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In vitro estimation of the phosphate-solubilizing potentiality of rhizosphere fungi isolated from New Valley Governorate, Egypt

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Abstract. Phosphate solubilization, production of IAA, and other related compounds by fungi interact with plants as part of its colonization, leading to growth promotion, induced resistance, and modification of basal plant defense mechanisms. In the current study, 77 rhizosphere fungal species belonging to 30 genera were isolated on Czapek's Dox agar from around 40 root samples of wheat- and pepper-soil collected from El-Kharga Oasis, New Valley Governorate, Egypt. Pepper-soil appertained a higher number of genera (22) and species (51) than wheat-soil. *Aspergillus*, *Penicillium,* and *Fusarium* were the most common genera recorded, and *Aspergillus flavus*, *A. terreus,* and *Penicillium chrysogenum* were the prevalent species in wheat-soil, while *A. niger*, *A. terreus,* and *Scopulariopsis fusca* were the wealthiest species in pepper-soil. Two hundred isolates were screened for their phosphate solubilization potential; only 31.0% of total isolates could solubilize phosphate with various degrees. The highest phosphate solubilization index (PSI) was shown in *P. chrysogenum* AUMC 14100, *A. niger* AUMC 14260, *P. chrysogenum* AUMC 14262, *A. brasiliensis* AUMC 14261, and *A. lacticoffeatus* AUMC 14257, recording PSI of 3.84, 3.74, 3.48, 3.17 and 3.11 respectively. The potentiality to solubilize phosphate by these five isolates was estimated in the liquid medium, *A. niger* AUMC 14260 could solubilize the maximum amount of phosphate (1754.7 µg/mL) after 20 days, while it reached its highest level in *A. brasiliensis* and *A. lacticoffeatus* (1745.8 µg/mL and 1679.1 µg/mL respectively) after 10 days. Therefore, these strains can be considered promising biofertilizers for application in agriculture.

Keywords: Wheat, pepper, Kharga Oasis, phosphate solubilization, *Aspergillus niger*, *Penicillium chrysogenum*

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1. Introduction

In recent decades, a significant consideration is being manipulated towards developing an integrated plant nutrition system for maintenance and enrichment of soil productivity through the balanced use of different sources of nutrients, including chemical, organic, and biofertilizers. The significant benefit of the plant nutrition system is the optimization of soil fertility and plant nutrient supply for sustainable crop productivity [1]. Globally, there are some problems associated with the use of chemical fertilizers such as high cost and low purchasing power that restrict their use in proper amounts, hampering crop production, loss of a sustainable amount of nitrogen through different mechanisms including ammonia volatilization, denitrification, and leaching losses causing environmental pollution problems [2,3]. Biofertilizers are eco-friendly organic products containing specific microorganisms, in concentrated forms, including fungi and bacteria derived from rhizosphere soil [4], and they have been proved to be effective and economical alternates of chemical fertilizers [5,6].

Phosphorous (P) is an essential constituent of nucleic acids, the energy molecule ATP, and membrane phospholipids representing about 0.2—0.8% of the plant dry weight, from which only 0.1% is available for plants in soil [7-10]. Some fungi such as *Aspergillus*, *Penicillium,* and *Trichoderma* can help, by their phosphate solubilization activities, in obtaining the monobasic

($H_2PO_4^-$) and dibasic ($H_2PO_4^{2-}$) phosphorus by plants [11,12]. Fungi are characterized by enormous potentiality to solubilize insoluble phosphate than bacteria on both solid and liquid cultures [13,14], particularly rhizosphere fungi, which have more potential of spreading through the soil than rhizobacteria [15,16]. Subculturing most phosphate-solubilizing bacteria resulted in the loss of phosphate solubilizing activity [17] compared with fungi that maintain their ability to leach P-containing rocks even after prolonged culturing [18].

Such abilities could be necessary for the industrial manufacturing of biofertilizers. Furthermore, a restricted number of published studies about phosphate solubilizing rhizosphere fungi and their use as biofertilizers. The current study was designed to isolate fungi from the rhizosphere of wheat and pepper plants in the New Valley and evaluate their potentiality for phosphate solubilization, leading to potent biofertilizers in agricultural applications.

2.Materials and methods

2.1. Collection of samples

Forty samples of rhizosphere soil around roots of wheat and pepper cultivated in Kharga Oasis, New Valley Governorate, Egypt, were collected monthly from January to April 2018 (for wheat) and June to October 2018 (for pepper). The samples were collected in sterile polyethylene bags and brought promptly to the laboratory, and maintained at 4 °C until fungal isolation.

2.2. Isolation of rhizosphere fungi

The dilution plate technique [19] was used for the isolation of fungi on Czapek's Dox agar (Cz) [20]. The isolation medium contained Rose Bengal (0.05 g/l) and chloramphenicol (250 mg/l) [21,22]. Five replicates plates were employed for each sample. All the plates were incubated at 25±2 °C for 7-15 days. The developing colonies were counted and isolated as pure cultures and preserved on Cz slants at 4 °C for further studies.

2.3. Screening of phosphate-solubilizing potentiality

All fungal isolates obtained were screened for their phosphate-solubilizing activity following the method of Pikovskaya [23] using Pikovskaya's (PVK) agar medium. After sterilization, the pH of the medium was adjusted to 7.2. The medium was inoculated with 50 µl spore suspension (prepared in 10% tween 80) containing 1 x 10^8 spore/mL obtained from 7-day-old cultures. The plates were then incubated for 7 days at 25±2 °C. The formation of clear zones around the fungal colonies indicates the fungal abilities to solubilize phosphate. The solubilizing phosphate index (PSI) was calculated according to the following equation:

$$\text{Phosphate solubilizing index (PSI)} = \frac{\text{Colony diameter+clear zone diameter}}{\text{Colony diameter}}$$

The results of phosphate solubilization index were expressed as high (PSI > 2.7), moderate (PSI (>2.2-2.7), low (PSI from >1-2.2), or negative (PSI = 1). The potential high phosphate solubilizer isolates were selected for further studies.

2.4. Quantification of phosphate solubilization in submerged fermentation

The high phosphate-solubilizing strains were inoculated separately in 500-mL Erlenmeyer conical flasks, each containing 100 mL of PVK broth medium (pH 7). Each flask was inoculated with 1 mL spore suspension obtained from 7-day-old culture and containing 1x10^6 spore/mL. The flasks were then incubated at 25±2 °C for 20 days in agitated conditions at 150 rpm. The concentration of solubilized phosphate and pH values were evaluated after the 5, 10, 15- and 20-day incubation period.

2.5. Assay of phosphate-solubilizing efficiency

After 5, 10, 15, and 20 days of incubation, the flask's contents were filtered, and the cell-free supernatants were obtained after centrifugation at 8000 rpm for 15 minutes the pH of the supernatants was measured, and the phosphate concentration was determined using chlorostannous acid reduced molybdophosphoric blue color method [24].

2.6. Reagents

1- Chloromolybdic reagent: 15 g of ammonium molybdate in 400 mL distilled water and 342 mL of concentrated HCl, and the total volume was made up to 1L.

2- Chlorostannous acid reagent: 10 g of $SnCl_2.2H_2O$ dissolved in 25 mL of concentrated HCl.

The reaction mixture was composed of 100 µL of the supernatant and 10 mL of chloromolybdic reagent. The reaction mixture was shacked well and diluted to 40 mL with distilled water. Five drops of chlorostannous acid reagent were added along the side and mixed properly. The final volume was completed to 50 mL with distilled water. The absorbance of the obtained blue color was measured at 660 nm against the blank. The concentration of the soluble phosphate was calculated using potassium dihydrogen phosphate (KH_2PO_4) as standard. The amount of soluble phosphate was expressed as µg/mL.

2.7. Statistical analysis

The data were introduced to one-way ANOVA using SPSS (version 21.0). Means were recorded for three replicates values and compared by Duncan's multiple range tests, and the statistical significance was evaluated.

3. Results and discussion

3.1. Rhizosphere fungi

Altogether seventy-seven species appertaining to thirty-one genera were isolated from forty samples of rhizosphere soil around wheat and pepper plants on Cz agar medium. *Aspergillus* was the most prevalent genus represented by 21 species comprising 35.65% and 58.61% of total fungi in wheat-soil and pepper-soil. *A. flavus* and *A. terreus* were the most common species from wheat-soil, constituting 45.97% and 33.22% of total *Aspergillus* and 16.39% and 11.84% total fungi, respectively. While *A. terreus* and *A. niger* were the most predominant species from pepper-soil, composing 30.13% and 22.65% of total *Aspergillus* and 17.66% and 13.27% of total fungi, respectively (Table 1).

Penicillium (6 species) was the runner of *Aspergillus* in wheat-soil comprising 38.28% of total fungi; however, it constituted 0.11% of total fungi in pepper-soil. Of *Penicillium*, *P. chrysogenum* and *P. waksmanii* were the most common in wheat-soil, encountering 45.6% and 21.25% of total *Penicillium* and 17.46% and 8.13% of total fungi, respectively. The remaining *Penicillium* species were fluctuated between 1.32% to 6.82% of total fungi (in wheat), while only one species (*P. oxalicum)* was isolated in low count from pepper-soil (Table 1).

Fusarium (6 species) ranked the third, comprised 13.64% and 1.64% of total fungi in wheat-soil and pepper-soil, respectively, and *Stachybotrys* represented by 6 species encountering 0.12% in wheat-soil and 5.2% in pepper-soil. *Mucor* and *Cunninghamella* were isolated from wheat-soil in moderate frequency, while the remaining (11 genera) were isolated in a low frequency of occurrence. On the other hand, *Neocosmospora* occurred moderately in 40% of total samples, and the remaining fungi (19 genera) were rarely isolated (Table 1).

Looking for fungal strains that have the potential to solubilize phosphate is the primary target of the current study, in which seventy-seven fungal species belonging to 30 genera were isolated from rhizosphere soil around the roots of 20 samples of each of wheat (35 species, 15 genera) and pepper collected from El-Kharga Oasis, New Valley Governorate, Egypt. In this respect, thirty-two species belonging to 19 genera of fungi were isolated from pepper near Vienna City, Austria, of which *Chaetomium globosum*, *Fusarium oxysporum*, *Gliocladium roseum*, *Mucor racemosus*, *Myrothecium verrucaria*, *Penicillium aurantiogriseum*, *P. expansum,* and *Trichoderma harzianum* were the most common genera [25]. The present findings showed that *Aspergillus*, *Fusarium*, *Penicillium* were the most common genera from which *Aspergillus flavus*, *A. terreus,* and *P. chrysogenum* (from wheat-soil) *A. niger,* and *Scopulariopsis fusca* (from pepper-soil) were the most prevalent species.

In agreement with these results, Abdel-Hafez et al. [26] studied the seasonal fluctuations of rhizosphere and rhizoplane fungi of Egyptian wheat plants. They stated that *Aspergillus niger*, *A. terreus*, *A. fumigatus*, *A. flavus,* and *Scopulariopsis brevicaulis* were the most common fungi. In other studies, *Aspergillus*, *Fusarium*, *Nigrospora*, *Penicillium*, *Pestaletiopsis,* and *Trichoderma* were isolated from rhizosphere soil of different healthy crop plants of India [27]. Also, *Alternaria alternata, Aspergillus fumigatus, A. niger, A. tamarii, Cochliobolus lunatus Cladosporium cladosporioides, Fusarium oxysporum, Gliocladium roseum, Humicola grisea,* and *P. chrysogenum* were frequently isolated as the most common species from wheat-cultivated soil in El-Kharga Oasis, New Valley [28]. Several studies also supported the current findings that obtained the same genera and species from rhizosphere soil [29,30]. Ismail et al. [31] isolated *Fusarium solani, F. oxysporum, Alternaria alternata, Macrophomina phaseolina, F. verticillioides, F. subglutinans, Acremonium strictum* from the root and stem samples collected from Assiut and Behera Governorates in Egypt.

Table 1. CFUs, percentage CFUs (calculated per total fungi in each soil type), and percentage frequency of occurrence (% F, calculated per 20 soil samples in each soil type) of fungi isolated from wheat-soil and pepper-soil collected from El-Kharga Oasis, New Valley Governorate, during January to April 2018 (for wheat) and during June to October 2018 (for pepper) on Czapek's Dox agar at 25±2 °C.

Fungal species	Wheat-soil			Pepper-soil		
	CFU	**%CFU**	**%F**	**CFU**	**%CFU**	**%F**
Acremonium rutilum W. Gams				100	0.11	5
Albifimbria verrucaria Lombard & Crous				200	0.23	5
Alternaria tenuissima (Kunze) Wiltshire	200	0.12	5			
***Aspergillus* P. Micheli ex Link**	**59600**	**35.65**	**80**	**52100**	**58.61**	**100 HH**
A. aureolatus Munt. -Cvetk. & Bata				900	1.01	20
A. aureoterreus Samson				3200	3.6	35
A. brasiliensis Varga, Frisvad & Samson	200	0.12	5			
A. chevalieri Thom & Church				100	0.11	5
A. cristatus Raper & Fennell				400	0.45	5
A. egyptiacus Moub. & Moustafa				200	0.23	10
A. flavipes (Bainier & R. Sartory) Thom & Church	8600	5.14	25			
A. flavus Link	27400	16.39	45	3000	3.37	40
A. lacticoffeatus Frisvad & Samson				100	0.11	5
A. neoniveus Samson	1400	0.84	5	200	0.23	5
A. nidulans (Eidam) Winter				8300	9.33	40
A. niger Tiegh.	1600	0.96	15	11800	13.27	75
A. ochraceous Wilh	400	0.24	5			
A. parasiticus Speare				1800	2.03	30
A. quadrilineatus Thom & Raper				800	0.9	10
A. speluneus Raper & Fennell				700	0.79	5
A. sulphureus Thom & Church				1500	1.63	10
A. sydowii (Bainier & Sartory) Thom & Church				3000	3.37	20
A. terreus Thom	19800	11.84	20	15700	17.66	95
A. versicolor (Vuill.) Tirab	200	0.12	5			
A. unguis (Émile-Weill & Gaudin) Thom & Raper				400	0.45	5
Bahusakala olivaceonigra (Berk. & Broome) *Subram*				300	0.34	5
***Bipolaris* Shoemaker**				**1800**	**2.03**	**15**
B. colocasiae (Tandon & Bhargava) Alcorn				1100	1.24	10
B. sorokiniana Shoemaker				700	0.79	5
Bisifusarium dimerum (Penz.) Lombard & Crous	5000	2.99	10			
***Chaetomium* Kunze**	**1000**	**0.6**	**15**			
C. globosum Kunze	400	0.24	10			
C. piluliferum J. Daniels	600	0.36	5			
Cochliobolus dactyloctenii Alcorn (1982)				300	0.34	5
Cosmospora butyri (Beyma) Gräfenhan, Seifert & Schroers				600	0.68	10
***Cunninghamella* Matr**	**400**	**0.24**	**10**	**2000**	**2.25**	**25**
C. echinulata Thaxt	200	0.12	5	1000	1.13	5
C. elegans Lendn.				1000	1.13	10
C. phaeospora Boedijn	200	0.12	5			

Fungal species	Wheat-soil			Pepper-soil		
	CFU	**%CFU**	**%F**	**CFU**	**%CFU**	**%F**
***Curvularia* Boedijn**	**600**	**0.36**	**10**	**5000**	**5.62**	**50**
C. lunata (Wakker) Boedijn	600	0.36	10	200	0.23	5
C. hawaiiensis (Bugnic. Ex M.B. Ellis) Manamgoda				200	0.23	10
C. spicifera (Bainier) Boedijn				4600	5.17	45
Exserohilum holmii (Luttr.) Leonard				200	0.23	10
***Fusarium* Link**	**22800**	**13.64**	**60**	**1500**	**1.69**	**20**
F. chlamydosporum Wollenw. & Reinking	4800	2.87	5			
F. incarnatum (Desm.) Sacc.	400	0.24	10	800	0.9	15
F. lateritium Nees	1400	0.84	5	700	0.79	5
F. oxysporum Schltdl.	15600	9.33	50			
F. pseudocircinatum O'donnell & Nirenberg	200	0.12	5			
F. udum E.J. Butler	400	0.24	5			
Gibberella nygamai Klaasen & Nelson	8200	4.90	10			
Haplotrichum croceum (Mont.) Partr. & Morgan-Jones				400	1.12	15
***Humicola* Traaen**				**400**	**0.45**	**10**
H. fuscoatra Traaen				300	0.34	5
H. grisea Traaen				100	0.11	5
Kernia sp. Nieuwl.	600	0.36	5			
Melanopsamma pomiformis (Pers.) Sacc.	200	0.12	5			
***Mucor* Fresenius**	**2800**	**1.67**	**35**			
M. circinellioides Tiegh.	600	0.36	5			
M. hiemalis Wehmer	2200	1.32	35			
Neocosmospora solani (Mart.) L. Lombard & Crous	1000	0.60	10	7200	8.04	45
Papulaspora irregularis Hotson				100	0.11	5
***Penicillium* Link**	**64000**	**38.28**	**90**	**100**	**0.11**	**5**
P. aurantiogriseum Dierckx	2200	1.32	10			
P. brevicompactum Dierckx	7600	4.55	20			
P. chrysogenum Thom	29200	17.46	50			
P. griseofulvum Dierckx	11400	6.82	15			
P. oxalicum Currie & Thom				100	0.11	5
P. waksmanii K.W. Zaleski	13600	8.13	30			
Plectospherella sp.				300	0.34	5
***Rhizopus* Ehrenb.**	**400**	**0.24**	**10**	**300**	**0.34**	**10**
R. arrhizus A. Fisch.				300	0.34	10
R. stolonifer (ehrenb.) Vuill.	400	0.24	10			
Sarocladium strictum (W. Gams) Summerbell				300	0.34	5
***Scopulariopsis* Bainier**				**10600**	**11.92**	**15**
S. brumptii Salv. -Duval				300	0.34	5
S. coprophila (Cooke & Massee) W. Gams				300	0.34	5
S. fusca Zach				10300	11.59	10
Septonema ochraceum Matsush	200	0.12	5			
***Stachybotrys* Corda**	**200**	**0.12**	**5**	**4700**	**5.3**	**15**

Fungal species	Wheat-soil			Pepper-soil		
	CFU	**%CFU**	**%F**	**CFU**	**%CFU**	**%F**
S. chartarum (Ehrenb.) S. Hughes	200	0.12	5			
S. havanensis Mercado & J. Mena				100	0.11	5
S. palmijunci Rifai				3200	3.6	10
S. sphaerosporus Morgan-Jones & R.C. Sinclair				700	0.79	5
S. variabilis H.F. Wang & T.Y. Zhang				200	0.23	5
Stachybotrys sp.				500	0.56	10
Talaromyces pinophilus Samson, Yilmaz, Frisvad & Seifert				400	0.45	5
Dark sterile mycelia				300	0.34	5
Total CFUs (256700)	**167200**			**89500**		
No. of genera (31)	**16**			**23**		
No. of species (77)	**35**			**52**		

CFU = Colony-forming Units.
% F (Frequency of occurrence)**:** 50-100% = high, 25-50% = moderate, < 25% = low frequency

3.2. Screening of phosphate-solubilizing potentiality

The abilities of two hundred and seven fungal isolates to solubilize phosphate were tested on Pikovskaya's agar medium. Based on the phosphate solubilization index (PSI) measurements, 31.0% of total isolates (62 isolates) showed positive results. Of which, 14 were high (PSI >2.7), 20 were moderate (PSI >2.2 to 2.7), and 28 were low phosphate-solubilizers. The highest phosphate-solubilizers were identified morphologically as *P. chrysogenum*, *A. niger*, *P. chrysogenum*, *A. brasiliensis,* and *A. lacticoffeatus*, recording PSI of 3.84, 3.74, 3.48, 3.17, and 3.11, respectively. These strains were deposited in the culture collection of Assiut University Mycological Centre with accession numbers *P. chrysogenum* AUMC 14100, *A. niger* AUMC 14260, *P. chrysogenum* AUMC 14262, *A. brasiliensis* AUMC 14261, and *A. lacticoffeatus* AUMC 14257, and they were selected for the quantification of solubilized phosphate in liquid medium (Table 2; Figure 1).

Regarding phosphate solubilization for 200 rhizosphere fungal isolates tested in the present study, only 62 isolates comprising 31.0% of total isolates showed positive results on solid medium. The variability of different isolates in phosphate solubilization efficiency may be attributed to the concentrations and rates of different organic acids produced by different fungal isolates [32]. Many fungal genera such as *Aspergillus*, *Penicillium*, and *Trichoderma* were recorded as phosphate solubilizers. They could be used to improve plant growth by synthesizing protons and secretion of organic acids, which are the significant contributors to the solubilization of metal compounds that lower the pH to dissolve bound phosphates in soil [33,34]. In this respect, 16 out of 290 yeast isolates collected from teff rhizosphere soil in Ethiopia showed positive results [35].

In the current study, five potent phosphate-solubilizing strains were recorded, namely *P. chrysogenum* AUMC 14100 and AUMC 14262, *A. niger* AUMC 14260, *A. brasiliensis* AUMC 14261, and *A. lacticoffeatus* AUMC 14257. These strains gave PSI of 3.84, 3.74, 3.48, 3.17, and 3.11, respectively. Following these findings, Mahadevamurthy et al. [27] tested 22 rhizosphere fungi for their phosphate-solubilizing abilities and found that only *Penicillium* sp. RF UOM 14 showed positive results. Also, Mendes et al. [36] detected that isolates of *Aspergillus niger* FS1, *Penicillium canescens* FS23, and *Eupenicillium ludwigii* could solubilize all forms of phosphate. Inconsistency with our results, El-Azouni [37] reported the maximum PSI of *A. niger* and *Penicillium italicum* were 3.15 and 2.42, respectively. Similar results were obtained in other studies [9,38]. The current results revealed that the fungal isolates with the highest phosphate solubilization index on PVK solid medium could not show the same trend in PVK broth. These findings were supported by Alam et al. [39] and Elias et al. [38], who reported that some fungal isolates showed a larger clear zone on PVK solid medium and gave low phosphate solubilization abilities in a liquid medium and *vice versa*. These results indicate that the isolates with high PSI do not necessarily give high solubilization efficiency in a liquid medium. Also, Nautiyal [40] estimated that plate technique is not enough for screening the potent phosphate solubilizers.

Phosphate solubilization, production of IAA, and other related compounds by fungi interact with plants as part of its colonization, leading to growth promotion, induced resistance, and modification of basal plant defense mechanisms [41,42]. It was stated that fungi need carbon-rich sources for the active production of organic acids used to solubilize soil-bound phosphate

[43]. Plant root exudates provide these carbon-rich sources, explaining the high potentiality of rhizosphere fungi in phosphate solubilization.

The tested strains could solubilize considerable phosphate concentrations in the current results, reaching 1754.7 µg/mL. *A. brasiliensis* AUMC 14261 showed a significant increase in phosphate solubilization after 10 days of incubation. However, it decreased after 15 and 20 days. Mahamuni et al. [44] noticed a decrease in phosphate solubilization at the end of the incubation period is inconsistent with the current findings. This decrease could have attributed to the accumulation of the soluble phosphate, which may obstruct further solubilization process, or shortage of carbon sources that decrease microbial activity and, as a result, decrease in the production of organic acids [45]. Furthermore, the production of organic phosphate compounds reduced the concentration of solubilized phosphate [46], and the fungal cells consumed soluble phosphate in their growth and metabolism [47].

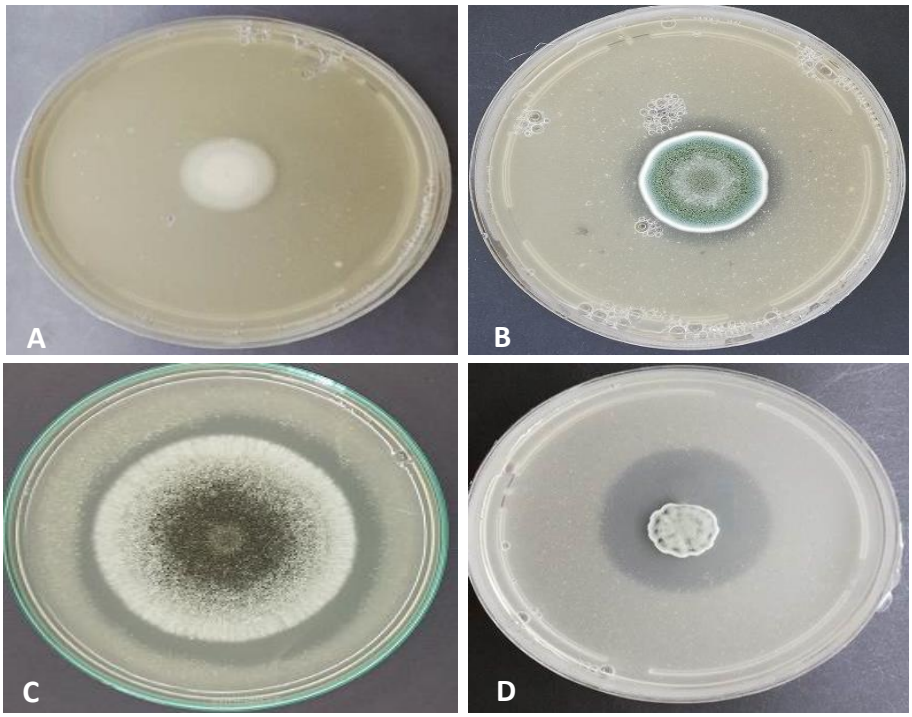

Figure 1. Screening of some fungal species for phosphate solubilization showing: A, negative result; B, weak activity; C, moderate activity, and D, high activity, based on measurements of phosphate solubilization index (PSI).

Table 2. Phosphate solubilization index (PSI) of fungi isolated from rhizosphere soil around wheat and pepper plants cultivated at El-Kharga Oasis, New Valley, monitored Pikovskaya's agar plates at 25±2 °C.

Fungal strains	No. of isolates	H	M	L	Negative
Acremonium rutilum	1				1
Albifimbria verrucaria	1				1
Alternaria tenuissima	1				1
Aspergillus aureolatus	4				4
A. aureoterreus	3			2	1
A. brasiliensis	1	1			
A. chevalieri	1			1	
A. cristatus	3		1		2
A. egyptiacus	2				2
A. flavipes	4			2	2
A. flavus	9			2	7
A. lacticoffeatus	1	1			
A. neoniveus	2			1	1
A. nidulans	8			2	6
A. niger	11	2	2	2	5
A. ochraceous	1			1	
A. parasiticus	3				3
A. quadrilineatus	2				2

Fungal strains	No. of isolates	H	M	L	Negative
A. speluneus	1				1
A. sulphureus	1			1	
A. sydowii	4		3		1
A. terreus	19		1	2	16
A. versicolor	1				1
A. unguis	1				1
Bahusakala olivaceonigra	1				1
Bipolaris colocasiae	1				1
B. sorokiniana	1				1
Bisifusarium dimerum	1				1
Chaetomium globosum	1				1
C. piluliferum	1				1
Cochliobolus dactyloctenii	1				1
Cosmospora butyri	1				1
Cunninghamella echinulata	4				4
C. elegans	2				2
C. phaeospora	1				1
Curvularia lunata	3			1	2
C. hawaiiensis	1				1
C. spicifera	4			1	3
Exserohilum holmii	2				2
Fusarium chlamydosporum	1				1
F. incarnatum	4		1	1	2
F. lateritium	2				2
F. oxysporum	8		1		7
F. pseudocircinatum	1				1
F. udum	1				1
Gibberella nygamai	1				1
Haplotrichum croceum	1				1
Humicola fuscoatra	2				2
H. grisea	1				1
Melanopsamma pomiformis	1				1
Mucor circinellioides	1				1
M. hiemalis	8				8
Neocosmospora solani	11		1		10
Papulaspora irregularis	1				1
Penicillium aurantiogriseum	2	2			
P. brevicompactum	4	1	2		1
P. chrysogenum	11	5	3	2	1
P. griseofulvum	5	1	3	1	
P. oxalicum	1			1	
P. waksmanii	5	1	2	2	
Plectospherella sp.	1				1
Rhizopus arrhizus	1				1
R. stolonifer	2				2
Sarocladium strictum	1				1
Septonema ochraceum	1				1
Scopulariopsis brumptii	1				1
S. coprophila	1				1
S. fusca	1				1
Stachybotrys chartarum	1				1
S. havanensis	1				1
S. palmijunci	1				1
S. sphaerosporus	1			1	
S. variabilis	1				1
Stachybotrys sp.	1				1
Talaromyces pinophilus	1			1	
Yunnania carbonaria	1			1	
Dark sterile mycelia	1				1
Total	**200**	**14**	**20**	**28**	**138**

H = high (> 2.7); **M** = moderate (>2.2-2.7); **L** = Low (>1-2.2); **negative** = 1

3.3. Assessment of phosphate-solubilizing potentiality

The phosphate-solubilizing potentiality of the most potent five strains *A. brasiliensis* AUMC 14261, *A. lacticoffeatus* AUMC 14257, *A. niger* AUMC 14260, and *P. chrysogenum* AUMC 14100 and AUMC 14262 was assayed *in vitro* using liquid Pikovskaya's medium. The current results revealed that all tested strains could significantly solubilize the insoluble phosphate in

the broth medium with a higher activity than control (P≤0.01). Phosphate solubilization potentiality of *A. niger* AUMC 14260 and *P. chrysogenum* AUMC 14100 and AUMC 14262 increased to the maximum levels (1754.7, 1371.8, and 1242.2 µg/mL respectively) after 20 days of incubation, while reached the highest values in *A. brasiliensis* AUMC 14261 and *A. lacticoffeatus* AUMC 14257 after 10 days (Table 3). The five strains showed a significant decrease in pH of the medium compared with control (P≤0.01) along the 20 days of incubation. The highest decrease in pH value (from 7.0 to 3.16) was recorded in *A. niger* after 10 days, while the maximum pH decrease for *P. chrysogenum* and *A. lacticoffeatus* was recorded after 15 days (from 7.0 to 6.28 and from 7.0 to 5.027 respectively), then the pH values slightly increased by 20 days (6.28 to 6.38 and 5.027 to 5.197) respectively (Table 3). In the present study, *Aspergillus niger* AUMC 14260 recorded the highest drop in pH values from initial pH = 7.0 to 3.16 after 10 days of incubation, while *P. chrysogenum* and *A. lacticoffeatus* recorded the maximum pH decrease after 15 days and increased after 20 days. The most known mechanism of phosphate solubilization is through the formation of organic acids, which are associated with a reduction in pH of the medium; after that, stability or increasing pH occurred with decreasing solubilization rate.

Table 3. Mean concentrations of solubilized phosphate (µg/mL) ± SD, after 5, 10. 15 and 20 days of incubation of fungal strains at 25±2 °C on PVK broth medium.

Fungal strains	After 5 days		After 10 days		After 15 days		After 20 days	
	pH	P (µg/mL)	pH	P (µg/mL)	pH	P (µg/mL)	pH	P (µg/mL)
Control	7.000^{a}±0.01	359.3^{e}±2.52	7.000^{a}±0.02	408.0^{e}±3.25	7.000^{a}±0.02	425.3^{d}±4.53	7.000^{a}±0.03	433.9^{d}±4.53
A. brasiliensis AUMC 14261	5.397^{c}±0.09**	1010.2^{a}±26.9**	5.193^{d}±0.06**	1745.8^{a}±101.8**	5.307^{c}±0.03**	1413.1^{ab}±152.3**	5.210^{c}±0.04**	1450.2^{b}±74.1**
A. lacticoffeatus AUMC 14257	5.143^{d}±0.13**	905.8^{b}±46.8**	5.123^{d}±0.11**	1679.1^{a}±138.8**	5.027^{d}±0.05**	1570.7^{a}±188.7**	5.197^{c}±0.08**	1673.3^{a}±65.7**
A. niger AUMC 14260	3.783^{e}±0.08**	913.6^{b}±49.5**	3.160^{e}±0.07**	1265.8^{b}±40.7**	4.653^{e}±0.07**	1478.7^{ab}±134.4**	4.980^{d}±0.06**	1754.7^{a}±96.1**
P. chrysogenum AUMC 14262	6.830^{b}±0.08**	645.8^{d}±31.5**	6.600^{c}±0.17**	701.3^{d}±66.7**	6.380^{b}±0.09**	1157.8^{c}±141.6**	6.350^{b}±0.06**	1242.2^{c}±99.0**
P. chrysogenum AUMC 14100	6.867^{ab}±0.06	823.6^{c}±69.4**	6.793^{b}±0.09**	1121.3^{c}±40.6**	6.280^{b}±0.07**	1229.8^{bc}±119.3**	6.383^{b}±0.10**	1371.8^{bc}±61.7**
F-test sig.	**	**	**	**	**	**	**	**

SD: Standard Deviation, *: Significant (P≤0.05), **: Significant (P≤0.01). Means followed by the same letter within the same column are not significantly different at 0.05 level of probability.

4. Conclusion

The current study met its primary objective in isolating and identifying fungal genera and species associated with the roots of both wheat and pepper plants and their phosphate solubilization potentiality, 30 genera, and 77 species collected. However, only 62 isolates belonging to 8 genera and 28 species could solubilize phosphate in PVK agar medium. This investigation could offer 5 fungal isolates related to *Aspergillus* and *Penicillium* species that candidate as highly phosphate solubilizers in both solid and liquid medium; consequently, they promised to be applied in agriculture as alternative biofertilizers after their studies *in vivo* for enhancing plant growth.

Conflicts of interest. The authors declare that there is no conflict of interest.

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