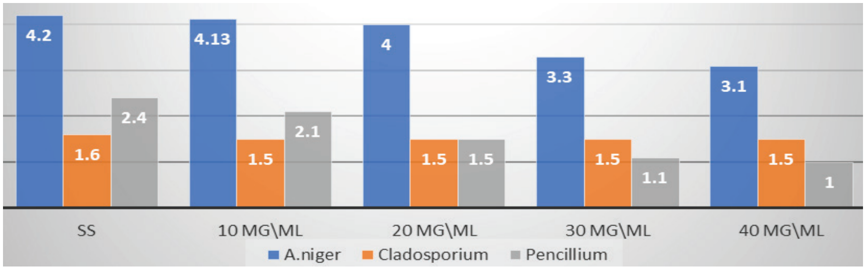


Fig. 5. Bioactivity of the aqueous extract of *Citrus sinensis* at concentrations of 10, 20, 30, and 40 mg/mL on *Aspergillus niger*, *Cladosporium cladosporioides* and *Penicillium melanoconidium*

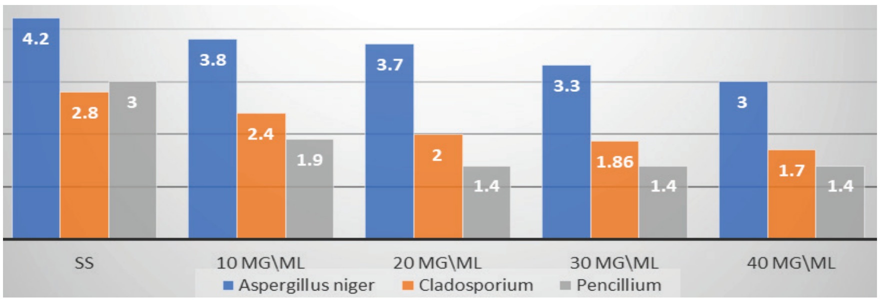


Fig. 6. Bioactivity of the aqueous extract of *Citrus aurantium* at concentrations of 10, 20, 30, and 40 mg/mL on *Aspergillus niger*, *Cladosporium cladosporioides* and *Penicillium melanoconidium*

Discussion

In this study, the dominant strains of yeast isolated from the soils of *Citrus sinensis* trees and *C. aurantium* trees were identified as *Saccharomyces cerevisiae* and *Pichia fermentans*. This is the first time that these strains were recorded in the soils of *C. sinensis* and *C. aurantium* trees in the gardens of Mosul University. Also *Aspergillus niger* is considered the most important and most isolated mold, isolated by a large percentage from the soil of orange trees. Both herbicidal and phytotoxic properties are tested in the most potent extracts made from *Aspergillus* species (Khattak et al., 2014). Raja et al. (2017) mentioned that one gram of soil contained a large number of fungi, some of which are intrusive, causing plant diseases, while the others were saprophytic, living on dead and rotting materials. Analyzing them through the balance of environmental conditions in the soil, he found that humidity is an important factor as it directly affects the fungal communities in a positive direction and to a certain extent. A significant number of keratinophilic fungi that have been identified from park soil are threats to public health, and youngsters are particularly vulnerable to the effects of these fungi (Pakshir et al., 2013). Abdel-Sater et al. (2016) isolated *Fusarium*, *Penicillium*, and *Aspergillus* from the soil of citrus trees (Azaz & Pekel, 2003; Balami et al., 2020) and also proved that *Aspergillius niger* and *A. ochtraceous* were isolated with a high frequency from the soil of these trees, and this is consistent with our results. *Aspergillus* is the most dominant genus in the soil of citrus trees. Younus & Hussain (2022) mentioned that the two fungi, *Aspergillus* and *Penicillium*, are the most abundant and dominant fungi in most soils of field crops, and they indicated the ability of each of these fungal genera to form several types of toxins, such as aflatoxins and ochratoxins, which are likely to represent a barrier to the growth of other fungi in the same environmental location (Hardan & Muhsen, 2024). Ali et al. (2014) mentioned that soil fungi are responsible for a large percentage of the damage to citrus trees, he also mentioned that the *Fusarium fungus* is almost present in all agricultural ecosystems that support citrus trees with high-frequency rates, we notice an increase in fungal growth in soils under the influence of *C. sinensis* followed by *C. aurantium*. It is possible that the secondary metabolite compounds in the precipitation, rich in organic matter, favor the growth of fungi compared to the control sample and this explains the appearance of *Fusarium* at a rate of 13% in the two citrus species (sweet and bitter orange). Bitter orange rootstock infected with *F. solani*, growing in a Mission, Texas, dooryard, displayed significant symptoms of dieback of the canopy, purple discoloration of the wood, wilt, tree decline, and desiccation of leaves and leaf drop.

Rasool et al. (2014) indicated that *Fusarium* is the most common fungus associated with diseases of citrus trees, and it is one of the most pathogenic (dry root rot disease), being also associated with post-harvest fruit rot. Evans et al. (2013) indicated that the fungi *Aspergillus*, *Penicillium*, *Fusarium*, *Cladosporium*, and *Ulocladium* are widespread in different soils, and they proved that pH (soil pH) has a direct effect on the growth of these fungi and that *Fuasrium* can grow well in pH more than 7 and cannot grow in pH less than 6, and *Aspergillus* grows well in pH = 5, while *Penicillium* cannot grow in pH more than 7. As there is a relationship between the spread and distribution of soil fungi and the physical and chemical properties of the soil, there is a direct relationship between high temperatures and fungal growth. There is an increase in the growth of fungi with high temperatures up to 25 °C, after which there is a decrease in the growth rate with a temperature increase of more than 25 °C. This can be attributed to the loss of the cell membrane's vital functions or the breakdown of some components. Cellularization, or lysis, of cells can occur under high-temperature conditions (Rohilla & Sallar, 2012; Moubasher, 2018; Balami et al., 2020; Rehman et al., 2023).

Conclusion

Experiments conducted in the soil microcosms of sweet and bitter orange trees evaluated the survival of two chosen yeast species (*Saccharomyces cerevisiae* and *Pichia fermentans*) using a molecular approach for fungal community profiling.. The most common mold in the soil of citrus trees is *Aspergillus niger*. There are clear changes in the numbers and types of fungi present in different soils due to their influence on plant residues and the inhibitory effects of the studied citrus trees varied considerably at different concentrations on the studied fungi. The aqueous extract of *C. aurantium* leaves was more effective against fungi than the aqueous extract of *C. sinensis* leaves. The aqueous extract of *C. aurantium* and *C. sinensis* leaves showed a gradual inhibitory effect against *Aspergillus niger*, which increased with increasing concentration. *Penicillium melanoconidium* was the most sensitive fungus to the aqueous extract of the *C. aurantium* leaves of the plant. *Cladosporium oxysporum* was more resistant to aqueous extracts of *Citrus sinensis* leaves.

We value the assistance provided by the University of Mosul's College of Science's Biology Department.

The authors whose names are listed above certify that they have no conflict of interest.

References

Abdel-Sater, M. A., Moubasher, A.-A. H., & Soliman, Z. S. M. (2016). Diversity of filamentous and yeast fungi in soil of citrus and grapevine plantations in the Assiut Region, Egypt. Czech Mycology, 68(2), 183–214.

Ahmed, A. S., Allawi, S. S., & Hassan, S. C. (2010). Effect of orange peel extract on some micro-organisms causing food spoilage and its role in shelf life of Iraqi bread. Iraqi Journal of Agricultural Sciences, 41(3), 105–115.

Ali, S. A., Snyder, J., Shehzad, M., & Khalid, A.-U.-R. (2014). Associating among fungi, bacteria, and phytoplasma in trees suffering citrus decline in Punjab. Journal of Agricultural Technology, 10(5), 1343–1352.

Al-Rejaboo, M. A., & Jalaluldeen, A. M. (2019). Studying the airborne fungi of some rooms in the internal sections of Mosul University campus and the possibility of using sage plants to control it. Journal of Advanced Pharmacy Education and Research, 9(3), 17–22.

Al-Rejaboo, M. A., Almola, Z. S., & Almashhadani, M. A. (2024). *In vitro* the effect of ethanol extracts of *Senna alexandrina* and *Achillea millefolium* on fungi pathogenic to plants and humans. Journal of Applied and Natural Science, 16(4), 1444–1455.

Al-Sumaidaiea, B. Z., & Al-Rejaboo, M. A. (2023). Effect of alcoholic extract of *Syntrichia intermedia* on some yeasts isolated from mobile phones and the user's finger. IOP Conference Series: Earth and Environmental Science, 1262(2), 022003.

Al-Zaidbaqy, O. I., & Hmawndi, N. G. (2019). Antifungal activity of leave's extractions of some forest trees against *Aspergillus niger* and *Rhizopus stolonifer*. Tikrit Journal for Agricultural Sciences, 15(2), 1–9.

Amenu, D., Desalegn, R., Nugusa, A., Tolera, C., & Tafesse, T. (2024). Evaluation of antifungal activity of plant extracts against plant pathogen associated with sweet orange (*Citrus sinensis* L.), in Jimma, Western Ethiopia. Discover Applied Sciences, 6(7), 357.

Aslam, S., Tahir, A., Aslam, M. F., Alam, M. W., Shedayi, A. A., & Sadia, S. (2017). Recent advances in molecular techniques for the identification of phytopathogenic fungi – a mini review. Journal of Plant Interactions, 12(1), 493–504.

Azaz, A. D., & Pekel, O. (2003). Comparison of soil fungi flora in burnt and unburnt forest soils in the vicinity of Kargicak (Alanya, Turkey). Turkish Journal of Botany, 26(6), 409–416.

Balami, S., Vašutová, M., Godbold, D., Kotas, P., & Cudlín, P. (2020). Soil fungal communities across land use types. iForest – Biogeosciences and Forestry, 13(6), 548–558.

Balamurugan, S. (2014). *In vitro* antifungal activity of *Citrus aurantifolia* Linn plant extracts against phytopathogenic fungi *Macrophomina phaseolina*. International Letters of Natural Sciences, 13, 70–74.

Benredjem, L. (2023). Antibacterial properties of leaves and peels extracts of *Citrus aurantifolia* cultivated in Algeria against multi-drug resistant *Staphylococcus aureus* originating from raw milk. Tropical Journal of Natural Product Research, 7(10), 4256–4261.

El Sawi, S. A., Ibrahim, M. E., El-Rokiek, K. G., & El-Din, S. A. S. (2019). Allelopathic potential of essential oils isolated from peels of three citrus species. Annals of Agricultural Sciences, 64(1), 89–94.

Evans, S., Hansen, R. W., Stone, H. M., & Schneegurt, M. A. (2013). Isolation and characterization of halotolerant soil fungi from the Great Salt Plains of Oklahoma (USA). Cryptogamie, Mycologie, 34(4), 329–341.

Hardan, S. M., & Muhsen, T. A. A. (2024). Isolation and diagnosis of some types of fungi using PCR technology for infection by (*Musca locala* L.) from some areas of Anbar Governorate. Journal of Bioscience and Applied Research, 10, 276–289.

Ibrahim, M. A., & Faisal, R. M. (2024). Molecular characterization of antibiotic resistance and virulence genes on plasmids of *Proteus mirabilis* isolated from urine samples of Hospitals in Mosul City, Iraq. Journal of Applied and Natural Science, 16(2), 830–841.

Juan-García, A. (2020). Introduction to the toxins special issue on toxicological effects of mycotoxin on target cells. Toxins, 12(7), 446.

Khaleel, A. M., Faisal, R. M., & Altaii, H. A. (2023). Using recombinant DNA technology in bacterial identification from vaginal swabs. Revista Bionatura, 8(3), 113.

Khattak, S. U., Iqbal, Z., Lutfullah, G., Bacha, N., Khan, A. A., Saeed, M., & Ali, M. (2014). Phytotoxic and herbicidal activities of *Aspergillus* and *Penicillium* species isolated from rhizosphere and soil. Pakistan Journal of Weed Science Research, 20(3), 293–303.

Koorem, K., Gazol, A., Öpik, M., Moora, M., Saks, Ü., Uibopuu, A., Sõber, V., & Zobel, M. (2014). Soil nutrient content influences the abundance of soil microbes but not plant biomass at the small-scale. PLoS One, 9(3), e91998.

Mahmood, A. A., & Almola, Z. S. (2024). The efficiency of *Bacillus subtilis* and *Thuja orientalis* leaves extracts as biocontrol agents of damping off in wheat (*Triticum aestivum*). Malaysian Journal of Microbiology, 20(4), 406–411.

Mailafia, S., Okoh, G. R., Olabode, H. O. K., & Osanupin, R. (2016). Isolation and identification of fungi associated with spoilt fruits vended in Gwagwalada market, Abuja, Nigeria. Veterinary World, 10(4), 393–397.

Maw, M. S. (2020). Isolation of fungi from soil samples and preliminary study of antimicrobial activitie. Maubin University Research Journal, 11, 311–326.

Mohammed, H., Abdel Aziz, M., & Yaacoub, R. (2019). Effect of coriander (*Coriandrum sativum*) extracts on growth of the photypathogenic fungi, *Fusarium oxysporum*, *Aspergillus* sp. and *Penicillium* sp. Arab Journal of Plant Protection, 37(4), 335–341.

Moubasher, A.-A. H., Abdel–Sater, M. A., & Soliman, Z. (2018). Biodiversity of filamentous and yeast fungi in citrus and grape fruits and juices in Assiut Area, Egypt. Journal of Microbiology, Biotechnology and Food Sciences, 7(4), 353–365.

Olorunfemi, I. E., Fasinmirin, J. T., & Akinola, F. F. (2018). Soil physico-chemical properties and fertility status of long-term land use and cover changes: A case study in forest vegetative zone of Nigeria. Eurasian Journal of Soil Science, 7(2), 133–150.

Pakshir, K., Rahimi Ghiasi, M., Zomorodian, K., & Gharavi, A. R. (2013). Isolation and molecular identification of keratinophilic fungi from public parks soil in Shiraz, Iran. BioMed Research International, 2013, 619576.

Prusky, D. B., & Wilson, R. A. (2018). Does increased nutritional carbon availability in fruit and foliar hosts contribute to modulation of pathogen colonization? Postharvest Biology and Technology, 145, 27–32.

Raja, M., Praveena, G., & William, S. J. (2017). Isolation and identification of fungi from soil in Loyola College campus, Chennai, India. International Journal of Current Microbiology and Applied Sciences, 6(2), 1789–1795.

Rasool, A., Zaheer, I., & Iram, S. (2014). Isolation and characterization of post harvest fungal pathogans of citrus varieties from the domestic markets of Rawalpindi and Islamabad. International Journal of Scientific and Engineering Research, 5(10), 408–418.

Ratana-Kumar, P. K., Hemanth, G., Niharika, P. S., & Kolli, S. K. (2015). Isolation and identification of soil mycoflora in agricultural fields at Tekkali Mandal in Srikakulam District. International Journal of Advances in Pharmacy, Biology and Chemistry, 4, 484–490.

Rehman, S. U., De Castro, F., Marini, P., Aprile, A., Benedetti, M., & Fanizzi, F. P. (2023). Vermi-biochar: A novel approach for reducing the environmental impact of heavy metals contamination in agricultural land. Sustainability, 15(12), 9380.

Rohilla, S. K., & Salar, R. K. (2012). Isolation and characterization of various fungal strains from agricultural soil contaminated with pesticides. Research Journal of Recent Science, 1, 297–303.

Su, W., Tang, C., Lin, J., Yu, M., Dai, Z., Luo, Y., Li, Y., & Xu, J. (2022). Recovery patterns of soil bacterial and fungal communities in Chinese boreal forests along a fire chronosequence. Science of the Total Environment, 805, 150372.

Timmer, L. W., Peever, T. L., Solel, Z., & Akimitsu, K. (2003). *Alternaria* diseases of citrus – Novel pathosystems. Phytopathologia Mediterranea, 42, 99–112.

Wang, H., Liu, Z., Duan, F., Chen, Y., Qiu, K., Xiong, Q., Lin, H., Zhang, J., & Tan, H. (2023). Isolation, identification, and antibacterial evaluation of endophytic fungi from Gannan navel orange. Frontiers in Microbiology, 14, 1172629.

Wei, Y., Li, L., Liu, Y., Xiang, S., Zhang, H., Yi, L., Shang, Y., & Xu, W. (2022). Identification techniques and detection methods of edible fungi species. Food Chemistry, 374, 131803.

Younus, A. H., & Hussain, H. T. (2022). Identification Fungi form soil of different places in Baghdad. Journal of the College of Basic Education, 21(89), 45–60.

Zhang, M., Wang, N., Zhang, J., Hu, Y., Cai, D., Guo, J., Wu, D., & Sun, G. (2019). Soil physico-chemical properties and the rhizosphere soil fungal community in a mulberry (*Morus alba* L.)/alfalfa (*Medicago sativa* L.) intercropping system. Forests, 10(2), 167.