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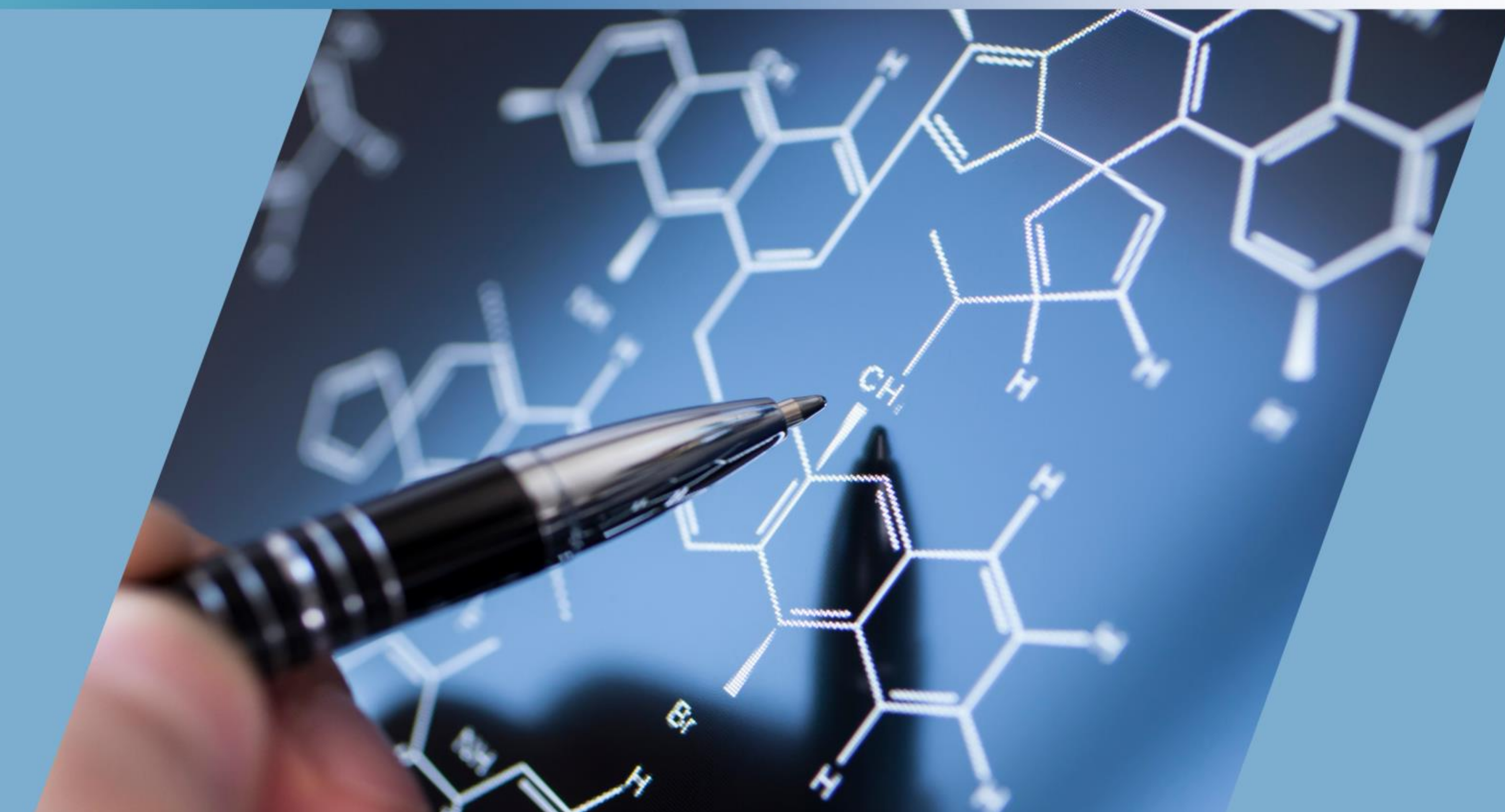

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Isolation and Study of the Phenotypic Characteristics of Some Soil-borne Fungi in two Different Locations in Omar AL-Mukhtar University, Albyda, Libya

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A B S T R A C T

Fungi assume a significant role within the terrestrial ecological system, as they are accountable for numerous crucial processes that contribute to the preservation of ecological equilibrium. Notably, they facilitate the recycling of soil organic matter and mineral elements. They are widely recognized for their role as a stimulator of plant development, a biocontrol agent for plant diseases, and participants in bioremediation processes. This study involved the isolation of fungi from agricultural soil previously employed at the Glasshouse facility at Omar AL-Mukhtar University, situated in Albayda City, eastern Libya. The investigation of soil fungus diversity in this region remains unexplored. This investigation involved the collection of soil samples from two distinct places within the institution. The soil dilution soil method and PDA agar medium were employed to isolate soil fungi. A notable disparity in fungal diversity was noted between the two sites, with the findings indicating that the predominant genera identified were associated with the Ascomycota family, while the proportions of Zygomycota were comparatively lower. The frequent species were in decrescent order: *Aspergillus*, *Penicillium* spp, and *Trichoderma* spp.

1 Introduction

The fungal communities present in soil are of significant importance in influencing plant communities and sustaining the functioning of the environment. These communities exhibit a strong correlation with both plant communities and soil attributes (Hicks *et al.*, 2021). Soil functions as a reservoir for several microbial communities found in plants and herbs, facilitating the production of carbon dioxide (CO_2) and nitrogen (N) cycles. Microbial composition changes the quality of soil through organic matter decomposition, nutrient recycling and biocontrol (Stefanis *et al.*, 2013). Typically, fungi remain inactive and exhibit modest growth, relying on a variety of organic compounds. In general, the concentration of microbes is greater near the roots of plants (rhizosphere), where its exudates are considered an important source of organic energy that enters from soils. Fungal organisms, particularly pathogenic fungi responsible for plant illnesses, are influenced by several living and non-living elements (Liu *et al.*, 2020). Fungi have a substantial impact on various aspects of human existence, including but not limited to their application in industry, agriculture, medicine, food industry, textiles, bioremediation, natural cycling, and as biofertilizers. This study aimed to investigate the distribution and genus-level identification of fungal isolates, whenever feasible, by analyzing macromorphological characteristics such as slow or rapid growth, topography,

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and micromorphological features, including hyphae, macroconidia, microconidia, chlamydospores, and other distinctive fungal structures Materials and Methods.

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2 Materials and Methods

2.1 Collection of soil samples

Two soil samples were obtained from different places within Omar AL-Mukhtar University in order to investigate the distribution of fungi. In this study the uppermost layer of soil was removed from each sample, measuring approximately 3 cm. Subsequently, three sub-samples were randomly extracted to a depth of 15 cm at each location, employing a sterile auger (Mailafia, et.al 2017). Soil samples were collected in each site, namely near the roots where the majority of microbial activity is concentrated (Burh, 2011 ; Han et al. 2023).

The fungal communities present in soil are of significant importance in laboratory settings, where they are stored in sterile polyethylene bags under aseptic conditions. Furthermore, the sub-samples from each site were combined to form a single compound sample that accurately represents the entire area. The soils were subjected to ambient temperature to facilitate the drying process. Once the samples had acquired a sufficient moisture content, they were subjected to sieving using a 2 mm screen to evaluate the soil characteristics.

2.2 Organic matter content of soil

It was determined according to Poudel (2020). A 1 gm of sieved soil was digested by chromic acid in the presence of 10 ml of $K_2Cr_2O_7$ and 20 ml H_2SO_4 (for oxidation of organic matter to carbon dioxide), while the excess of chromic acid was titrated against standard ferrous sulfate solution using diphenylamine as an indicator.

2.3 pH value of soil

The soil pH was determined using a Beckman pH meter. The pH of the soil was calculated by quantifying the addition of 5 ml of distilled water to 1 g of soil, as described by (Zhang et al. 2021; Li et al. 2023).

2.4 Isolation and purification

To isolate fungi, the soil dilution plating technique was employed, which involved combining 10 g of soil sample with 100 ml of sterile distilled water, followed by agitation on a shaker at a speed of 100 rpm for a duration of 10 minutes. The soil was diluted to a concentration of 10-3. Subsequently, 1 ml of the resulting diluted soil solution, ranging from 10-1 to 10-3, was pipetted into a petri dish. This process was repeated three times. Approximately 9 milliliters of Potato Dextrose Agar were introduced into the petri dish containing diluted soil, gently swirled, and allowed to undergo solidification. Daily examinations were conducted on the soil plates, while fungal colonies were subsequently subcultured onto PDA. This study conducted a single spore isolation procedure on a fresh PDA medium to acquire pure fungal culture isolates (Noman et al. 2018; Soltani et al., 2022).

2.5 Identification of Fungi

Similar to the study conducted by Raja *et al.* (2022), fungal isolates were classified at both the genus and species levels, whenever feasible, based on morphological analysis, which involved examining colonies for characteristics such as rapid or slow growth, topography, texture, surface pigmentation, as well as micromorphological features including hyphae, macroconidia, microconidia, chlamydospores, and other distinctive fungal structures.

3 Results

According to the findings shown in Table 1, the soil organic matter content was determined to be 3.3% for cultivated sandy soil in the Faculty of Science region and 4.3% for cultivated sandy loam soil in the Faculty of Agriculture region. The soil samples from both locations had alkaline pH values, as indicated in Table 1. Where soil pH and organic matter content had no significant differences between the two locations.

Table (1) Characteristics of the soil samples and plant used for isolatio

Soil No	Locations of soil samples	Particle size distribution				pH	Organic matter %	Plant under cultivation
		Sand %	Silt %	Clay %	Texture			
1	Faculty of science	64.60	25.6	9.8	Sandy Loam	7.6	3.3	*Phagnallon rupestre*
2	Faculty of agriculture	65.66	21.64	12.69	Sandy Loam	7.9	4.3	*Portulaca oleracea*

The primary objective of this study was to isolate soil fungus from two distinct locations within Omar AL-Mukhtar University. Thirteen fungal isolates were obtained from the soil samples. The majority of species within the genus were classified as *Aspergillus*. The identified soil fungus, as presented in Table 2, include *Aspergillus niger*, *Aspergillus* spp., *Fusarium* sp., *Trichoderma* ssp., *Penicillium* spp., and *Rhizopus* sp. The species *Aspergillus* had the highest abundance in both sites, followed by *Penicillium* ssp. and *Trichoderma* ssp.

Table (2) The colony morphology of different species isolated from two different locations in Omar AL-Mukhtar University

Soil No	Size	Color	Nature of hyphae	Conidia shape	Species	Divisions
1	Large	Black	Septate	Globose	*Aspergillus niger*	Ascomycota
	Small	Green	Septate	Oval	*Aspergillus* sp.	Ascomycota
	Medium	White	Septate	Microconidia: Oval (one or two cells	*Fusarium* sp.	Ascomycota
				Macroconidia: (more than two cells)		
				Chlamydospores: Oval		
	Medium	White	Septate	Globose	*Trichoderma* sp.	Ascomycota
	Large	Green	Septate	Globose	*Trichoderma* sp.	Ascomycota
		Green	Septate	Oval	*Penicillium* sp.	Ascomycota
2	Large	Black	Septate	Oval	*Aspergillus niger*	Ascomycota
	Medium	Green	Septate	Oval	*Aspergillus* sp.	Ascomycota
	Small	Brown	Septate	Globose	*Aspergillus* sp.	Ascomycota
	Medium	Green	Septate	Oval	*Penicillium* sp.	Ascomycota
	Medium	Yellow-green	Septate	Oval	*Penicillium* sp.	Ascomycota
	Large	Green	Septate	Globose	*Trichoderma* sp.	Ascomycota
	Medium	Brown	Aseptate	Globose	*Rhizopus* sp.	Zygomycota

4 Discussion

Soil is a complex surface composed of mineral and organic elements that exist in solid, liquid, and gaseous phases, forming several layers. The composition and pH levels of soil are influenced by the processes of weathering and erosion on rock (Raja et al., 2022). The community structure of soil fungus is significantly influenced by soil pH. A previous study has indicated that alterations in fungal communities within the rhizosphere can occur in response to several environmental conditions, such as pH levels, temperature fluctuations, and nutrient availability (Timling et al., 2012). Deslippe et al. (2012) observed that the fungal community structure varied between mineral and organic soils, maybe due to differences in nutrient content and carbon ratio in the organic soil. In a study conducted by Wahegaonkar et al. (2011), a total of 45 genera were identified and spread over 85 species within agricultural soils.

In a study conducted by Gaddeyya in 2012, a total of 15 species belonging to six genera were isolated from agricultural areas. The most common isolates were *Trichoderma harzianum*, *Trichoderma viride*, *Aspergillus flavus*, *Fusarium solani*, and *Fusarium oxysporum*, which aligns with the present findings.

5 Conclusions

In brief, this study has provided a comprehension of the range of soil fungi present in various locations inside Omar AL-Mukhtar University. The pH level of soil is widely recognized as a significant determinant in the establishment of fungal communities. Furthermore, there exist other unmeasured environmental elements that could potentially influence the development of fungal communities in the soil, including climate and vegetation type. These factors will impact the composition of soil fungus populations. Hence, it is imperative to conduct further studies to validate the impact of vegetation cover on the dispersal of fungi.

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