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Isolation and Characterization of Phosphate Solubilizing Fungus in *Vitro*

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ABSTRACT

Series of laboratory (in *vitro*) examinations were carried out to recognize the most potent fungi isolates and optimum culture conditions helps in solubilizing sparing phosphate in soil. Therefore, the present study aimed to isolate and screening of phosphate solubilizing fungi (PSF), study the contribution of PSF on solubilization of tri-calcium phosphate through excretion of acid and alkaline phosphatase and determine the optimal conditions for phosphate solubilization. From 12 fungal isolates, only four were chosen depending on their capacity to convert tri-calcium – P into soluble one. In this regard, *Aspergillus niger*, *Aspergillus flavus, Aspergillus ficuum* and *Fusarium oxysporum* were efficient in producing halo zone around the colonies on petri dish. *Aspergillus niger* was the most efficient phosphate solubilizer on Pikovskaya's agar plates (PVK) achieving solubilization index (PSI) 2.4, followed by *Aspergillus flavus* with a SI= 2.3. The perfect activities of selected fungal isolates were detected with incubation periods 7 days and temperature 30°C for maximum phosphate solubilization, acid and alkaline phosphatase activities, pH 7.0 for maximum phosphate solubilization, pH 6.0 for maximum acid phosphatase activity while pH 8.0 was suitable for maximum alkaline phosphatase activity for both *A. niger* and *A.flavus*.

KEYWORDS

Phosphate Solubilization, Tri-Calcium Phosphate, P – Ase Enzyme, P-Solubilizing Fungi (PSF).

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INTRODUCTION

Phosphorus (P) plays an essential biochemical role in photosynthesis, respiration, energy storage and transfer, cell division, cell vastness and some other processes in the living plant **(Sagervanshi *et al*.,2012)** . Egyptian soils have low available phosphorus that cannot meet the demands of plant **(Reghab *et al*., 2010)**. Due to limited availability of chemical –P fertilizers under alkaline conditions **(Podile and Kishore, 2006)**, the fungi could be used as safe, less expensive and ecofriendly agents that helps in converting insoluble –P into soluble one **(Coutinho *et al*., 2012)**. Maize yield and P uptake were enhanced by *Aspergillus* inoculation **(Xiao *et al*., 2013)**.

Organic P compounds undergo mineralization, and the resulting P is taken up as nutrient by plants. In this regard, numerous soil microbes or rhizosphere microflora possess the ability to transform organic P into soluble forms of P **(Rodriguez *et al*., 2006)**. This mineralization process is mediated by the enzymes especially phosphatases **(Aseri *et al*., 2009)** and phytases **(Maougal *et al*., 2014)**, released by the soil microbes. **Richardson and Simpson (2011)** pointed out that, A wide domain of fungi (e.g. *Aspergillus* and *Penicillium* spp. and bacteria (e.g. *Actinomycetes, Pseudomonas,* and *Bacillus* spp) produce the enzymes involved in organic P-mineralization. Phosphorus mineralization is an enzymatic process, involving phosphatases, which catalyze a variety of reactions capable of releasing phosphate from organic phosphorus compounds into the soil solution; phosphatases are released by microbes extracellular into the soil solution. Mineralization of organic phosphate affected by several factors such as temperature (the optimum being between 18°C to 40°C), soil pH (the optimum occurring at soil pH 6.5), moisture, aeration, type of crop cultivated, the presence of growing plants, and the addition of fertilizer phosphate **(Hyland *et al*., 2005)**. Microorganisms play a fundamental role in the biogeochemical cycling of phosphorus in natural ecosystems. As phosphate solubilization is a prime process for plant growth, the importance of phosphate solubilizing microorganisms is well recognized **(Velazquezand Rodriguez-Barrueco, 2007)**. Inacbation periods, temperatures and pH are vital factors for various activities of microorganisms. Finally, correlations were developed between phosphate solubilization potential and associated factors, namely, incubation periods, temperatures and pH.

The present study aimed to isolate and recognize the most potent fungi that have the highest activity under the examined optimum conditions (in *vitro*).

MATERIALS AND METHODS

Collection of soil samples

Soil samples were collected from different locations, i.e., Kulj (Great Cairo), Ras Sidr (South Saini Governorate), and Inshas (Sharkia Governorate). Soil sample was collected from the upper surface layer (0-15 cm). The soil samples were transferred to the laboratory in sterile polyethylene bags under aseptic conditions, and reserved in refrigerator at 5 °C. Air dried soil was sieved through a 2-mm sieve.

Isolation of fungal species

Fungal strains were isolated from different soil samples on **Dox's agar medium (Dox, 1910) (g/l):** Sucrose, 20.0g; $NaNO_3$, 2.0g; KH_2PO_4, 1.0g; $MgSO_4.7H_2O$, 0.5g; KCl, 0.5g; $FeSO_4.5H_2O$, 0.001g; agar, 15.0g . 10 g of each soil sample was added to 90ml of 0.85 % w/v of physiological saline. Ten – fold dilution was made to drop 1ml of (10^{-1}-10^{-7}) on plates containing Dox's agar medium. The plates were spread and incubated at 28°C for 7 days. After incubation period, growing fungal isolates were purified by repeated culturing and maintained on slants at 4°C to be ready for identification.

Isolates identification

Fungal isolates were Qualitatively identified by studying the cultural characters, i.e. colour, shape of colony, surface and reverse pigmentation, growth rate and texture of the colony as well as the microscopically structure (septate or non- septate hyphae, structure of hyphae and conidia) on Sabouraud's dextrose agar medium. Fungal isolates were identified according to **Pitt (1979 and Pitt and Hocking (1985 and 1997)** by Microbiology Department, Faculty of Science, Ain Shams University.

Screening of P-solubilizing Fungal isolates

Qualitative method:

Twelve fungal isolates were screened on selective **Pikovskaysa's agar medium (PVK) (*Pikovskaysa*,1948) (g/l):** 0.5g $(NH_4)_2SO_4$, 0.5g $MgSO_4.7H_2O$, 0.3g NaCl, 0.3g KCl, 0.03g $FeSO_4.7H_2O$, 0.02g $MnSO_4.H_2O$, 10.0g $Ca_3(PO_4)_2$, 10.0g Glucose and 15.0g Agar. Sterilized Pikovskaya's medium was poured into sterilized Petri dishes, after solidification on the dishes; a pinpoint inoculation of fungal strains was placed on the center of plates under aseptic conditions. The dishes were incubated at 28°C for 7 days. Solubilization index (SI) was calculated using the following equation **(Edi – premono, 1996)**.

$$\text{Solubilizing index} = \frac{\text{Colony diameter+ Halo zone diameter}}{\text{Colony diameter}}$$

Quantitative method:

Fungal isolates with high ratio value of clear zone and maximum phosphate solubilization index (PSI) were used to solubilize tri-calcium phosphate (Quantitative method). Each flask was inoculated with 1 ml of spore suspension (1.0×10^6 spores/ml) of selected fungi. The flasks were incubated at 28 °C with rotary sheker at 200 rpm for 7 days. The cultures were harvested by filtration with Whatman paper No. 42. The supernatants were analyzed for available phosphate according to **Olsen *et al.* (1954)** and alkaline and acid phosphatase activity was estimated as described by **Tabatabai and Bremner (1969)**.

Optimization of culture conditions for phosphate solubilization

Sterilized PVK broth medium was exposed to different incubation periods, temperature and pH. The un-inoculated autoclaved medium with phosphate substrate was incubated under similar conditions to serve as controls to all investigated parameters.

A set of triplicate culture flasks for each fungal isolate were established, each containing 50 mL of sterilized PVK broth medium at pH 7.0. Each flask was inoculated with 1 ml of (10^6 spores) of most potent fungal isolates (*A.niger* and *A.flavus*) for phosphatase enzymes production and incubated at 28°C at different incubation periods (3, 5, 7, 9, 11 and 14 days), respectively. Similar set of culture flasks were exposed to different temperatures of 20, 25, 30, 35, 40 and 45 °C.

In addition, the PVK broth medium was individually adjusted at different values of pH by using 6N HCL or 6N NaOH. The medium was inoculated with 1 ml of (10^6 spores) for both selected *A.niger* and *A.flavus*, and then were incubated at 30°C for 7 days.

Statistical Analysis: Experiments were carried out in triplicates. Statistical analysis was performed with Microsoft excel 2007. Difference on statistical analysis of data were considered Significant at $P<0.05$. Comparisons between results were based on least significant difference (LSD) values derived from the analysis of variance. Tests with $p\leq0.05$ were considered statistically significant **(Snedcor and Cochran, 1980)**.

RESULTS

Isolation of fungal species

Twelve fungal isolates were obtained as a result of the isolation and purification processes. Each fungal isolate was identified based on morophological features.

These fungi are nine *Aspergillus* species, one *Fusarium* species, one *Penicillium* species and one *Alternaria* species.

Screening of P-solubilizing fungi

Qualitative Method:

Twelve fungal isolates of *Aspergillus niger, A. flavus, A. japonicas, A. terricola, A. terreus, A. ficuum, A. flavipes, A. awamori, A. fumigatus, Fusarium oxysporum, Pencillium oxalicum* and *Alternaria alternata* were assessed for phosphate solubilization Plate method on the basis of a clearance of zone on PVK medium at 28 °C for 3 days was observed **(Table 1)**.

From the 12 isolated fungi, only four showed a positive zone of P solubilization on PVK agar medium. Halo zone around fungal colonies was appeared **(Fig. 1)**. The four isolates that displayed the highest ratio of clear zone / colony diameter were selected and considered as the more efficient P solubilizing fungi (PSF). The zone of P solubilization appeared on the third day of incubation. Continuous observation of the halo zone formation indicates phosphate solubilizing ability which was in increasing up to the 7 days of inoculation. Zone diameter was 7.2, 7.8, 6.3 and 3.8 cm for *A. niger*, *A. flavus*, *A. ficuum* and *F. oxysporum,* respectively **(Table 2)**.

Table (1) : *Screening of fungi for tri-calcium phosphate solubilization [Positive means halo zone formation around the colony].*

Isolate No.	Name of isolated fungi	TCP Solubilization
1	*Aspergillus niger*	**Positive**
2	*Aspergillus flavus*	**Positive**
3	*Aspergillus japonicas*	ND
4	*Aspergillus terricola*	ND
5	*Aspergillus terreus*	ND
6	*Aspergillus ficuum*	**Positive**
7	*Aspergillus flavipes*	ND
8	*Aspergillus awamori*	ND
9	*Aspergillus fumigatus*	ND
10	*Fusarium oxysporum*	**Positive**
11	*Penicillium oxalicum*	ND
12	*Alternaria alternate*	ND

ND not detected

Table (2) : *Estimation of solubilization index of tested fungal isolates.*

Fungal isolates	Colony diameter (cm)	Halo zone diameter(cm)	Solubilization index (SI)
Aspergillus niger	5.1	7.2	2.4
Aspergillus flavus	5.8	7.8	2.3
Aspergillus ficuum	6.6	6.3	2.1
Fusarium oxysporum	3.1	3.8	2.2
LSD $_{(0.05)}$	**0.05**	**1.32**	

Calculated means is for triplicate measurements ± SD

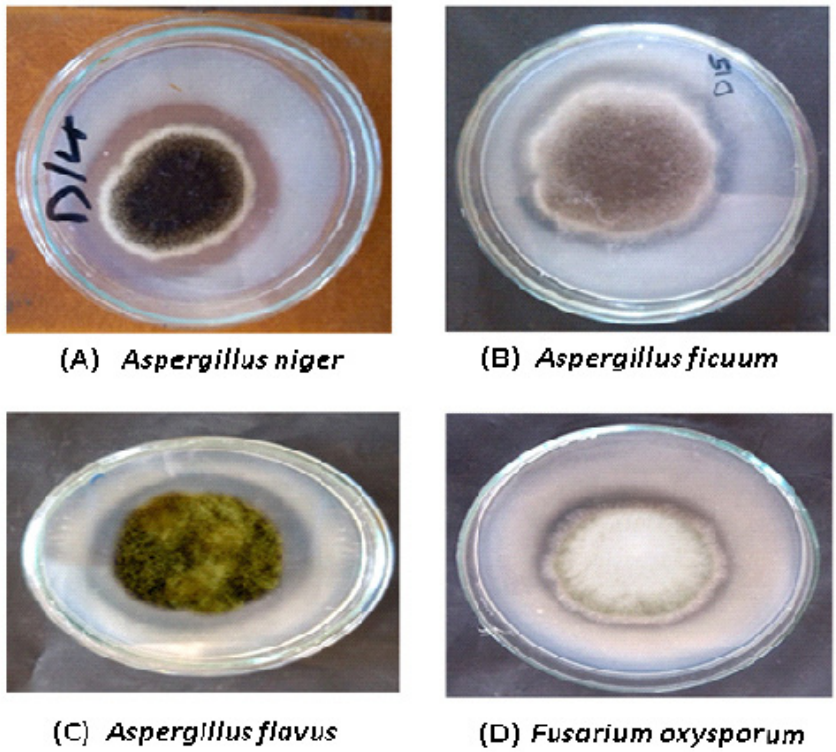

Fig. (1): Clearing zones around colonies of (A) Aspergillus niger, (B) Aspergillus ficuum, (C) Aspergillus flavus, (D) Fusarium oxysporum.

Quantitative Assay

Among four selected strains, *A.niger* was the most efficient solubilizer, where SI= 2.4, followed by *A. flavus, A.ficuum* and *F. oxysporum* **(Table 2)**. According to its high SI, *A.niger* and *A.flavus* were selected for further examinations on qualitative and quantitative scales. *A.niger* achieved 73.8 µg/ml soluble -P followed by *A. flavus* (72.6 µg/ml). Acid phosphatase enzyme activity of 30.0 µg/ml, 23.5 µg/ml, 17.5 µg/ml and 12.6 µg/ml were detected by *A. niger*, *A. flavus*, *A. ficuum* and *F. oxysporum*, respectively. While alkaline phosphatase enzyme activities were 45.7 µg/ml, 43.8 µg/ml, 34.7 µg/ml and 21.5 µg/ml for the same sequence **(Table 3).**

Table (3) : *Acid and alkaline phosphatase activities of fungal isolates.*

Fungal Isolates	Soluble phosphate (µg/ml)	Acid phosphatase Activity (µg/ml)	Alkaline phosphatase activity (µg/ml)
A. niger	73.8	30.0	45.7
A. flavus	72.6	23.5	43.8
A. ficuum	73.5	17.5	34.7
F. oxysporum	50.4	12.6	21.5
LSD (0.05)	**3.25**	**1.54**	**1.51**

Calculated means is for triplicate measurements ± SD

Effect of incubation periods on P solubilization .

Increasing phosphate solubilization was increased at fifth day of incubation period and reached to the maximum after 7 days **(Table 4)**. The Maximum phosphate availability by *A. niger* and *A. flavus* (56.53 and 43.23 µg/ml), respectively were observed after 7 days followed by 9 days (54.16 and 42.26 µg/ml), respectively as compared to un-inoculated treatment. A decline occurred in PS from 9 days until the end of incubation period.

Table (4) : *Effect of different incubation periods on phosphate solubilization (µg/ml) released by A. niger and A. flavus.*

Phosphate solubilization (µg/ml)							
Fungal sp.	**Incubation periods (days)**						**Mean**
	3	**5**	**7**	**9**	**11**	**14**	
Un-inoculated	8.45	8.60	10.10	9.57	9.46	10.18	**9.39**
A. niger	33.20	41.13	56.53	54.16	52.46	50.53	**48.00**
A.flavus	24.86	37.13	43.23	42.56	40.73	32.53	**36.67**
Mean	**22.17**	**28.95**	**36.62**	**35.43**	**34.22**	**31.08**	
LSD 0.05: T: 0.39 ; F: 0.28 ; T*F:0.68							

Calculated means is for triplicate measurements ± SD

*Notes: T: treatment ; F: fungi and TF : treatment * fungi*

Effect of incubation periods on phosphatase activity

Maximum production of acid phosphatase enzyme by *A. niger* and *A. flavus* (46.70 and 41.20 µg/ml) were obtained after 7 days followed by 9 days (32.30 and 32.26 µg/ml respectively as compared to control (0.95 µg/ml **(Table 5)**. At the same time, the maximum alkaline phosphatase enzyme production by *A. niger* and *A. flavus* (67.13 and 53.13 µg/ml), respectively were observed at 7 days followed by 9 days (45.86 and 43.70 µg/ml) respectively.

Table (5) : *Effect of different incubation periods on acid and alkaline phosphatase activities (µg/ml) induced by A. niger and A. flavus.*

Acid phosphatase activity (µg/ml)							
Fungal sp.	**Incubation periods (days)**						**Mean**
	3	**5**	**7**	**9**	**11**	**14**	
Un-inoculated	0.93	0.94	0.95	0.92	0.91	0.90	**0.92**
A. niger	13.16	22.70	46.70	32.30	23.63	18.50	**26.16**
A.flavus	12.60	32.26	41.20	32.26	21.66	18.06	**26.34**
Mean	**8.89**	**18.63**	**29.62**	**21.82**	**15.40**	**12.48**	
$LSD_{0.05}$: T: 0.56 ; F: 0.39 ; T*F:0.97							
Alkaline phosphatase activity (µg/ml)							
Un-inoculated	0.92	0.93	0.94	0.91	0.90	0.90	**0.91**
A. niger	18.80	38.60	67.13	45.86	42.86	38.86	**36.18**
A. flavus	14.33	39.36	53.13	43.70	35.13	24.13	**34.96**
Mean	**11.35**	**26.29**	**40.40**	**30.15**	**26.29**	**21.29**	
$LSD_{0.05}$: T:1.43 ; F: 1.01 ; T*F:2.48							

Calculated means is for triplicate measurements ± SD

*Notes: T: treatment ; F: fungi and TF : treatment * fungi*

Effect of different temperatures on phosphate solubilization

The maximum phosphate solubilization occurred at 30°C by *A. niger* and *A. flavus* (58.53 and 48.50 µg/ml) compared to un-inoculated ones (10.42 µg/ml).A decrease in P solubilization occurred at temperatures above or below 30°C. It was noticed that both *A. niger* and *A. flavus* were able to grow at temperature higher than 40°C but produce lower available P. Maximum phosphate solubilization by *A. niger* and *A. flavus* (58.53 and 48.50 µg/ml), respectively were observed at 30°C followed by 35°C (48.16 and 44.63 µg/ml), 25°C (46.26 and 35.86 µg/ml), 40°C (39.23 and 40.80 µg/ml), 20°C (34.16 and 28.90 µg/ml) and 45°C (32.26 and 31.66 µg/ml), respectively as compared to un-inoculated treatments (8.20, 8.52, 10.42, 10.12, 9.32, 9.02 at 20, 25, 30, 35, 40 and temperature 45°C, respectively **(Table 6)**.

Table (6) : *Effect of different temperatures on phosphate solubilization (µg/ml) produced by A. niger and A. flavus.*

Phosphate solubilization (µg/ml)							
Fungal sp.	**Temperatures (˚C)**						**Mean**
	20	**25**	**30**	**35**	**40**	**45**	
Un-inoculated	8.20	8.52	10.42	10.12	9.32	9.02	**9.26**
A. niger	34.16	46.26	58.53	48.16	39.23	32.26	**43.10**
A.flavus	28.90	35.86	48.50	44.63	40.80	31.66	**38.39**
Mean	**23.75**	**30.22**	**39.15**	**34.30**	**29.78**	**24.31**	
$LSD_{0.05}$: T: 0.78; F: 0.55; T*F: 1.35							

Calculated means is for triplicate measurements ± SD

*Notes: T: treatment ; F: fungi and TF : treatment * fungi*

Effect of different temperatures on phosphatase activity.

In general, the maximum production of acid and alkaline phosphatase activities was assayed at 30°C **Table (7)**. Then a decrease occurred at temperatures either above or below 30°C. Maximum productions of acid phosphatase enzyme by *A. niger* and *A. flavus* (41.23 and 39.80 µg/ml), respectively were observed at 30°C followed by 35°C (22.0 and 31.63 µg/ml), respectively as compared to un-inoculated treatments (0.74 µg/ml). While the maximum productions of alkaline phosphatase enzyme by *A. niger* and *A. flavus* (55.56 and 46.83 µg/ml), respectively were obtained at 30°C followed by 35°C (38.50 and 37.96 µg/ml), respectively as compared to un-inoculated treatments (0.74 µg/ml).

Table (7) : *Effect of different temperatures on acid and alkaline phosphatase activity (µg/ml) released by A. niger and A. flavus.*

Acid phosphatase activity (µg/ml)							
Fungal sp.	**Temperatures (˚C)**						**Mean**
	20	**25**	**30**	**35**	**40**	**45**	
Un-inoculated	0.73	0.74	0.74	0.72	0.70	0.70	**0.72**
A. niger	15.00	22.30	41.23	22.00	19.06	15.50	**22.51**
A. flavus	11.13	24.70	39.80	31.63	26.30	21.80	**25.89**
Mean	**8.95**	**15.91**	**27.25**	**18.11**	**15.35**	**12.66**	
$LSD_{0.05}$: T: 0.73 ; F: 0.52 ; TF:1.27							
Alkaline phosphatase activity (µg/ml)							
Un-inoculated	0.73	0.74	0.75	0.72	0.72	0.71	**0.72**
A. niger	19.73	27.60	55.56	38.50	24.16	17.03	**30.43**
A.flavus	18.26	34.80	46.83	37.96	32.50	30.03	**33.39**
Mean	**12.90**	**21.04**	**34.38**	**25.72**	**19.12**	**15.92**	
$LSD_{0.05}$: T:1.30; F: 0.92; TF:2.25							

Calculated means is for triplicate measurements ± SD

*Notes: T: treatment ; F: fungi and TF : treatment * fungi*

Effect of pH on P solubilization

Maximum phosphate solubilization by *A. niger* and *A. flavus* (92.33 and 92.66 µg/ml) were observed at pH 7 followed by pH 6 (91.33 and 80.56 µg/ml) as compared to corresponding un-inoculated treatments **(Table 8)**.

Table (8) : *Effect of different pH on phosphate solubilization (µg/ml) induced by A. niger and A. flavus.*

Phosphate solubilization (µg/ml)							
fungal sp.	pH						Mean
	4	5	6	7	8	9	
Un-inoculated	9.96	10.33	10.45	11.48	9.83	8.79	**10.14**
A. niger	81.36	83.10	91.33	92.33	79.20	65.50	**82.13**
A. flavus	71.06	71.80	80.56	92.66	66.76	50.43	**72.21**
Mean	**54.131**	**55.078**	**60.786**	**65.493**	**51.933**	**41.576**	
$LSD_{0.05}$: T: 0.75; F: 0.53; TF:1.30							

Calculated means is for triplicate measurements ± SD

*Notes: T: treatment ; F: fungi and TF : treatment * fungi*

Effect of pH on phosphatase activity.

Maximum acid phosphatase activity by *A. niger* and *A. flavus* was detected at pH 6 (83.20 and 69.16 µg/ml). A decline in acid P-ase occurred from pH 7 to pH 9, while the maximum production of alkaline P-ase activity accounted for *A. niger* and *A. flavus* (90.03 and 70.13 µg/ml) was at pH 8 compared to control treatment (0.8 µg/ml). A decline in phosphatase occurred at pH 6 to pH 4 **(Table 9)**.

Table (9) : *Effect of different pH on acid and alkaline phosphatase activity (µg/ml) produced by A. niger and A. flavus.*

Acid phosphatase activity (µg/ml)							
Fungal sp.	pH						Mean
	4	5	6	7	8	9	
Un-inoculated	0.84	0.85	0.86	0.83	0.82	0.80	**0.83**
A. niger	46.10	57.23	83.20	77.33	74.46	58.36	**66.11**
A. flavus	39.26	42.93	69.16	64.53	51.30	51.43	**53.10**
Mean	**28.73**	**33.67**	**51.07**	**47.56**	**42.19**	**36.86**	
$LSD_{0.05}$: T: 0.46; F: 0.32; TF: 0.79							
Alkaline phosphatase activity (µg/ml)							
Un-inoculated	0.84	0.86	0.87	0.88	0.84	0.83	**0.85**
A. niger	56.33	69.36	82.80	88.86	90.03	81.50	**78.15**
A. flavus	51.53	57.36	58.86	60.16	70.13	74.26	**62.05**
Mean	**36.23**	**42.52**	**47.51**	**49.96**	**53.66**	**52.19**	
$LSD_{0.05}$: T: 3.09; F: 2.18; TF: 5.35							

Calculated means is for triplicate measurements ± SD

*Notes: T: treatment ; F: fungi and TF : treatment * fungi*

DISCUSSION

Hefnawy *et al.* (2014) found that out of all 20 fungi isolated from the salinity soils, only two fungi showed significant zone of P solubilization (*Penicillium oxalicum* and *Penicillium expansum*). **Yasser *et al.* (2014)** reported that *Aspergillus, Penicillium*

and *Trichoderma* were the most important PSF genera isolated from different localities and different habitats in Beni-suef governorate, after 4 days of incubation PVK agar plate, these results are in agreement with our results. At the same time **Gupta *et al.* (2007)** found that the continuous observation of the halo zone was in increasing order up to the 7th day. **Chuang *et al.* (2007)**, **Oniya *et al.* (2015) and Verma and Ekka (2015)** also isolated P-solubilizing fungi such as *A. niger* and *Penicillium spp* from various rhizospheric soil samples.

***El-Azounil* (2008)** reported that SI of *A. niger* was greater than *P. italicum* and also this kind of comparable results have been reported by many investigators **(Afzal *et al.*, 2005 and Gupta *et al.*, 2007)**. Also, **Iman (2008)** reported that SI of the test PSF strains (*P. italicum* and *A. niger*) were 2.42 and 3.15, respectively. Conversely, **Mahamuni *et al.* (2012)** reported that SI for different fungal strains isolated from sugarcane and sugar beet ranged from 1.13 to 1.59. Also, **Das *et al.* (2013)** reported that among PSM, *Trichoderma asperellum* was most efficient phosphate solubilizer on Pikovskaya's agar plates with SI=1.87, followed by *T. harzianum*, *T. viride, T. citrinoviride* with a SI of 1.79, 1.68 and 1.62 respectively, while, *A. niger* and *A. flavus* showed SI value of 1.58 and 1.52 respectively and the least was noted by *P. funiculosum* with a SI value of 1.41, these results are in agreement with the present study. **Achal *et al.* (2007)** whom reported that the *Aspergillus sp* showed highest available phosphate when tri-calcium phosphate was used and also showed maximum acid phosphatase and alkaline phosphatase. **Iqbal *et al* . (2016)** reported that, the phosphate solubilizing index (PSI) of fives rhizobacterial isolate ranged from 2.33 to 3.17 and maximum PSI was observed by S5. S5 isolates showed lowest available phosphate than S3 and S1. However S5 showed maximum acid phosphatase acid phosphatase and alkaline phosphatase than S3 and S1. **Deepa *et al.* (2010)** found that the acid phosphatase activity of *P. chrysogenum* and *T. viride* were more, they released less amount of phosphorus which is due to the non-specific binding activity of the phosphatase**.** PSF play an important role in phosphate solubilization through extrusion of acid phosphatases and phytases enzymes **(Aseri *et al.*, 2009).**

Pandey *et al.* (2008) who observed themaximum solubilization of phosphate occurring at day 15 of incubation for TCP under controlled conditions. ***Vyas et al.* (2007)** reported that a significant increase with the prolongation of incubation period from 3 to 9 days, followed by a significant decline after 12 days of incubation. **Narsian and Patel (2000)** reported that the maximum release of P from China and Udaipur RPs and Sonrai and Hirapur RPs by *A. aculeatus* after 8 and 14 days of incubation. The decrease in P content with the advance of incubation period could be attributed to the utilization of P by fungal species resulting in the fluctuating levels of P release **(Deepa *et al.*, 2010).**

The optimum incubation temperature for maximum values of P solubilization and maximum production of acid and alkaline phosphatase activities by both *A. niger* and *A. flavus* were at 30 °C .The present finding is in accordance with the study where in 30°C resulted in maximum phosphate solubilization by *A. niger* PSF4 (**Bhattacharya *et al.*, 2015**). Similarly, other workers found 30°C to be best temperature for phosphate solubilization by *A. niger* (**Saber *et al.*, 2009**). Although many researchers reported phosphate solubilization at different temperatures, most of them suggested 25°C to be the optimal temperature for phosphate solubilization (**Sayer and Gadd, 1998**). Higher or lower than optimal temperature, the content of soluble P decreased, the results are in similar to those of **(*Barroso and Nahas*, 2005).**

At pH values higher or lower than the optimal pH, the content of soluble phosphorus decreased (**Saber *et al.*, 2009**). **Xiao *et al.* (2008)** reported that there was remarkable reduction in the content of soluble P at higher or lower than the optimal pH, the

obtained result in this work are in agreement with the other studies.

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مجلة
التقنيات النووية
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عزل وتوصيف للفطريات المذيبة للفوسفات فى المعمل

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أجريت سلسلة من التجارب المخبرية (في المعمل) للتعرف على أكثر عزلات الفطريات فعالية ، والظروف المثلى التى تساعد في اذابه الفوسفات في التربة. ولذلك ، هدفت الدراسة الحالية إلى عزل وفحص الفطريات الفوسفاتية (PSF) ، ودراسة مساهمة هذه الفطريات في إذابة ثلاثي فوسفات الكالسيوم من خلال إفراز الفوسفاتيزالحمضى والقلوى وتحديد الظروف المثلى لاذابه الفوسفات. من ال 12 عزلة فطرية ، تم اختيار أربعة فقط اعتمادا على قدرتها على تحويل ثلاثي فوسفات الكالسيوم إلى فوسفات قابل للذوبان . وفي هذا الصدد ، كان اسبيرجلس نيجر ، اسبيرجلس فلافس ، اسبيرجلس فيكوم وفيوزاريم أوكسيسبوريوم الاكثر فعالية في إنتاج منطقة الهالة حول المستعمرات على طبق بتري.وكان اسبيرجلس نيجر الاكثر فعالية لتحقيق مؤشر الإذابة PSI 2.4 ، يليه اسبيرجلس فلافس مع SI = 2.3 ثم تم الكشف عن الأنشطة المثالية للعزلات الفطرية المختارة وجدت أن الظروف المثلى عند فترة تحضين 7 ايام وعند درجه حراره 30 درجة مئوية لإنتاج أعلى قيم للفوسفات المذاب وافراز انزيم الفوسفاتيز الحمضي والقلوي واظهرت النتائج ايضا ان الرقم الهيدروجيني 7 اعطى اعلى قيمه للفوسفات المذاب وعند الرقم الهيدروجيني 6 اعطى أعلى قيمه لإفراز انزيم الفوسفاتيز الحمضي اما الرقم الهيدروجيني 8 فقد اعطى أعلى قيمه لإفراز انزيم الفوسفاتيز القلوي لكلا من اسبيرجلس نيجر واسبيرجلس فلافس.

الكلمات الرئيسية: اذابة الفوسفات ، ثلاثى فوسفات الكالسيوم ، انزيم الفوسفاتيز والفطريات المذيبة للفوسفات .

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