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Screening and Identification of the Rhizosphere Fungal Communities Associated with Land Reclamation in Egypt

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Abstract: Soil fungi are a wide range of microorganisms that play an essential role in enhancing the available nutrients in the soil for plants. In the current study, to study the fungal association with newly reclaimed land in Egypt, 22 composite soil samples were screened and characterized from citrus and olive orchard soil in contrast to a control soil that had never been cultivated (a nearby desert). The isolates were identified and tested for P solubilization and IAA production to highlight their potential as biofertilizers while the sampled soil was characterized. The physicochemical characteristics of the orchard's soil sample had a high relative mean moisture content, and the C/N ratio were 45.24% and 16.8% compared with desert lands of 32.80% and 8.12%, respectively, while a higher pH was recorded for desert lands. A total of 272 fungal isolates yielded 27 filamentous fungal species. Based on ITS molecular identification, the 27 isolates belonged to phyla Ascomycota, from eight genera. Twelve species were positive in producing a phosphate clearance zone around the fungal colony growth, while ten species were able to release IAA in vitro with different tryptophan concentrations under different pH values. When known pathogenic fungi were excluded, *Aspergillus tubingensis* and *A. fumigatus* were the highest IAA producers and can solubilize phosphorus. The screening and identification of the fungal diversity of the newly reclaimed land provided insights into potential phosphate solubilizers and plant phytohormones producers (i.e., IAA). Overall, the obtained results can provide primary knowledge that indicates the great potential fungal ability to develop biofertilizers for application in improving the production of immature soil for agriculture reclamation processes and practices.

Keywords: reclaimed lands; internal transcribed spacer (ITS); phylogeny; phosphate solubilization; Indole acetic acid (IAA)

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1. Introduction

A major global challenge is the decline of agricultural resources. Consequently, new multifaceted sustainable agriculture strategies are required to meet the growing demand for crops [1]. A wide range of microbial communities naturally live in soil, interact with each other, and affect soil health and plant growth [2]. In addition, soil microorganisms play a significant role in the evolution of soil ecosystems as well as the formation of soil properties. At the same time, some species of bacteria and fungi can contribute to nutrient bioavailability and aggregate formation, especially in newly reclaimed soils [3]. During soil reclamation by the plantation process, organic amendments widely used, such as livestock manure, biosolids, and crop residues, can modify the soil characteristics such as physical and chemical properties (moisture content, pH, carbon, and nitrogen contents) and the various occurrence of heterotrophic microbial communities [4]. Soil fungi play an essential role in the decomposition and mineralization of soil organic matter as well

as the cycling of nutrients, protecting the environment, and improving plant and animal health [5]. Therefore, it is crucial to accurately identify fungal species in various fields, such as biodiversity conservation management, ecological monitoring, etc.

Microorganisms in the rhizosphere can influence plant biodiversity in an ecosystem directly or indirectly. Several organisms participate in these processes, resulting in various interactions between plants, symbionts, and antagonists [6,7]. Living and nonliving components contribute to the composition and structure of rhizosphere communities; it has been demonstrated in several studies that soil type has a significant impact on the diversity of bacteria and fungi in the root zone [8]. Subsequently, the impact of the plant type on the microbial diversity in the rhizosphere of cultivated and wild plant species [9,10]. Microbial diversity, abundance, and activity all influence the sustainability of agricultural lands and production systems. Therefore, it is crucial to understand the complex interrelationships between the microbial communities within rhizospheres to select sustainable crop rotations and management practices [11,12].

To identify soil fungal species, barcode sequences using DNA internally transcribed spacer ITS region, which is the primary barcode of fungal species [6], provide a reliable method for studying biodiversity and identifying cryptic fungal species. However, fungi are characterized by various morphologies, ecologies, and life cycles [13–15]. Consequently, previous research on soil fungal diversity aimed to study their functions, such as organic matter decomposition, plant growth, and pesticide degradation [16]. They are characterized mainly by the production of siderophores [17], the production of extracellular enzymes, phosphate solubilization [18,19], and indoleacetic acid (IAA) [20].

In the present study, we screened and identified fungal species from soil samples from two newly reclaimed sites compared with an uncultivated site based on their fungal community. The main objective was to determine the fungal communities associated with land reclamation for agriculture in order to understand and expand our knowledge of this ecological system and examine the influence of specific soil fungal diversity on the cultivation of horticulture trees in the area. In addition, the detected fungal diversity was tested as potential biofertilizers.

2. Materials and Methods

2.1. Sampling Locations

A total of 22 composite samples were collected (i.e., 11 composite samples collected twice) in July at average temperatures of 34 °C during the daytime. Samples were obtained from newly reclaimed orchards cultivated with citrus and olives. Two composite sample types (i.e., rhizosphere and uncultivated) for each plant species were collected from each orchard located near the Cairo-Alexandria Road (30°16′5″ N and 30°39′38″ E), along with triplicate composite samples from a nearby desert area (30°15′19″ N and 30°38′38″ E; Figure S1). For orchard composite samples, for each tree species, 5 random soil samples were collected and mixed from the root rhizosphere area of healthy trees at least 1 m distance apart at a depth of 5–10 cm from the surface alongside the tree trunk (Rhizosphere samples); an additional 5 random samples were mixed from the area between two trees of the same type at the same depth (uncultivated samples). Approximately 50 g of each composite soil sample was stored at 4 °C in autoclaved plastic zip-lock bags until processing [21] (Table 1, Figure 1). Within a week after the last sample was collected, samples were processed simultaneously.

Table 1. A list of sample codes describing the land type, plantation, and collection area for each sample type.

Reclaimed Lands	Plantation	Sample Area	Code	Composite Samples	Replicates
Citrus Orchard (A)	Citrus trees 1	Rhizosphere (R)	AR1	5	2x
		Uncultivated (U)	AU1	5	2x
	Citrus trees 2	Rhizosphere (R)	AR2	5	2x
		Uncultivated (U)	AU2	5	2x
Olive Orchard (B)	Olives trees 1	Rhizosphere (R)	BR1	5	2x
		Uncultivated (U)	BU1	5	2x
	Olives trees 2	Rhizosphere (R)	BR2	5	2x
		Uncultivated (U)	BU2	5	2x
Desert Lands (D)	No plantations	Rhizosphere (R)	DU1	5	2x
			DU2	5	2x
			DU3	5	2x

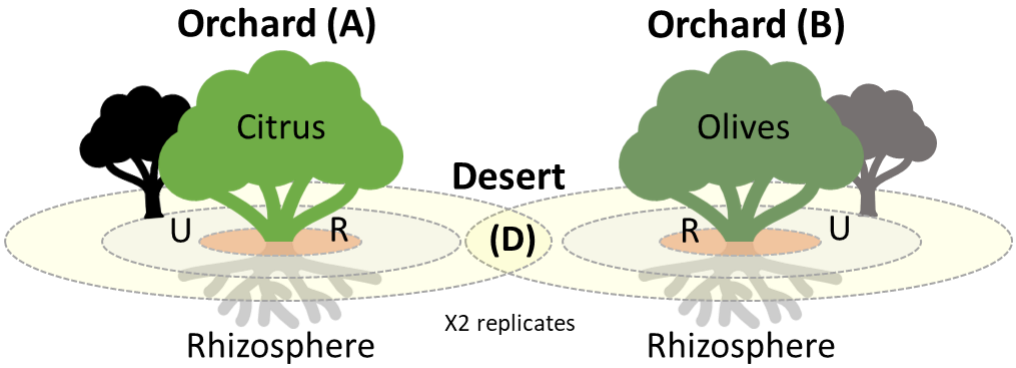

Figure 1. Graph illustrating sample selection from land types, both reclaimed, and desert lands. Each land type was sampled beneath the plant trunk (i.e., rhizosphere; R) and from the uncultivated area (U) between trees. All samples were collected at the same depth in duplicates.

2.2. Soil Physio-Chemical Characterization

All of the soil samples were analyzed in triplicates in all of the following procedures. A fresh soil sample was oven-dried at 105 °C for 12 h, and then ground. An average of 25 mg from each soil sample was used to determine sample elements ratios following the manufacturer's instructions [22]. Based on chloroform-fumigation-extraction, microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN) were calculated as MBC = EC/0.45 and MBN = EN/0.54, where EC and EN is the differences in organic carbon and nitrogen between chloroform-fumigated and unfumigated soil samples, respectively, extracted with 0.5 M K_2SO_4 [23]. Soil pH was determined using a pH meter using a soil-to-water ratio of 1:2.5, and soil moisture content (Mc) was measured gravimetrically. A total carbon analyzer (TC) and a total nitrogen analyzer (TN) were used, as well as the alkali N-proliferation method for alkali nitrogen (AN) testing [24]. To measure dissolved organic carbon (DOC), high-purity water was used to extract DOC and assayed using an automated TOC-VCPH analyzer. Soil organic carbon (SOC) was measured using a non-dispersive infrared method [25].

2.3. Fungal Isolation

Fungal isolation was carried out by preparing a decimal dilution from the soil samples. One gram of the soil sample was suspended in 10 mL of physiological solution to make a serial dilution up to 5-fold. 1 mL of each suspension/dilution was inoculated into Petri dishes containing Dichloran Rose Bengal Chloramphenicol (DRBC) agar medium (#CM0727, Oxoid, Lenexa, KS, USA) and incubated at 25 °C for 5–10 days [26,27]. All

obtained morphotypes were counted to detect colony forming unit (CFU; Table 2). The morphological observation for the screened fungi in the current study was performed on Potato Dextrose Agar (PDA; #1022, Condalab, Madrid, Spain) and incubated at 25 °C for 7 days [28] incubated at 25 °C for fungal single colonies differentiation and then subjected to morphological and molecular identification.

Table 2. A list of the successfully obtained fungi expressed as CFU/g from each sample.

Soil Samples Code	Vegetation	Sample Area	Total CFU/g
AR1	Citrus trees	Rhizosphere	3.8×10^3
AU1		Uncultivated	1.6×10^3
AR2	Citrus trees	Rhizosphere	4.4×10^3
AU2		Uncultivated	1.8×10^3
BR1	Olives trees	Rhizosphere	4.8×10^3
BU1		Uncultivated	2.7×10^3
BR2	Olives trees	Rhizosphere	3.0×10^3
BU2		Uncultivated	2.2×10^3
DU1	Desert	Uncultivated	1.2×10^2
DU2			1.1×10^2
DU3			0.7×10^2

2.4. DNA Extraction, PCR Amplification, and Sequencing

Genomic DNA was obtained from the fungal cultures using sterile spatulas and extracted using DNeasy® PlantMini Kit (Qiagen, Hilden, Germany) following the manufacturer's protocol. Polymerase chain reaction (PCR) amplification for the nuclear ribosomal internal transcribed spacer (ITS) region was amplified with primer ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) [29].

PCR reaction was conducted in a 25 µL volume including 12.5 µL of OnePCR™ master mix (Genedirex®, Taiwan), 1 µL of each primer (forward and reverse, each of 10 µM), and 1 µL of extracted DNA (100 ng/µL). The thermal conditions were as follows: an initial denaturation for 5 min at 95 °C, followed by 35 cycles of denaturation for 1 min at 94 °C, annealing for 30 s at 55 °C, extension for 90 s at 72 °C, and a final extension step at 72 °C for 10 min. Subsequently, all PCR products were checked using gel electrophoresis in 1.5% agarose, and the purified fragments were then directly subjected to Sanger sequencing (Macrogen Inc., Seoul, Republic of Korea).

2.5. Sequence Alignment and Data Analysis

DNA fragments in both directions were sequenced with sufficient overlap. After sequencing, the chromatograms obtained were evaluated, assembled, and edited using Geneious R10 [30]. Consensus sequences were subject to BLAST tool identification in the NCBI database using default parameters. The sequences were aligned using the MAFFT aligner [31]. Pairwise genetic distance among sequences was calculated using maximum likelihood methods implemented in Geneious R10. The obtained sequences were assembled and aligned using Geneious Prime, then analyzed with BLAST searches in GenBank for species identification using the NCBI online Blast tool (https://blast.ncbi.nlm.nih.gov/Blast.cgi, accessed on 10 March 2022).

The sequences retrieved were revised and checked against the GenBank nucleotide database (https://www.ncbi.nlm.nih.gov/nuccore, accessed on 10 March 2022). Datasets included the newly generated sequences, along with the NCBI-deposited sequences of the identified sequences from the current study. Phylogenetic inference was conducted using maximum likelihood (ML) and Bayesian inference (BI). The ML analyses were performed

using FastTree V2 [32] with 500 bootstrap replicates and default parameters. The BI analysis was performed by MrBayes 3.2 [33] where two parallel analyses of four chains were run for 1,000,000 generations.

2.6. Phosphate-Solubilizing Test

All of the obtained fungal strains were subjected to testing of their ability to solubilize phosphate [34] on PVK medium (Himedia M520) incubated at 25 °C for 3–7 days. The phosphate solubilization ability was determined by measuring the size of the fungal phosphate-solubilizing zone.

2.7. Indole Acetic Acid (IAA) Production

The production of IAA in the isolated fungal strains was determined [35]. A 5 mm in diameter fungal hyphae was obtained from a fresh culture of 3 days incubation at 25 °C on PDA, then placed in glass tubes containing 10 mL of PDB with different concentrations of tryptophan (0.1, 0.5, 1, and 1.5%) and different pH values (pH 5.0, 7.0, and 9.0) incubated at 28 °C in a rotary shaker at 150 rpm for 7 days. The cultures were centrifuged at 8000 rpm for 5 min, and 1 mL of supernatant from each tube was mixed with 4 mL of Salkowski's reagent, followed by incubation in the dark at room temperature for 30 min. A pink-colored appearance indicated IAA production, calculated using a standard curve of standard IAA (0–100 g mL^{-1}) at 530 nm measured with a UV spectrophotometer [36]. The test outputs were subject to three-way ANOVA using SAS software.

3. Results

3.1. Fungal Screening and Isolation

The results of the screened soil samples in the current study were a total of 272 fungal isolates obtained during the isolation from 22 soil samples cultivated on DRBC media after 3–7 days at 25 °C. An example of the cultivated plates is shown in Figure 2. To detect the fungal community occurrence relating to the colony forming unit CFU/g the average fungal abundance was found to be around 10^3 from orchards and 10^2 from the desert (Table 2).

Figure 2. An example of the cultivated fungi on DRBC media from the current study. Plates from the reclaimed soil samples (**A**), and the desert soil samples (**B**).

3.2. Species Abundance

Based on the morphological characteristics of the pure culture on PDA media, an example of the pure culture is shown in Figure 3. In total, 272 isolates of fungi were isolated from the orchards, and desert soil samples that were collected from two different localities with two types of plantations and a desert control sample, resulting in 87 fungal strains obtained from the rhizosphere of olives reclaimed land and 49 from the uncultivated plot from the same orchard. A total of 82 fungal strains were obtained from the rhizosphere of citrus reclaimed land, 34 from the uncultivated plot from the same orchard, and 29 fungal strains were obtained from the control desert land. The 272 fungal isolates yielded 27 filamentous species belonging to phyla Ascomycota from eight genera. Most of the fungal isolates were identified at the species level as Aspergillus, Penicillium, Epicoccum, Fusarium, Ulocladium, Trichoderma, Cladosporium, and Chaetomium. The taxa isolated from the soil samples and the percentage occurrence from each locality are listed in Table 3. The dominant genus was Aspergillus with nine species, Fusarium with five species, followed by three species from every three genera of Penicillium, Ulocladium, and Trichoderma, while the lowest number was one species for the genus Epicoccum and Chaetomium.

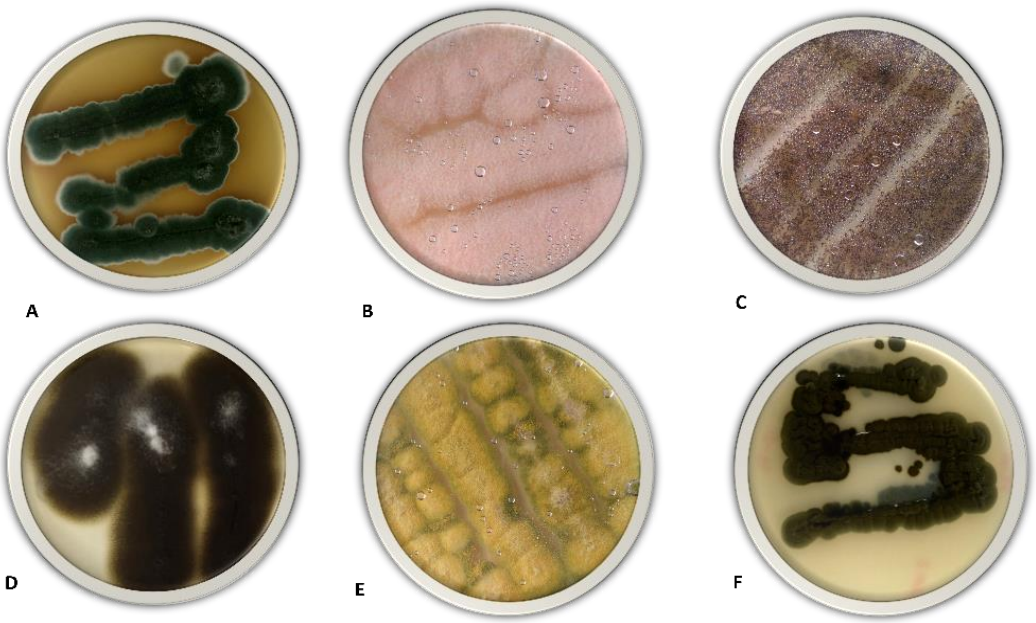

Figure 3. The morphological growth of the pure colonies isolated on PDA media from the soil samples: *Penicillium oxalicum* (**A**); *Fusarium acuminatum* (**B**), *Aspergillus niger* (**C**); *Chaetomium globosum* (**D**); *Penicillium digitatum* (**E**); *Cladosporium herbarum* (**F**).

Table 3. A list of samples distribution and frequency of the identified fungi in soil by location, crop, and percentage frequency of isolates.

Species Code	Species Name	Reclaimed Land				Desert Land
		Olives Trees		Citrus Trees		
		R%	U%	R%	U%	
SFSA1	*Aspergillus tubingensis*	7.69	4.08	-	-	-
SFSA2	*Aspergillus flavus*	8.97	-	-	-	6.89
SFSA3	*Aspergillus aculeatus*	6.41	6.12	8.53	-	6.89
SFSA4	*Aspergillus niger*	2.56	6.12	6.09	8.82	-
SFSA5	*Aspergillus ochraceus*	-	-	-	-	13.79

Table 3. *Cont.*

Species Code	Species Name	Reclaimed Land				Desert Land
		Olives Trees		Citrus Trees		
		R%	U%	R%	U%	
SFSA6	*Aspergillus chevalieri*	-	-	-	-	10.34
SFSA7	*Aspergillus fumigatus*	-	-	8.53	-	6.89
SFSA8	*Aspergillus terreus*	7.69	-	-	-	-
SFSA9	*Aspergillus* sp.	3.84	4.08	4.87	23.52	-
SFSA10	*Penicillium oxalicum*	10.25	8.16	7.31	5.88	-
SFSA11	*Penicillium digitatum*	-	-	6.09	8.82	6.89
SFSA12	*Penicillium citrinum*	3.84	10.20	7.31	11.76	-
SFSA13	*Epicoccum nigrum*	5.12	6.12	-	-	-
SFSA14	*Fusarium oxysporum*	6.41	14.28	4.87	8.82	6.89
SFSA15	*Fusarium acuminatum*	-	-	6.09	8.82	-
SFSA16	*Fusarium brachygibbosum*	-	-	4.87	-	-
SFSA17	*Fusarium solani*	-	-	7.31	2.94	-
SFSA18	*Fusarium equiseti*	2.56	8.16	-	-	-
SFSA19	*Ulocladium atrum*	2.56		-	8.82	-
SFSA20	*Ulocladium* sp.	-	-	-	-	13.79
SFSA21	*Trichoderma harzianum*	11.53	14.28	6.09	-	-
SFSA22	*Trichoderma stromaticum*	8.97	6.12	4.87	5.88	-
SFSA23	*Trichoderma* sp.	3.84	4.08	4.87	-	-
SFSA24	*Cladosporium herbarum*	-	-	-	-	10.34
SFSA25	*Cladosporium oxysporum*	-	-	7.31	5.88	-
SFSA26	*Cladosporium velox*	-	-	-	-	6.89
SFSA27	*Chaetomium globosum*	7.69	6.12	4.87	-	10.34

R = rhizosphere; U = uncultivated area.

3.3. Soil Physio-Chemical Characterization

Based on the soil physicochemical analysis profile, reclaimed sites had a significant impact on physicochemical properties in comparison with the desert location as follows: soil samples from the two orchard's locations A and B had a high relative mean moisture content, which was 45.24% higher than the desert site 32.80% (D). The C/N ratio, considered a key factor influencing the soil fungal communities, was 16.8% in the reclaimed location, higher than in the desert location at 8.12%. The mean pH value in the desert location was 6.9 to 7.3, higher than in the reclaimed locations, which were 5.4 to 5.9. Soil content of the following elements in the reclaimed locations TC, TN, and SOC were 21.02, 3.06, and 9.07 g/kg, while AN, DOC, BMC, and BMN were 325.03, 22.03, 490.07, and 58.09 mg/kg; respectively. The records from the desert location were 14.06, 2.01, and 25.03 g/kg for TC, TN, and SOC, and were 235.09, 75.06, 220.03, and 37.07 mg/kg for AN, DOC, BMC, and BMN, respectively.

3.4. Molecular Characterization and Phylogeny

Internal transcribed spacer ITS region was sequenced to identify the fungal isolates based on phylogenetics (Figure 4). All ITS sequences were sequenced across all isolates representing the 27 identified fungal species. The BLAST results by the ITS differentiated all the samples to the species level (Table 3). In detail, samples from 1 to 27 matched with a

pairwise identity of 100% to the top hit best-matched sequences retrieved from the NCBI database when aligned with identified sequences for all samples. The alignments were 790 bp, of which 214 bp were phylogenetically informative after removing the ambiguously aligned regions. The relationship between all reference isolates could distinguish species and genera clearly. Except for the Aspergillus species, two species, *A. fumigatus* clustered with Penicillium species (bootstrap value = 0.59), and another *A. chevalieri* (monophyletic with *A. cristatus* and *A. caperatus*) were separated from the other Aspergillus species. All other Aspergillus species were clustered with a high bootstrap value (0.88). All other species were identified by phylogeny and supported by the BLAST results (Figure 4).

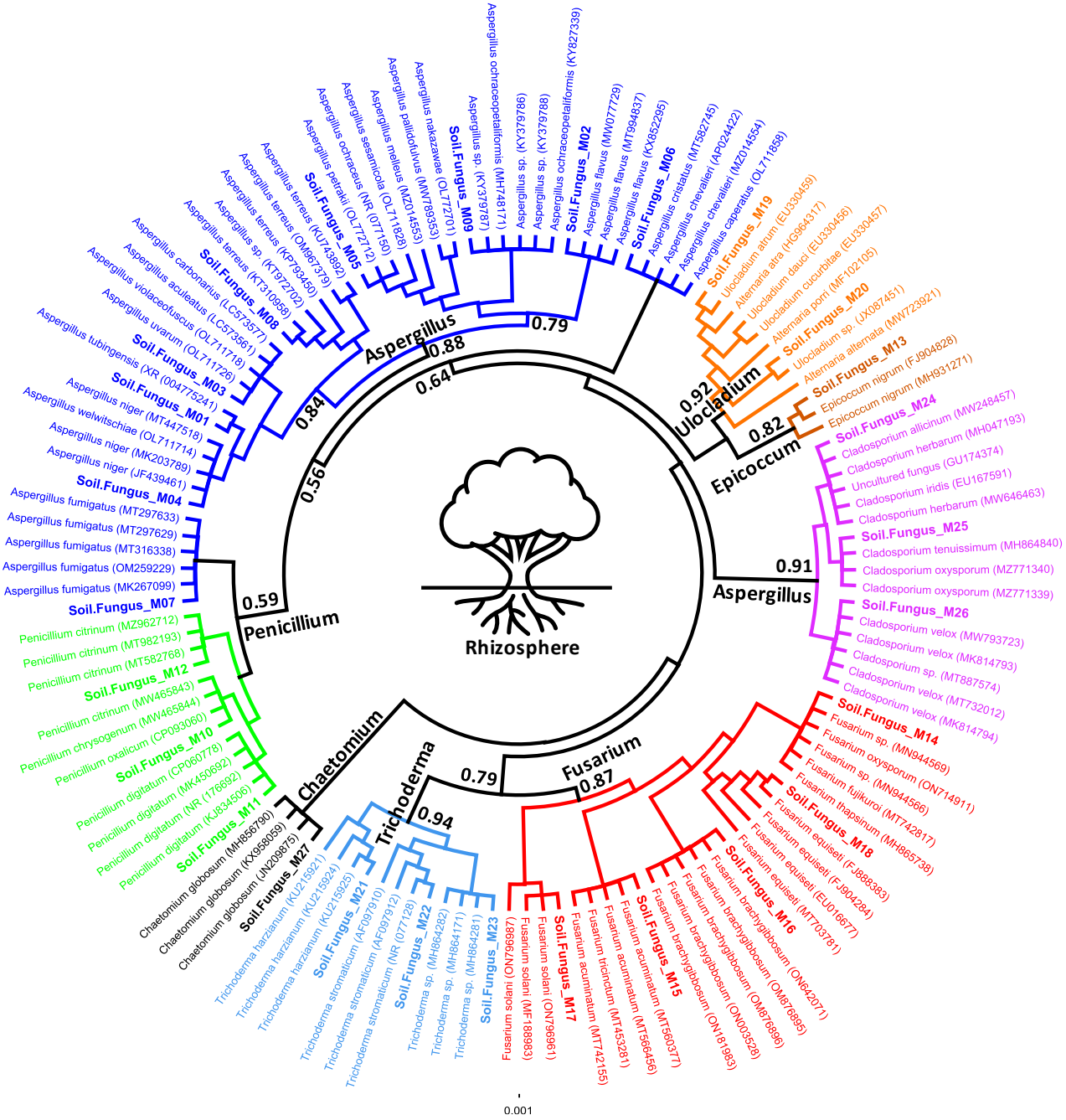

Figure 4. The phylogenetic tree for the fungal isolates species from the current study includes similar internal transcribed spacer data recorded for each species in the NCBI database. Different clusters were named by genus name, and samples were coded as "Soil.Fungus_M#" and serialized by number in the same order as Table 3.

3.5. Phosphate-Solubilizing Test

The 27 identified fungal strains obtained from the current study were subjected to test their ability for P solubilization. The results showed that 12 strains could produce a different halo size around the colony growth (Figure 5), indicating their potential ability for phosphate solubilization on PVK media, while the rest of the species recorded negative results. In detail, the most significant clearance zone ranged from 6.7 to 6 mm for species *Penicillium citrinum*, *Aspergillus aculeatus*, and *Penicillium oxalicum*; followed by a reduction from 5.6 to 5.3 mm for species *Aspergillus tubingensis*, *Chaetomium globosum*, and *Aspergillus niger*; then reduction from 4.1 to 3.4 mm for species *Fusarium oxysporum*, *Aspergillus terreus*, *Trichoderma harzianum*, and *Aspergillus flavus*; and the lowest halo zone ranged from 2.9 to 2.4 mm for species *Aspergillus fumigatus*, and *Aspergillus chevalieri*.

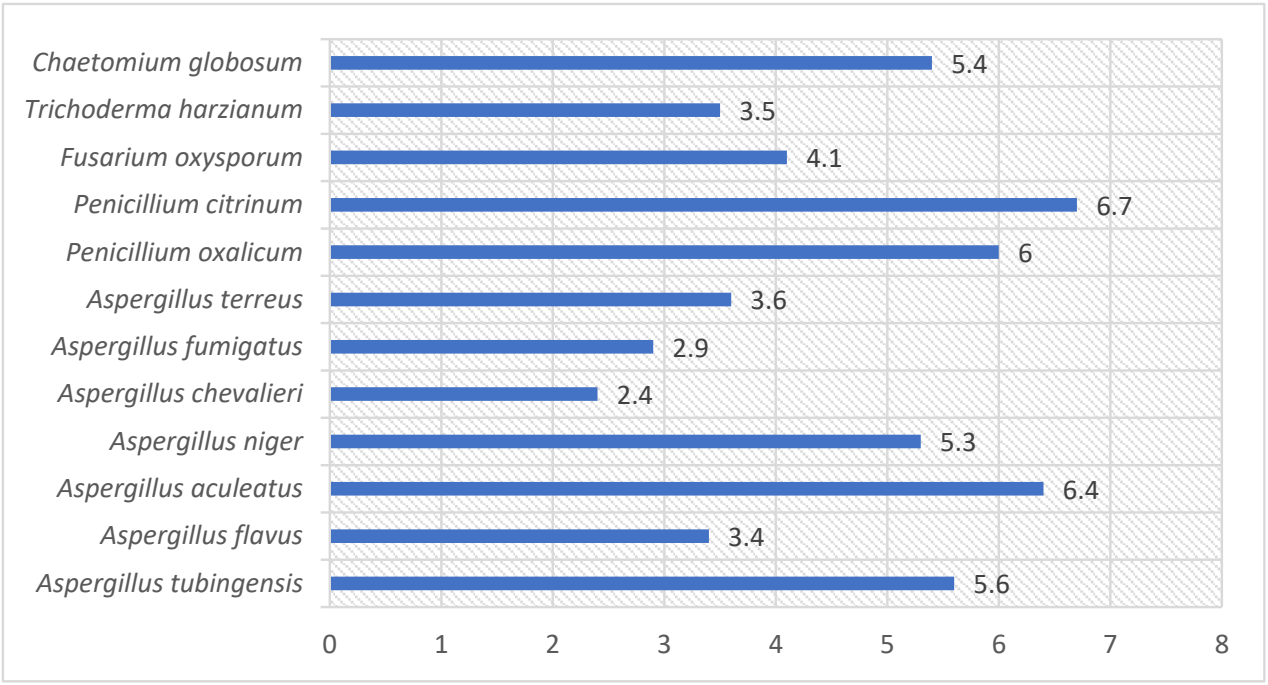

Figure 5. Phosphate solubilization ability (mm) of the fungal strains after 7 days of incubation.

3.6. Indole Acetic Acid (IAA) Production

The ability to produce IAA in vitro from the obtained fungal species in this study resulted in a positive for ten species that vary in the amount of IAA production, with different tryptophan concentrations at different pH values (Table 4). Based on the ANOVA analysis, the production of IAA is significantly different between the tested fungal isolates based on tryptophan concentrations ($p < 0.0001$), but not significantly affected by different pH levels ($p = 0.3171$). The concentration of 1.5% tryptophan at pH 7.0 recorded the highest production of 260 µg/mL of IAA for strain *Aspergillus tubingensis*, 85 µg/mL for *Aspergillus niger*, and the lowest recorded at this concentration is 33 µg/mL for *Fusarium oxysporum*.

Table 4. The IAA production under different concentrations of tryptophan at different pH levels.

Fungal Species *	IAA Concentrations (µg/mL) *											
	pH5 NS				pH7 NS				pH9 NS			
	0.1% b	0.5% b	1.0% a	1.5% a	0.1% b	0.5% b	1.0% a	1.5% a	0.1% b	0.5% b	1.0% a	1.5% a
A. tubingensis a	27	75	240	176	10	126	215	260	63	25	73	84
A. flavus a	45	31	33	85	52	92	198	210	98	84	92	72
A. niger ab	76	75	72	89	95	87	124	85	59	79	75	73
A. fumigatus a	41	78	210	97	37	85	197	212	88	43	76	79
A. terreus c	16	22	35	83	43	7	36	39	35	56	79	85
P. oxalicum c	13	22	34	62	11	19	23	40	35	45	76	82
P. citrinum c	7	21	32	79	6	26	45	66	35	67	77	80
F. oxysporum c	22	31	34	75	23	19	40	33	35	53	57	61
T. harzianum bc	45	54	43	89	18	30	48	55	39	73	78	92
C. globosum c	32	31	34	22	19	43	79	50	62	76	63	78

Color gradient (min: dark blue; max: dark red). For each factor, significant groups are labeled by superscripted letters. * $p < 0.0001$, NS = not significant.

4. Discussion

The current study aimed to screen and identify the rhizosphere-associated fungal communities from newly reclaimed land and uncultivated land in terms of taxa composition and relative abundance following a specific sampling design. A multi-plant sampling from the two different land types was the main feature of the analysis by using samples from the rhizosphere area in the newly cultivated reclaimed lands, from two orchards cultivated with orange and olive trees, and other samples were controlled by uncultivated areas from the same location. Meanwhile, uncultivated locations (desert land as general control) were used to omit desert-related fungal-presented species from the current analysis. In order to reveal as many relevant species associated with the plant rhizosphere as possible, different sampling approaches were applied [21]. Based on ITS sequence identification and analysis, twenty-seven species were identified using DNA barcoding with 98–100% identity. The ITS rDNA region sequence typically identifies fungi species isolated from environmental sources [37]. Consequently, it has been widely used for detecting the soil fungal community and as an improvement over classical identification. Furthermore, a phylogenetic analysis can be performed using ITS rRNA genes since they are universally distributed, functionally constant, sufficiently conserved, and of adequate length to reveal evolutionary relationships [38].

Cultivation-dependent techniques applied to the soil samples of rhizosphere associated with the two planting types revealed highly variable counts that changed between the two planting positions over citrus and olive trees, as the highest viable counts were 3.8×10^3 to 3.8×10^3 CFU/g, respectively. In contrast, the fungal community composition differed significantly among the uncultivated location as the count varied between 0.7 and 1.2×10^2 CFU/g [39]. The soil fungal community isolated within the three studied locations was assigned to phyla Ascomycota. A total of 27 filamentous fungal species belonging to eight genera were obtained from the analyses of 22 composite soil samples. Ten isolates resulted positive for in vitro phosphate solubilization ability, and twelve isolates resulted positive for in vitro IAA production in various pH conditions. In some cases, phosphate solubilization, IAA production, and other compounds produced by the fungus may interact with plants during colonization, promoting growth, inducing resistance, and modifying plant defenses [40,41].

In the past, numerous studies have identified and described rhizosphere and non-rhizosphere fungi, including species belonging to Aspergillus and Penicillium genera [22]. The most frequently identified genera were assigned to Aspergillus, isolated from all the studied soil samples. The *A. tubingensis*, *A. terreus*, *Aspergillus sp.*, and *A. niger* were more frequent in reclaimed soil from both studied locations' rhizosphere and the surface; *A. flavus*, *A. aculeatus*, and *A. fumigatus* were found in both land types, reclaimed and the

uncultivated, while *A. ochraceus*, and *A. chevaliers* were exclusively found in the control soil, which has never been cultivated. Only *Aspergillus terreus* was found to be associated with the olive rhizosphere plantation. The second most frequently isolated genera were assigned to Fusarium. The *F. oxysporum* was isolated from all the studied soil samples, *F. acuminatum*, and *F. solani* were found only in the citrus orchard soil. In contrast, *F. equiseti* were exclusively isolated from olive orchard soil, and *F. brachygibbosum* was found to be associated with the citrus rhizosphere plantation. Genera Penicillium also was isolated from all soil samples, *P. oxalicum*, and *P. citrinum* were isolated from reclaimed land from both citrus and olive soil, while *P. digitatum* were isolated from citrus soil and the control soil which has never been cultivated. All Trichoderma species, *T. harzianum*, *T. stromaticum*, and *Trichoderma* sp. were isolated from both reclaimed soils from olive and citrus orchards with no occurrence in desert soil. *Cladosporium herbarum*, and *C. velox* were isolated only from the control desert land, while *C. oxysporum* was found in citrus orchards only. *Epicoccum nigrum* was isolated only from olive orchard soil samples, while for genera Ulocladium, *U. atrum* was isolated from olive rhizosphere samples and the citrus orchard surface. Otherwise, another *Ulocladium* sp. was isolated from the control land, and *Chaetomium globosum* occurred in the citrus rhizosphere samples, all olive orchard samples, and the control soil samples. The results came in agreement with the results of different studies that revealed the identity of the same genera of fungi [42–45].

The diversity of fungal communities is widely related to the changes in soil physiochemical properties such as pH, water, C and N ratio. Consequently, the natural fungal communities' changes result from soil-plant-microorganism interactions after land reclamation. Soil-altering ecosystems are affected by two main factors: the addition of nutrients and the decomposition of root exudates. These factors provide the soil with soluble nutrients such as C, N, and iron [46]. In land reclamation, soil pH, carbon-nitrogen ratio, and moisture content are closely related to the composition, diversity, and richness of microbial communities [47]. In soil fungal communities, pH, Mc, TC, TN, DOC, AN and SOC vary with pH, TC, AN, and SOC levels. In particular, the community structure of Ascomycota, Basidiomycota, and Zygomycota affected by the soil physicochemical properties perform better in environments with high contents of soil nutrients. Our results were similar to those that investigated the diversity of fungal communities in reclaimed soil, which was higher than the diversity in uncultivated soil with high contents of Mc, C/N, TC and TN. In similar research on a large scale, soil yeast and fungal community composition abundance of different natural lands in Germany (i.e., cambisols, luvisols, and arenosols in forest systems; and leptosols, stagnosols, histosols, and gleysols for grassland systems) were significantly related to soil properties, including pH, N content, and C/N ratio [48]. Accordingly, many fungi thrived in high-moisture, low-pH environments, as many fungi in the marsh and reclaimed soil demonstrate a complex soil fungal community [49].

Due to its role in plant growth and development, phosphorus is one of the most essential macro elements for plants. Crop production is limited by P deficiency in many agricultural soils worldwide. Therefore, chemical phosphate fertilizers are widely used in agriculture to improve crop yields [50]. Despite its expensive nature, chemical phosphate fertilizer is extremely harmful to the environment. As a result, agrochemical inputs must be dramatically reduced and replaced with more ecological and natural products, such as rock phosphate [51]. In this study, among the 27 identified fungal species, 12 species exhibited the ability to solubilize phosphate in vitro on PVK media. The most successful genera were Aspergillus, Penicillium, and Chaetomium; while similar observations have been made by scientists working in tropical Indian habitats [19,49]. A maximum level of phosphate solubilizing activity was detected in vitro for Aspergillus and Penicillium species isolated from rhizosphere soil [50]. Apart from commonly reported phosphate solubilizers, some other interesting genera like Chaetomium and Syncephalastrum have been reported [43]. *Chaetomium globosum* showed a maximum P-solubilizing indicating that those isolates may be utilized as biofertilizers to increase crop yield in arid regions.

Phytohormones such as indole acetic acid (IAA), which are plant growth promoters, are key factors in crop growth promotion. This usually occurs in microorganisms and is commonly established in the rhizosphere. Therefore, the inoculation of plants by targeting microorganisms at higher concentrations benefits overall crop production [52,53]. In the current study, eleven fungal isolates tested positive for their ability to produce IAA in vitro, which can be used in the future for the improvement of plant growth; the highest amount of production was assigned to *Aspergillus tubingensis*, *A. fumigatus*, and *A. flavus*, followed by *Trichoderma harzianum*, *Penicillium oxalicum*, *P. citrinum*, and *Chaetomium globosum*. The ability of endophytic filamentous fungi to produce IAA in vitro has also been described in non-agricultural plants, such as *Epicoccum nigrum* [54], *Aspergillus japonicus*, and *A. niger* [49,50] to improve plant growth parameters, as well as from cereals, like *T. harzianum* [55,56] and *F. oxysporum* in maize [57]. In addition, *Pseudomonas syringae*, which causes bacterial speck disease, was suppressed by *Aspergillus terreus* isolated from tomato plants that produced IAA [58].

Based on the literature, all of the reported fungal strains (SFSA1–4, 7–8, 10, 12, 14, 21, and 27) have a putative plant host [59]. *Aspergillus niger, Aspergillus tubingensis, Penicillium citrinum, Trichoderma harzianum, Aspergillus terreus, and Aspergillus fumigatus* never been reported as plant pathogens. However, unlike *Aspergillus aculeatus, Aspergillus flavus, Fusarium oxysporum, Chaetomium globosum, and Penicillium oxalicum* were reported as plant pathogens [59]. Thus, they were excluded from the study recommendation list of potential fungal biofertilizers. Regardless of the isolation source, and complemented by IAA production and phosphate solubilization abilities, three Aspergillus species, *A. tubingensis* (SFSA1) and *A. fumigatus* (SFSA7) are highly recommended as potential biofertilizers for horticulture plants in newly reclaimed lands. In comparison, *A. niger* (SFSA4) comes in third, since it was found in the uncultivated areas rather than in the rhizosphere of the tree, in addition to its modest ability to produce IAA and solubilize phosphate. In order to profile its safety level and usage recommendations for agricultural use, additional pathogenicity tests will need to be conducted on plants and humans before they can be used in agriculture.

5. Conclusions

The current study successfully characterized the soil fungal diversity from rhizosphere and non-rhizosphere soil. The fungal isolates from newly reclaimed land, in comparison with isolates from uncultivated land, were identified based on the results of the morphological and molecular analyses. The isolates were identified at the species level; all belong to phyla Ascomycota and represent eight different genera. Some isolated strains proved their potential ability for P solubilization and IAA production in vitro. Overall, the findings demonstrated that the soil physicochemical properties significantly influenced soil fungal community composition. The nutrients and soil fungal community are related to the reclamation processes for agriculture. The obtained data suggest that the colonization of fungi in the rhizosphere and non-rhizosphere of the studied locations is independent of the P-solubilizing activity and IAA production. Based on the reviewed literature, the pathogenic and non-pathogenic fungal populations were distinguished in association with the vegetation type of the studied orchards (i.e., Citrus and Olives). In conclusion, two non-pathogenic, IAA producers and P-solubilizing Aspergillus species, *A. tubingensis* (SFSA1) and *A. fumigatus* (SFSA7) can be recommended for use as biofertilizers in newly reclaimed lands. The findings of the current study can serve as a baseline for the future development of fungal-based antagonistic on soil microbiome, plant growth, and biocontrol research.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agriculture13010215/s1, Figure S1: A map for the sampling location in Egypt: (A) shows the cultivated orchards, (B) shows the near desert area.

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