Identification Fungi from Soil of different places in Baghdad

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Summary:

The present study deals with isolation and characterization of fungi from different soil samples including: agricultural, fields, gardens, petroleum and cattle soil) from Baghdad . The fungi have been isolated and identified by using two methods: direct isolation (soil plate method) and soil dilution method, using sabouraud's dextrose agar (SDA). also the fungi have been examined microscopically with Lacto phenol cotton blue dye ,to determine the genus of the fungi isolates. The results were: 89 fungal isolates have been identified, and the results have shown that the highest percentage of fungi isolates was *penicilium* with 38.2%, followed by *Mucur* with 23.59%. The third one in the isolation percentage was *alternaria* with 14.6%, while *yeast, Rhizopus, Fusarium* and *Aspergillus* had the isolation percentages: 8.98% , 3.37% , 6.74% and 4.49% respectively.

Introduction:

The biodiversity refers to the variability of life on the earth, including all of the living species of animals, plants and microorganisms. Fungi are a major component of biodiversity; they are essential for the survival of other organisms and are crucial in global ecological processes [1].Soil microbial communities are among the most complex and diverse assemblages in the biosphere and they have important role in all of the ecosystem services provided by soils [2]. Plant roots release amino acids, carbohydrates and organic acids into the rhizosphere which stimulate the growth of a diverse microbial community [3].Microbial communities, particularly bacteria and fungi constitute an essential component of biological characteristics in soil ecosystems. It has been estimated that there is a minimum of 7, 12,000 fungal species worldwide [4] , only 5-13 % of the total estimated global fungal species have been described . The actual number of fungi is still unknown [5].Soil fungi described as the major decomposers in the soil ecosystem. They also provide us with very useful pharmaceutical products, such as antibiotics and other valuable substances, including enzymes, organic acids, pigments and secondary metabolites used in the food industry and fermentation [6].Soil fungi and bacteria have important roles in various biological cycles (BGC), also they influence on above ground

ecosystems by contributing to plant nutrition, plant health, soil structure and soil fertility [7]. Fungi among other soil microorganisms are important in both of the formation and stabilization of soil aggregates [8].the saprophytic lignin decomposing basidiomycete has the ability to efficiently bind and stabilize soil particles into water stable aggregates, and this is because of the fungus secretes a large amounts of water insoluble extracellular compounds that acts as binding agents of soil particles [9]. Despite of the importance of microorganisms in soil processes, several soil-borne microbial species are known to cause plant diseases [10].

Due to the importance of fungi, this research aimed to determine the species that could be isolated from soil, and which one was the most common.

Material and methods:

Collection of soil samples:

The soil samples have been collected from eight different places in Baghdad, including (gardens, petroleum soil, cattle soil , agricultural field soil ,agricultural soil contaminated with pesticides and stockyard soil). The soil was taken at 10 cm depth, they were sieved and air dried for 3-5 days at 18 °C, then the samples were kept at 10 °C until used [11].

Media preparation:

Sabouraud's dextrose agar media was prepared by dissolving (62 gm) in (1 liter) of distilled water, autoclaved for 15 minutes at 121°C , after cooling it to (47°C), chloramphinicol was added, then the media have been poured into petri dishes and let to solidify at room temperature, and used in the following steps.

Isolation of fungi: Two methods of isolation have been used, soil plate method and soil dilution method, by using sabouraud's dextrose agar media.

1- **Soil plate method:**

0.005 gram of each soil sample have been scattered on the bottom of sterile petri dish ,then a molten cooled (40-45°C) media of sabouraud's dextros**e** agar have been added , rotated gently to disperse the soil particles in the medium. They let to solidify, and incubated at 28 ± 20 °C in dark for 3-7 days to read the results [12].

2- soil dilution method:

One gram from each soil samples have been suspended in 9 ml sterile distilled water to prepare the microbial suspension. Four dilutions 10^{-1},10^{-2},10^{-3} and 10^{-4} have been prepared from the microbial suspension. 1 ml from each dilution was added to a sterile petri dish , then molten cooled

(40-45°C) media of sabouraud's dextrose agar have been added , rotated gently to disperse the soil solution into the media, (triplicate of each dilution have been prepared) .the petri dishes were incubated at 28± 2 ^{0} C, for 4-7 days ,then the grown fungi colonies have been counted and identified [13].the appearance percentage of each genus have been recorded, according to the following equation :

$$\text{Appearance percentage of the fungi genus} = \frac{\text{no. of fungi genus isolates}}{\text{Total no. of fungi genera isolates}} * 100\ \%$$

Identification of the soil fungi:

Fungi species have been identified by observing the morphology of fungi, colony features (Colour and Texture) . Also, they have been examined microscopically by staining with lactophenol cotton blue dye, observed under a compound microscope for the conidia, conidiophores and arrangement of spores. The identification of fungi was done with the help of literature [3], [13]

Results and discussion:

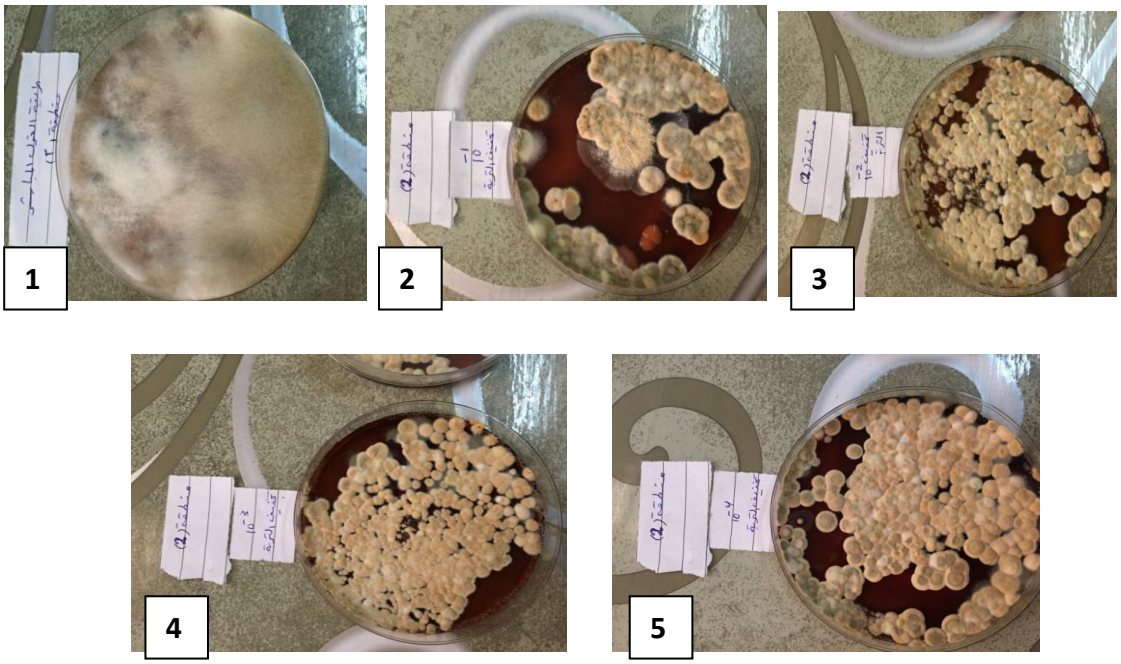
Eighty nine isolates of fungi and yeast have been isolated from 8 different soil samples, and all of these isolates have been identified, as in the table 1, F.(1: a, b, c, d, e, f , g and h).

Soil sample	Source of soil sample	Direct isolation	Dilution 10^{-1}	Dilution 10^{-2}	Dilution 10^{-3}	Dilution 10^{-4}
1	Garden soil	*1-Penicillium chrysogenum* *2-Rhizopus stolanifer* *3-Alternaria teunissima*	*Penicillium chrysogenum*	*Penicillium chrysogenum*	*Penicillium chrysogenum*	*Penicillium chrysogenum*
2	Garden soil	*1- Mucur hiemalis*	*1- Penicillium chrysogenum* *2-Candida albican*	*1-Penicillium chrysogenum* *2-Mucur hiemalis*	*1-Penicillium chrysogenum* *2-Mucur hiemalis*	*1-Penicillium chrysogenum* *2-Mucur hiemalis*
3	Cattle soil	*1- Mucur hiemalis* *2- Penicillium chrysogenum*	1. *Mucur hiemalis* 2. *Fusarium oxysporum*	*1-Mucur hiemalis* *2- Penicillium chrysogenum*	*1-Mucur hiemalis* *2- Penicillium chrysogenum*	*1-Mucur hiemalis* *2- Penicillium chrysogenum*

4	Garden soil	*1- Mucur hiemalis* *2- Penicillium chrysogenum* *3-Alternaria alternaria*	*1- Penicillium chrysogenum* *2- Mucur hiemalis*	*1- Penicillium chrysogenum* *2- Mucur hiemalis* *3- Candida albican*	*1- Penicillium chrysogenum* *2- Candida albican* *3- Alternaria alternaria* *4- Aspergillus flavus*	*1- Penicillium chrysogenum* *2- Candida albican*
5	Agricultural soil contaminated with pesticides	*Mucur hiemalis*	*1-Aspergillus niger* *2-Pencilium chrysogenum* *3-Fusarium oxysporum* *4-Mucur hiemalis*	*1-Aspergillus flavus* *2-Alternaria alternaria* *3-Mucur hiemalis* *4-Fusarium oxysporum* *5- Aspergillus niger*	*1-Alternaria alternaria* *2-Pencilium chrysogenum*	*1-Alternaria alternaria* *2-Pencilium chrysogenum* *3- Fusarium solani* *4- Candida albican*
6	Agricultural field	*Mucur hiemalis*	*Mucur hiemalis*	1. *Mucur hiemalis* 2. *pencilium chrysogenum*	*Penicillium chrysogenum*	*Penicillium chrysogenum*
7	Petroleum soil	*1-Mucur hiemalis* *2-Alternaria alternaria* *3-Candida albican* *4- Penicillium chrysogenum*	*1- Penicillium chrysogenum* *2-Rhizopus stolanifer* *3-Alternaria alternaria*	*1-Alternaria alternaria* *2- Penicillium chrysogenum* *3-Fusarium oxysporum*	*1-Alternaria alternaria* *2-Penicillium chrysogenum*	*Penicillium chrysogenum*
8	Stockyard soil	*1-Pencilium chrysogenum* *2-Rhizopus stolanifer*	*1-Penicillium chrysogenum* *2-Alternaria alternaria* *3-alternaria teunissima* *4-Fusarium oxysporum*	*1-Pencilium chrysogenum* *2-Candida albican* *3-Mucur hiemalis*	*1-Pencilium chrysogenum* *2-Candida albican* *3-Mucur hiemalis* *4-Alternaria teunissima*	*pencilium chrysogenum*

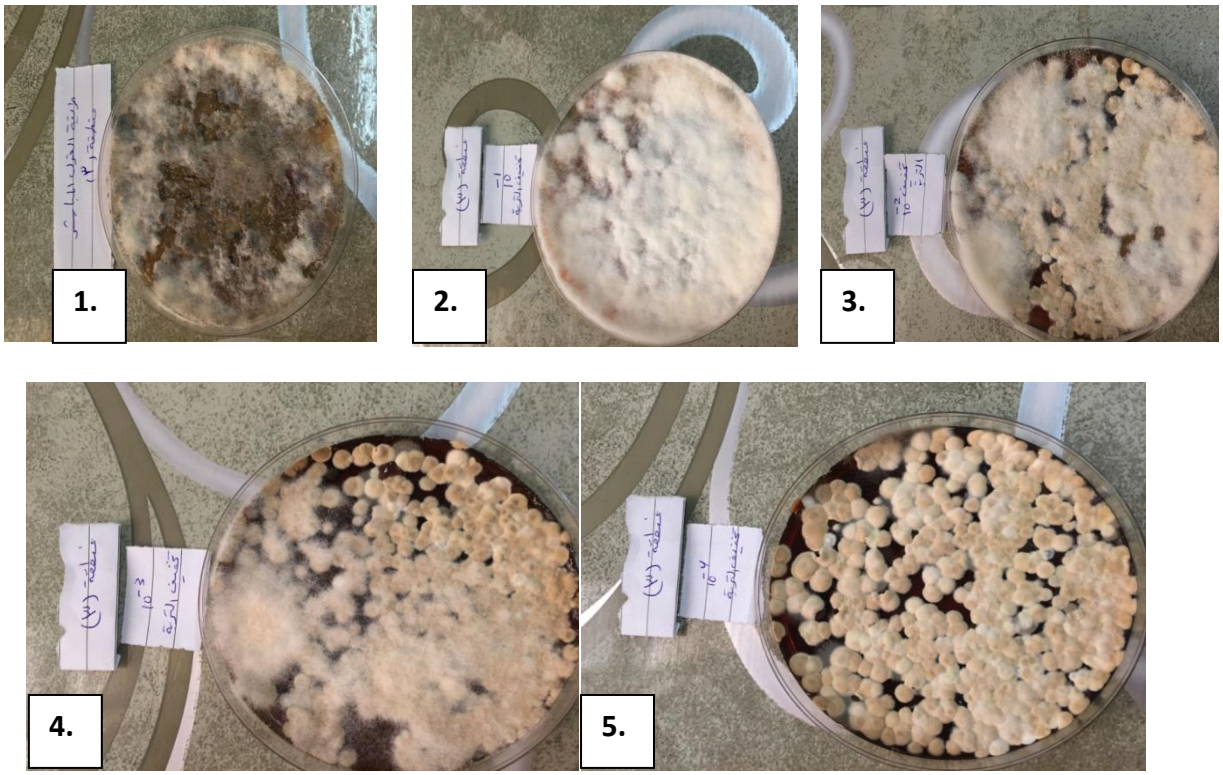
Table 1: the isolates of fungi from different soil samples

a. Isolation from

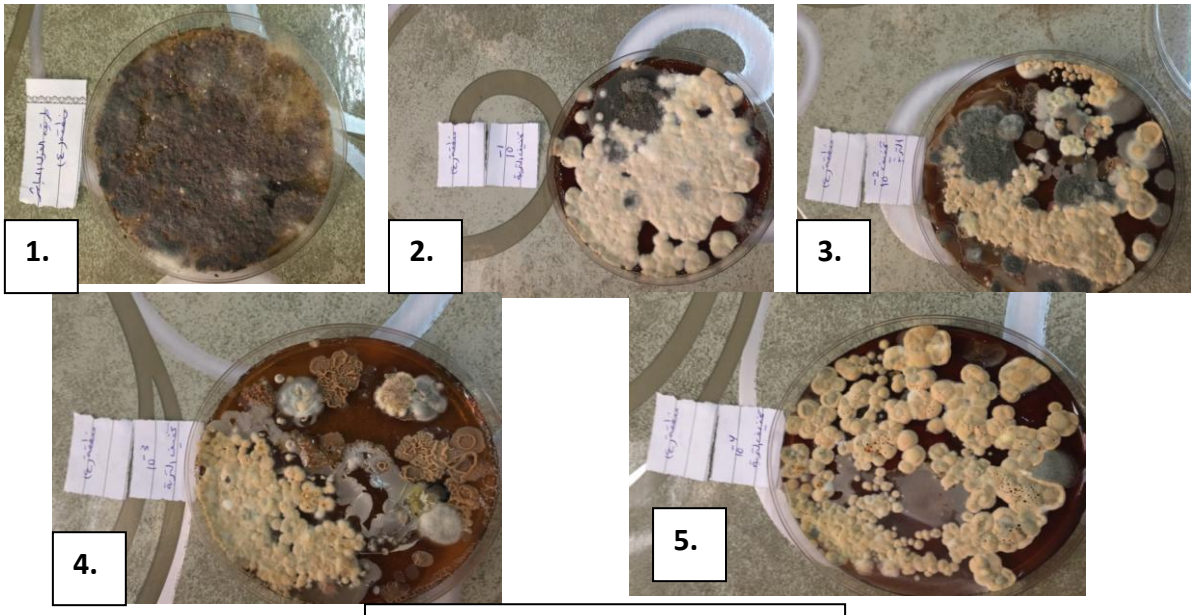


b. Isolation from

Figure 1:a and b: isolation from sites 1 and 2 respectively.1:direct isolation from soil.2,3,4and 5:dilution isolation method with conc.10^{-1},10^{-2},$10^{-3}$and 10^{-4}respectively.

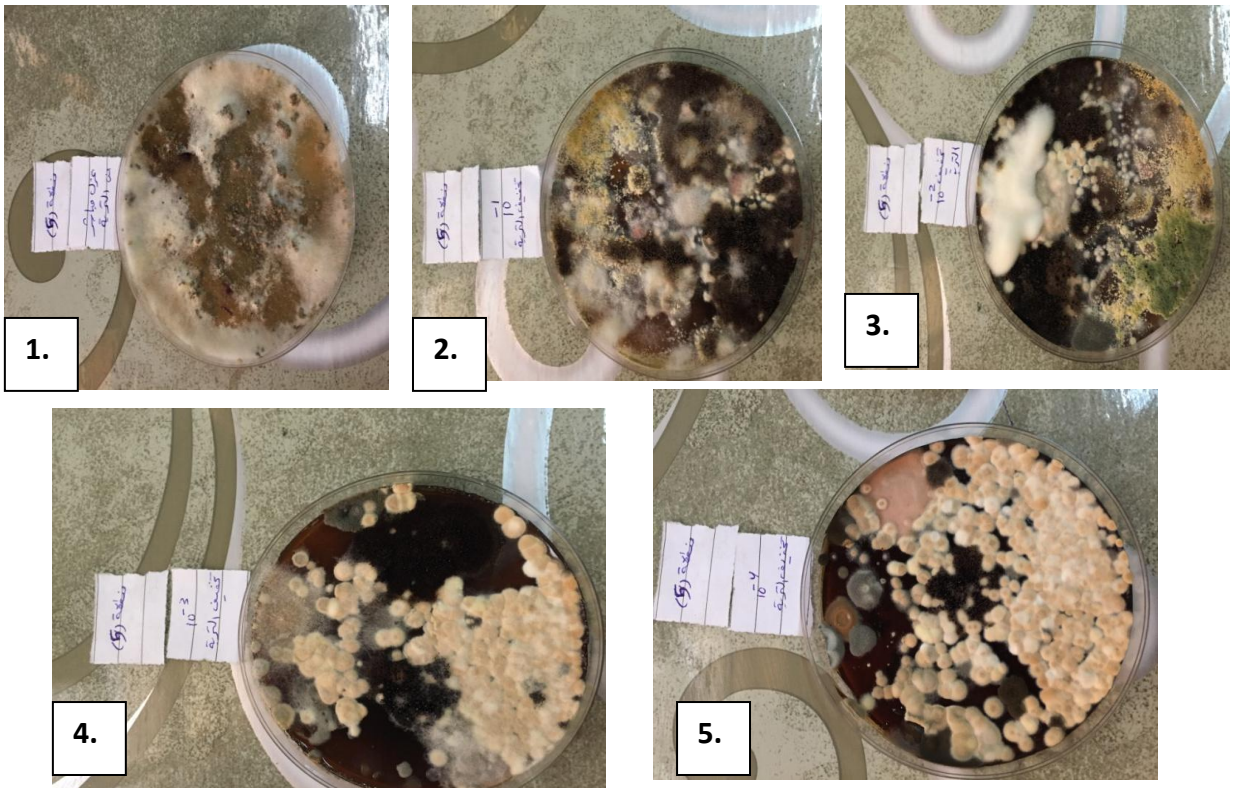


c. Isolation from site 3

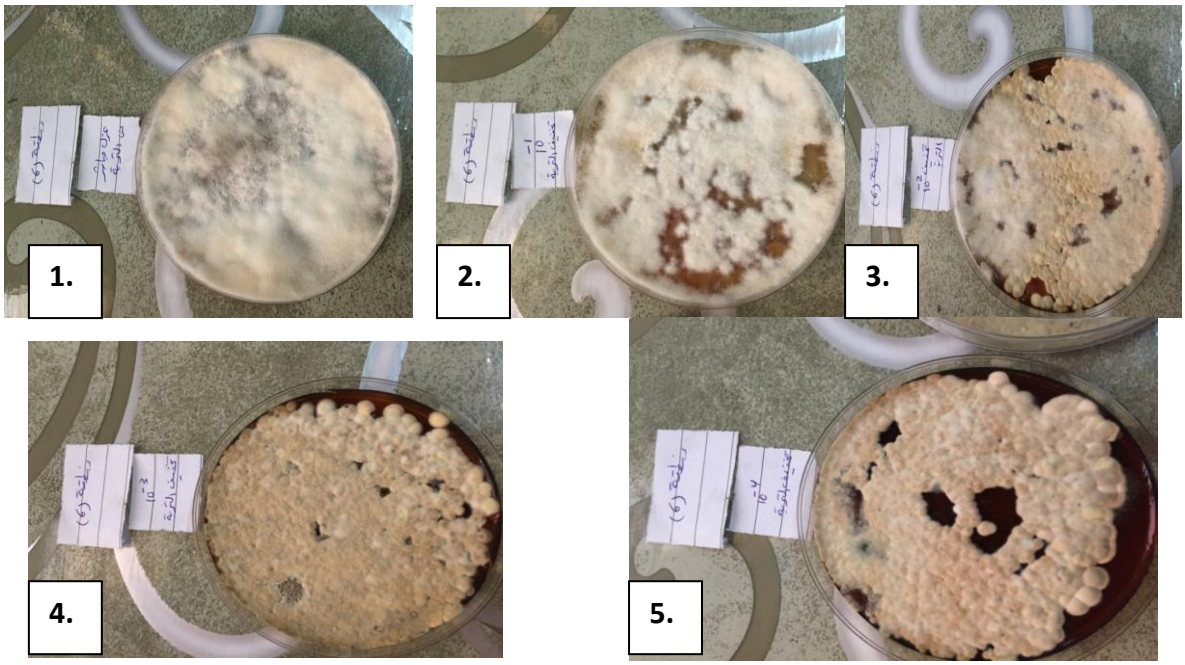


d. Isolation from site 4

Figure 1:c and d: isolation from sites 3 and 4 respectively.1:direct isolation from soil.2,3,4and 5:dilution isolation method with conc.10^{-1},10^{-2},$10^{-3}$and 10^{-4}respectively.

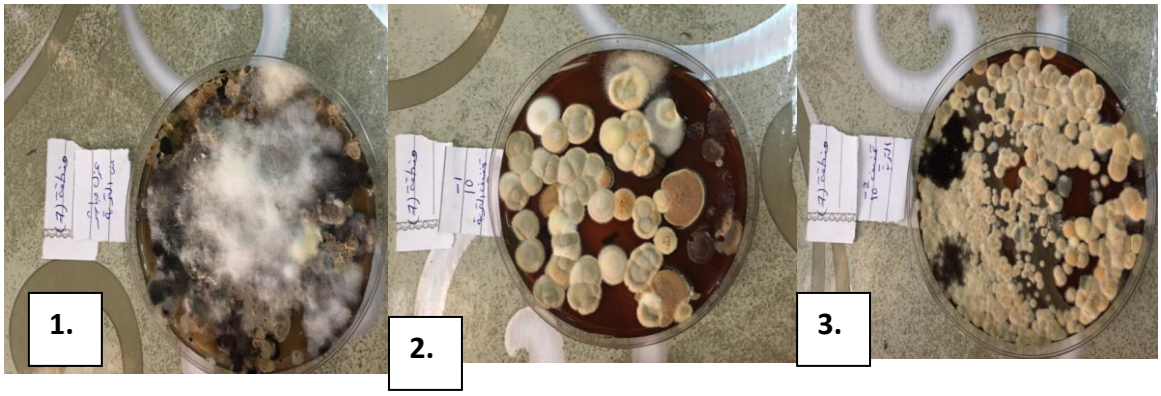


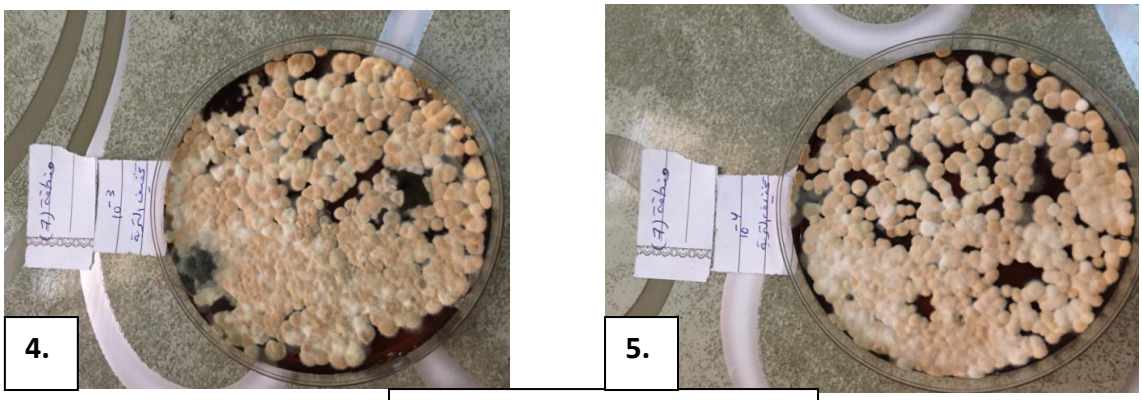
e. Isolation from site 5



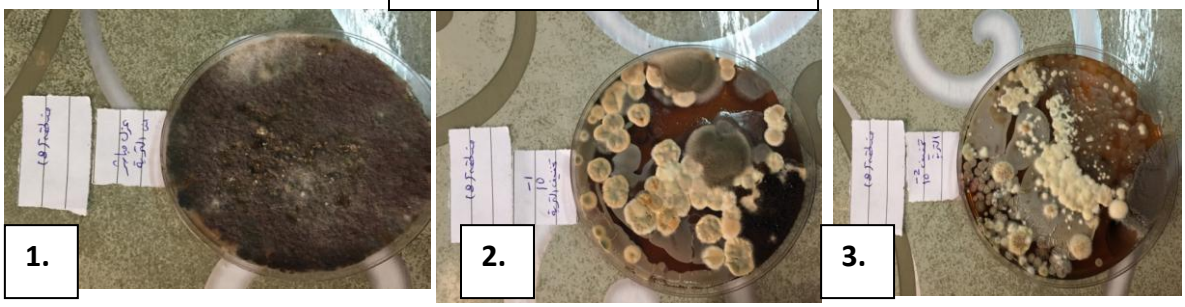
f. Isolation from site 6

Figure 1:e and f: isolation from sites 5 and 6 respectively.1:direct isolation from soil.2,3,4and 5:dilution isolation method with conc.10^{-1},10^{-2},$10^{-3}$and 10^{-4}respectively.





g.Isolation from site 7



h.Isolation from site 8

Figure 1:g and h: isolation from sites 7 and 8 respectively.1:direct isolation from soil.2,3,4and 5:dilution isolation method with conc.10^{-1},10^{-2},$10^{-3}$and 10^{-4}respectively.

The results of fungi isolation percentage according to the genus that have been isolated , are listed in the table 2,table 3, F. 2:

Table 2: The percentage of fungi genera isolates from the soil samples:

No.	Fungi genus	No. of isolates	Percentage of fungi isolates
1	*pencilium*	34	38.2%
2	*Mucur*	21	23.59%
3	*Alternaria*	13	14.6%
4	*Candida*	8	8.98%
5	*Rhizopus*	3	3.37%
6	*Fusarium*	6	6.74%
7	*Aspergillus*	4	4.49%

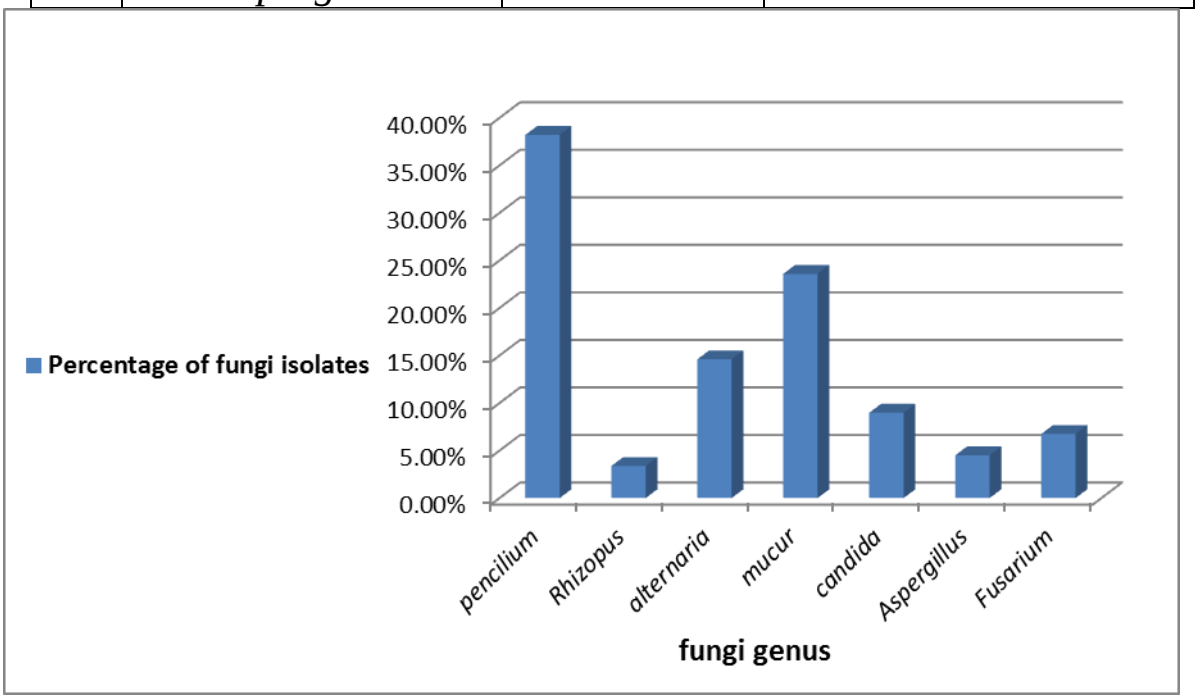


Figure 2: the percentage of fungi genera that have been isolated from soil samples.

Table 3: classification of fungal isolates according to their classes:

	class	species		No. of isolates
1-	Zygomycetes	*Mucor hiemalis*	21	24
		Rhizopus stolanifer	3	
2	Deuteromycetes	*Alternaria teunissima*	3	19
		Alternaria alternaria	10	
		Fusarium oxysporum	4	
		Fusarium solani	2	
3	Ascomycetes	*Penicillium chrysogenum*	34	46
		Aspergillus niger	2	
		Aspergillus flavus	2	
		Candida albicans	8	

The results of fungi isolation from different soil samples (table 2) have shown that the highest percentage of fungi isolates was *pencilium* with 38.2%, followed by *Mucur* with 23.59%. The third one in the isolation percentage was *alternaria* with 14.6%.While *yeast, Rhizopus, Fusarium* and *Aspergillus* had the isolation percentages: 8.98% , 3.37% , 6.74% and 4.49% respectively. There were other strains could not be identified because they lack of the sporulating structures under presently used incubation conditions.

In a study of fungal diversity in different crop fields at Salur Mandal [12] ,they found that *Aspergillus fumigates, Aspergillus niger*, *Aspergillus flavus* and *Penicillium chrysogenum* had a high occurrence in all of the crop fields. They also mentioned that *Aspergillus* and *Penicillium* were dominant in all agricultural fields. In our research, *Penicillium* isolation results constituent with that result, it had the highest isolation percentage 38.2% .this may be due to the fact that *Penicillium* spp. have a high sporelation capacity and producing fungal and bacterial antibiotics, also *Aspergillus* spp. can produce different kinds of toxins such as aflotoxins, achrotoxins etc. , these toxins may prevent the growth of other fungal species.

In our results , *Alternaria* was the third genus in the isolation percentage from the soil samples that have been tested. This is similar to the results of a study of fungi diversity in india[1], they mentioned that *Alternaria* was dominant in three types of soils (agricultural land, barren land and gardens) after *Aspergillus*.

Other study [14] ,found that *Alternaria, Aspergillus,* and *Fusarium* were predominant genera in agricultural soil contaminated with pesticides, this finding agreed with our results from soil sample 4.The fast growing of these genera reveals that these fungal strains are much adapted to this soil type, and could be used for the degradation to the common pesticides.
some of the fungi species have been reported as common isolates from pesticides polluted soil and used for biodegradation of xenobiotics[15] .

Aspergillus flavus and *A.niger* that have been isolated in the present study ,were previously isolated as insect pathogens by several author[16],[17]. *Aspergillus* among various genera of hyphomycetes have been reported with its ability to distribute easily in different soil environments, this due to the adaptation ability of this genus easily to different environment [1]

In a study of isolation and identification of soilborne fungi in fields irrigated by GAP [18],they found that the genera with the greatest number of species were *Penicillium* (24 species), *Aspergillus* (20 species), *Acremonium* (nine species) in the soil plate method. The most widely distributed and abundant colony forming taxa in the soil plate method were *Penicillium* (1464 colonies), *Aspergillus* (770 colonies),*Acremonium* (275 colonies), *Rhizopus Ehrenb.* (136 colonies) and *Stachybotrys Corda* (102 colonies).

Other research [19], mentioned that: *A. niger, A. fumigatus, A. flavus, Trichoderma* sp.,*Rhizopus* sp. and *Penicillium* sp. were isolated from the soil contaminated with degraded paper. This investigation may lead to the development of strains of soil fungi that would be used locally for the biodegradation of cellulose materials. These organisms are so recommended for management of solid wastes containing cellulose.

In other researchers study of soil fungi diversity in different lands [6], they mentioned that: most soil fungi in all of the tested lands belong to the classes Zygomycetes and Deuteromycetes , and they are very common in the agricultural soil.while in our results, we found that ascomycete was the most isolated class with (47 isolates), Zygomycetes and Deuteromycetes classes had (24 and 19 isolates) respectively.

Also, in a study of determination of the soil Microflora of in Nigeria [20],they isolated *Rhizopus oryzae, Aspergillus niger, Aspergillus flavus, Mucor hiemalis, Penicillium chrysogenum, Fusarium oxysporium,* and *Trichophyton rubrum.* And these results were almost similar to our results of fungi isolation.

In a study of isolation and identification the types of microorganisms in soil habitat of a young oil palm plantation [10] ,they found that: the fungal isolates were identified as *Mucor* sp., *Penicillium* sp. and *Aspergillus* sp.

Two actinomycete isolates were identified as *Streptomyces* spp. Also they mentioned that there was no previous report founded on the pathogenicity of all the identified isolates towards oil palm. However, *Aspergillus* sp. could be pathogenic to other plant crops.

Fusarium solani was the most common species recovered from the peat soil samples which are not surprising , because *F. solani* is one of the most common species inhabit different types of soil ,and it has been isolated from numerous soils in sub-tropical, semi-arid and grassland soils, cultivated soils ,forested area and sandy soils [21], [22], [23] .

The genus *Alternaria* includes a diverse assemblage of species that occur worldwide in a variety of habitats [24].this fact constitute with our results, *Alternaria* was the third genus in the percentage of fungi isolation .

Alternaria have been recorded with their role in degradation and manufacturing products causes commercial losses worldwide. Also as plant pathogens, decomposers of food stuffs, contribute as a pathogens causes spoilage of 25%–50% of agricultural output.

The relationships between *Alternaria* toxins and cancer development have been found in epidemiological studies [25].

There is relationship between soil fungi and the soil environment properties like physical and chemical properties. also , some soil fungi related positively with the P, Ca and Mg content in the soil[4].the maximum species and numbers of soil fungi were found in soil collected in the rainy season[26].

Another research showed that the effect of microbial diversity on microbial functions in the soil depends on the function measured. Some functions increased (substrate induced respiration, SIR) with decreasing microbial diversity in soil, others were not affected (thymidine and leucine incorporation, NO3 accumulation, respiratory growth response), and some declined when microbial diversity was small (short-term respiration from added grass, potential nitrification rates[27].

No relationship exists between microbial diversity and decomposition of organic matter, and a reduction in any group of species has little effect on overall soil process because the surviving microorganisms can carry out the decomposition of organic matter [28],[29].

The fungal diversity of any soil depends on a large number of factors of the soil such as pH, organic contents, and moisture. And there was no much of a variance in the three types of soil (agricultural fields , garden and barren land soil), the diversity was found to be higher in the unattended barren land as compared to the agricultural fields and garden soils[1].

Because of the importance of the soil fungi category, it should receive much more attention, and more researches should be done to find any promising species that could be use in the industry, medicine and agriculture .also to preserve any important species that might be disappeared.

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تشخيص الفطريات من التربة من مناطق مختلفة من بغداد

كيمياوي.الاء حسين يونس
الجامعة التكنولوجية
العلوم التطبيقية
فرع التقنيات الاحيائية

م.م.هبة ثامر حسين
الجامعة التكنولوجية
العلوم التطبيقية
فرع التقنيات الاحيائية

الخلاصة:

اهتمت الدراسة الحالية بعزل وتشخيص الفطريات من نماذج مختلفة للتربة من بغداد وتضمنت: (التربة الزراعية,حدائق ,تربة نفطية,تربة من اماكن تربية المواشي والدواجن).تم عزل الفطريات بالطريقة المباشرة, وطريقة العزل بأستخدام التخافيف على وسط السباروید دكستروز اكار (SDA) . شخصت العزلات عيانيا ومجهريا باستخدام صبغة Lacto phenol cotton blue

لتشخيص الفطريات المعزولة .اظهرت نتائج العزل والتشخيص وجود ٨٩ عزلة فطرية, وان اعلى نسبة مئوية للفطريات المعزولة كانت لـــ *penicilium* بنسبة 38.2%, يليها *Mucur* *بنسبة* .23.59%,بينما جاءت*alternaria* *بالمرتبة الثالثة بنسبة*14.6%. وكانت نسبة كل من *Yeast* و*Rhizopus* و*Fusarium* و *Aspergillus* هي8.98% و3.37% و6.74% و 4.49% على التوالي.