DOI: 10.21276/haya.2017.2.1.3

Haya: The Saudi Journal of Life Sciences **ISSN 2415-623X (Print)**
Scholars Middle East Publishers **ISSN 2415-6221 (Online)**
Dubai, United Arab Emirates
Website: http://scholarsmepub.com/

Research Article

Bioleaching of Abu Tartur Phosphate Ore by Using *Aspergillus niger*

T.A. Elbarbary[1], M.A. Hafez[2], I.A. Ibrahim[1], S.A. Abd EL-Halim[3], H.M. Sharada[3], Y. M. Abdel-Fatah[1]
[1]Central Metallurgical Research and Development Institute, Egypt
[2]Botany and Microbiology Department, Faculty of Science, Al-Azher University, Egypt
[3]Biochemistry Department, Faculty of Science, Helwan University, Egypt

***Corresponding Author:**
Mohamed Ali El-Badry Hafez Amin
Email: mohamed.helwanuni@yahoo.com

Abstract: The use of phosphate solubilizing microorganism to dissolve phosphate content of phosphate ore instead of conventionally methods is an ecologically safe and economically reasonable. The objective of the present study is to study the factors affecting on dissolution of phosphate content in Abu Tartur phosphate ore by using fungus.The serial dilution method was performed to inoculums solutions to achieve microorganisms isolation which obtaining one fungus that has ability to dissolve phosphate ore and is identified by 18 sRNA as *Aspergillus niger*. The optimum conditions of bioleaching of Abu Tartur phosphate ore were 7 days incubation period at glucose-yeast extract medium, $2x10^6$ SFU of *A.niger* for 50 ml medium, 0.5% Abu Tartur phosphate ore incubated at 30 °C. different carbon and nitrogen sources were evaluated which peptone was the best nitrogen source, glucose the best carbon source, the best diameter of conical flask base for bioleaching process was above 4.5 cm , no significant effect of addition factor, also there was decreasing in pH and increased in redox potential, initial pH 6, shaking flask is at 150 rpm. The efficiency of *A. niger* for phosphate content dissolution of Abu Tartur phosphate ore by applying all optimum conditions was 100%.
Keywords: *Aspergillus niger*, Phosphate ore, bioleaching.

INTRODUCTION

Phosphorus is one of the most important essential elements for crop production. Application of phosphate fertilizers can enhance agricultural production in soils with low phosphate availability, especially in the tropical and subtropical region at present. Phosphate rock is the major source of phosphorus in nature and is being used as the raw-material for manufacturing commercial phosphate fertilizers (90%) and elemental phosphorus (10%) used in the chemical and food industries. The rock consists of insoluble calcium phosphate, generally known as apatite. The general formula of apatite is $Ca_5(PO_4)_3$ (OH,F,Cl) and depending on the end member, apatite can be named as hydroxyl apatite, fluorapatite or chloroapatite [1].

Conventionally, rock phosphate is chemically processed with sulfuric acid or phosphoric acid into phosphate fertilizer. This process makes the fertilizer more expensive and contributes to environmental pollution [2]. As an alternative to the direct use of sulfuric or phosphoric acid, microorganisms may be considered a source of solubilizing agents for insoluble mineral phosphates [3]. The phosphate solublizing microorganisms have been considered as plant growth promoters as the inoculation of soil with these microorganisms increase the crop yield. Inoculation of soil with phosphate solublizing microorganisms yields crop similar to those obtained by addition of soluble phosphate [4]. Filamentous fungi are widely used as producers of organic acids, particularly black *Aspergilli* and some species of *Penicillium*, these species have been tested for solubilization of rock phosphate and have been reported for various properties of biotechnological importance, such as, biocontrol, biodegradation, phosphate solubilization and P fertilizer [5-9] dissolution of phosphorus from Abu Tarture Egyptian ore which has low solubility level was applied by two different fungal isolates *penicillium chrysogenum* and *Aspergillus niger* to increase availability of phosphorous [10].

In this work, the factors affecting on dissolution of phosphate content in Abu Tartur phosphate ore were evaluated by using *A.niger* to reach to maximum dissolution of P_2O_5.

MATERIAL AND METHODS

Abu Tartur phosphate ore sample was collected in plastic bags from phosphate mine present in Safaga and Elkosir on the red sea cost in Egyptian eastern desert. Chemical composition of the studied phosphate sample is determined by using XRD analysis.

Isolation of fungal species

The fungal species isolated from Abu Tartur phosphate ore according to the serial dilution technique as described by [11]. 100µl of each dilution is placed on the surface of sterile agar plate of pikoveskey's agar medium. After 4 days of incubation at 30°C, only one fungus isolated.

Culture media

Different types of culture media were used in the practical study of this work, which are:

Modified Czapek's Dox medium [12]

It contains (g\l): $NaNO_3$, 2; $Ca_3(PO_4)_2$, 1; $MgSO_4.7H_2O$, 0.5; KCl, 0.5; $FeSO_4$. $5H_2O$ traces; sucrose, 30 and 1 liter distilled water.

Glucose-yeast extract medium [13]

It contains (g\l): Glucose, 10 g; Peptone, 5.0 g; Yeast extract, 3.0g; Agar, 20.0 g, Distilled water, 1.0 l and adjust pH to 6.8 before autoclaving, autoclave at 121°C for 15 min. The above media are solidified by adding 15g agar per liter. They are autoclaved for 20 min at 1.5 Atm. Pressure.

Modified ammonium medium [14]

It contains (g\l): Sucrose, 20; $Ca_3(PO4)_2$, 5; $MgSO_4.7H_2O$, 1; NH_4Cl, 2 and 1 liter distilled water.

Modified 9 k medium

It contains (g\l): Solution A; 3 g/l $(NH_4)_2SO_4$, 0.5 g/l $MgSO_4.7H_2O$, 0.5 g/l K_2HPO_4, 0.1g/l KCl, 0.01 g/l Ca $(NO_3)_2$, 1 g/l glucose, 0.3g/l yeast extract. These components are dissolved in 700 ml bidistilled water and are sterilized by autoclave at 121°c for 15 min.

Solution B; 44.2 g/l $FeSO_4.7H_2O$ is dissolved in 10 ml (1N) H_2SO_4 and 290 ml bidistilled water then sterilized by filtration system and is added to solution A after cooled it. Initial pH value is adjusted to 2.5 with about 50% H_2SO_4.

Pikovskaya's medium [15]

It contains (g/l): 0.5 g/l Yeast extract, 10 g/l Dextrose, 5 g/l Tri calcium phosphate, 0.5 g/l Ammonium sulphate, 0.2 g/l Potassium chloride, 0.1 g/l Magnesium sulphate, 0.0001 g/l Manganese sulphate and 0.0001 g/l Ferrous sulphate. Suspend 16.3 grams in 1000 ml distilled water. Heat if necessary to dissolve the medium completely and sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Dispense as desired. This medium is solidified by adding 15 g agar per liter.

Phosphate solublization evaluation by isolated fungus

Pikovskaya's Agar Medium

The fungus isolate was evaluated for its ability to phosphate solublization. 100µl of spore suspension of fungus is placed in the center of Pikovskaya's medium agar plate and put in incubator at 30°C. The solublization activity was detected by the presence of clear zone around the fungal colony.

Characterization of phosphate solubilizing fungus isolate

DNA isolation and PCR conditions

DNA extraction was done by use protocol of Gene Jet Plant genomic DNA purification Kit (Thermo). PCR by using Maxima Hot Start PCR Master Mix (Thermo) #K0221and Primer of ITS1 5-TCCGTAGGTGAACCTGCGG-3 and ITS4 5-TCCTCCGCTTATTGATATGC-3 (White et al. 1990). Initial denaturation 95°C for 10 min, Denaturation 95°C for 30 sec, annealing 55°C for 1 min Extension 72°C for 1min Final extension 72°C for 15min Number of cycles 35. PCR clean up for the PCR product using GeneJET™ PCR Purification Kit (Thermo)

Sequencing is done to the PCR product on GATC Company by use ABI 3730xl DNA sequence by using forward and reverse primers.

Experiment method

Prepare 50 ml of glucose yeast extract medium in 100 ml conical flask then put 0.25 gm sterilized Abu Tartur phosphate ore on 50 ml of sterilized glucose yeast extract medium and inoculated by 100 µl of spores *A. niger* and also compare with control then incubated in shaking incubator at 30 °C and 160 rpm, take 5 ml from filtrate of sample with fungus and control. The amount of soluble phosphate in the culture filtrate was evaluated every 1 day and determined calorimetrically according to the method described by Olsen [16].

Effect of different growth parameter on phosphate solublization

Different culture media, Czapek's Dox, glucose-yeast extract, modified 9K and Richard's, separately supplemented with 0.25gm Abu Tartur phosphate ore for 50 ml medium. Each flask was inoculated with $2x10^6$ SFU and incubated at 30 °C. The amount of soluble phosphate in the culture filtrate is determined. The previous conditions were conducted on glucose-yeast extract medium supplemented with Abu Tartur rock phosphate for *Aspergillus niger* at different incubation periods, incubation temperatures, ore concentrations, fungus concentration, carbon, and nitrogen sources, initial pH, number of shaking flask, addition of medium, fungus and both of them during experiment, diameter of conical flask base.

Organic Acid Production screening:

A. niger isolated was subjected for organic acid production. One disk of fungal isolate was inoculated on petri plates containing mineral agar organic acid indicator [17] and incubated for five days for the formation of yellow zone around the mycelial growth.

The medium used was Czapek's agar medium with 1% bromocresol green as organic acid indicator.

Detection of organic acids produced by *A. niger* by using HPLC

A.niger was grown on glucose-yeast extract medium supplemented with 0.5 % w/v of Abu Tartur phosphate ore as main phosphorus source. Flasks of each sample were incubated for 7days at 30°C.The culture filtrate was centrifuged at 9000 rpm for 10 min. Organic acids were determined in the culture filtrate in the presence of 0.5 % (w/v) Abu Tartur phosphate ore and in its absence (control) using HPLC (GBC) LC - 1445 international research centre, Cairo, Egypt. Organic acids were identified by using authentic samples of the produced acids under the same condition of sample determination.

The HPLC analysis

The HPLC analysis was performed on clarity chromatography data system, the HPLC system consisted of two pressure pumps (Sykam S1122 delivery system), the injection port with a 2ml loop (Sykam S 5111 Injector valve bracket), a UV detector (Jasko – UV – 2070 Plus, Intelkigent UV/Visible detector, Japan). For chromatographic separation, C-18 column (Thermo Hypersil Keystone, 5 µm, 250×4.6 mm) was used. Potassium dihydrogen phosphate buffer (pH 2.8) was used as mobile phase and flow rate is adjusted to 1 ml /min. Sample volume (20 µl) was injected with the help of a micro syringe, the run time was adjusted to 10 min and UV absorbance was determined at 214 nm. Autochrom 3000 software is the data acquisition system. Authentic sample of oxalic, citric and fumaric acids were used (Sigma). The results obtained from HPLC analysis of the samples were monitored using the above mentioned authentic samples.

RESULTS AND DISCUSSION

Chemical composition of Abu Tartur phosphate ore

XRD analysis of the Abu Tartur phosphate ore sample demonstrated the presence of insoluble P_2O_5 is 24.5 % and the other element presence in this ore was Ca 39.5 %, L.O.I. 12.32 %, SiO_2 with 7% , SO 5.07%, Fe_2O_3 with 6.6%, Al_2O_3 2.02%and other traces elements (Table 1).

Table-1: Chemical Analysis of Abu Tartur Phosphate Ore.

Elements	Percentage %	Elements	Percentage %
P_2O_5	24.5	Na_2O	0.194
Fe_2O_3	6.6	Al_2O_3	2.025
Ca	39.5	MgO	1.750
SiO_2	7.0	MnO	0.503
SO_2	5.072	K_2O	0.254
Cl	0.064	Cr_2O_3	0.052
L.O.I	12.32	F	0.157

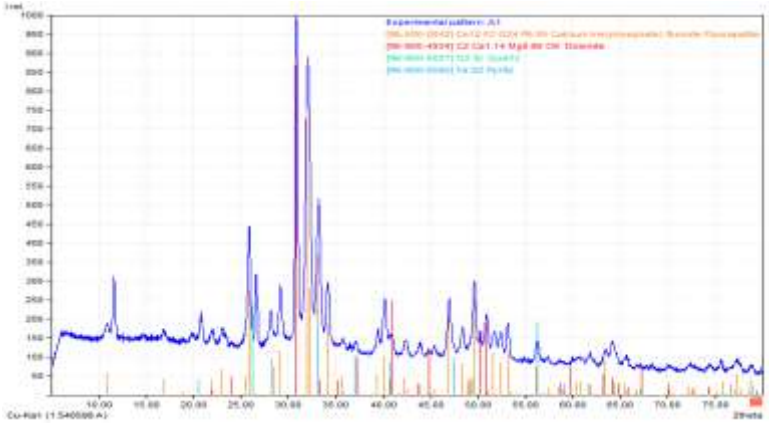

Fig-1: XRD Analysis of Abu Tartur Phosphate Ore

Identification of fungal isolate

Molecular identification of the selected isolate 18S rRNA sequencing was a powerful tool for rapid identification and phylogenetic analysis of fungal species. The obtained 550 bp 18S rRNA nucleotide sequence was compared with available 18S ribosomal sequences in the NCBI database using BLASTN (Table 4). The MPF-8 isolate had been enrolled into a cluster containing *Aspergillus* sp. and was found to be closely related to *Aspergillus niger* strain HPA8 with 99% sequence similarity (Figure 2). Hence it was designated as *Aspergillus niger* isolate. The submitted nucleotide sequence was provided a GenBank accession number KM386638.

Table-2: The Aligned Sequence Data Obtained for *Aspergillus Niger* (~ 550 bp).

1	gccgcttgtc	ngccgccggg	ggggcgcctc	tgccccccgg	gcccgtgccc	gccgganacc
61	ccaacacgaa	cactgtctga	aagcgtgcag	tctgagttga	ttgaatgcaa	tcagttaaaa
121	ctttcaacaa	tggatctctt	ggttccggca	tcgatgaaga	acgcagcgaa	atgcgataac
181	taatgtgaat	tgcagaattc	agtgaatcat	cgagtctttg	aacgcacatt	gcgcccccctg
241	gtattccggg	gggcatgcct	gtccgagcgt	cattgctgcc	ctcaagcccg	gcttgtgtgt
301	tgggtcgccg	tccccctctc	cgggggggacg	ggcccgaaag	gcagcggcgg	caccgcgtcc
361	gatcctcgag	cgtatggggc	tttgtcacat	gctctgtagg	attggccggc	gcctgccgac
421	gttttccaac	cattctttcc	aggttgacct	cggatcaggt	agggataccc	gctgaactta
481	agcatatcaa	taagcggagg	nnnnttncng	agtgnnggtc	cgttgggccc	aactcnatcc
541	nngtctatag	taccctgttg	cttcngcngg	ccgcc		

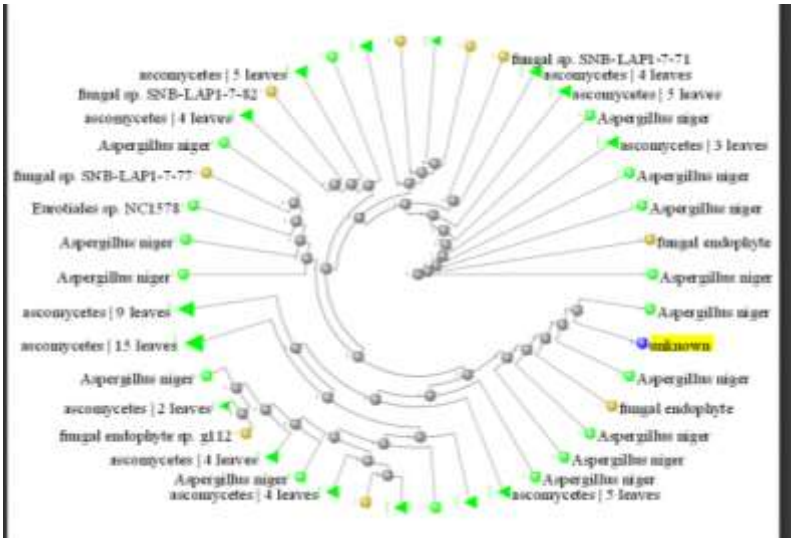

Fig-2: Identification of Fungus Isolated from Abu Tartur Phosphate Ore.

Detection the ability of *Aspergillus niger* for phosphate solublization by using Pikovaskay's agar medium

In our study, isolation of fungi from Abu Tartur phosphate ore and tested for their ability on dissolution of phosphate content in ore. One fungus was isolated from this ore and had ability to dissolve of phosphate content in this ore which form clear zone around fungal colony on PVK agar medium and was identified by 18 sRNA as *Aspergillus niger*. Production of organic acids by *Aspergillus niger* and it's diffusion into the medium are recorded by formation clear zone around the fungal colony on pikovaskay's agar medium and this refers to the solublization of $Ca_3(PO4)_2$ by fungus, Figure (3) and this agree with Kang et al. [18], Gupta et al. [19].

Solubilization of rock phosphate depends on its structural complexity, particle size and organic acids produced through fungus [20].

Fig-3: Solubilization of Tricalcium Phosphate of PVK Agar Medium by *A. niger*.

Effect of incubation period on phosphate solublization by *A. niger*

Using glucose-yeast extract medium in presence 0.25 g Abu Tartur phosphate ore for 50 ml of yeast extract medium and inoculated with $2x10^6$ SFU of *A. Niger* and measuring P_2O_5 each 2 days, pH and redox potential. The results revealed that maximum phosphate solublization was recovered after 7 days which reaching to 100 % with decreasing pH value and increasing electric potential value and little changes have occurred above this period. Therefore, there were no significant changes in phosphate solublization with increasing incubation period above 7days, Figure (4). This correlated with growth curve of *A.niger* that begins the log phase from third day until seventh day from growth then enter in stationary phase up to fourteenth day of growth that produce all acids in these phase which may be in agreement with results obtained by Darmwal *et al*. [21] who proved that *A. niger* was found to be the best phosphate solublizer among several tested fungi and bacteria and that the maximum amount solublized from P_2O_5 was reached after 7to 10 days of incubation period. Also, El-Badry *et al*., [10] showed that phosphate ore dissolution by *A. niger* isolate show that phosphate dissolution a maximum value reached after 4 day with 47.8 % phosphate dissolution

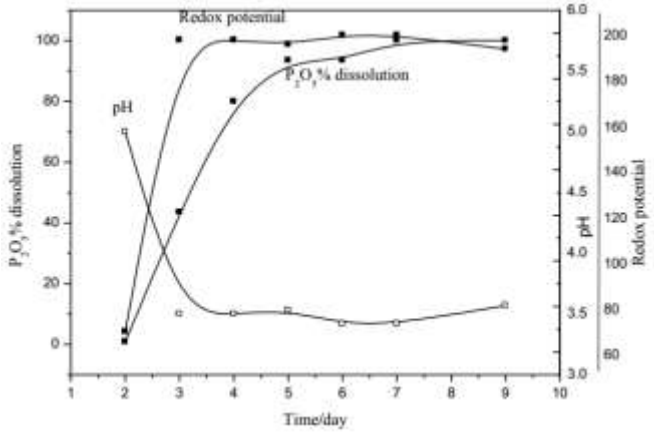

Fig-4: The Effect of Incubation Period on Dissolution of P2O5 Content of Ore

Effect of different media on phosphate solublization by *A. niger*

Four different types of media (glucose yeast extract, sucrose ammonium, 9 k and czapek's Dox) were used each contained 0.25 g Abu Tartur phosphate ore for 50 ml of medium and inoculated with $2x10^6$ SFU of *A. niger* and incubated at 30 °C and 160 rpm and measuring P_2O_5, pH and redox potential each 2day. The results revealed that maximum phosphate solublization was recovered with glucose-yeast extract medium which reaches to 100% after 7 days while, the minimum phosphate solublization occurred with 9k liquid medium and Czapek's Dox medium. The results are monitored with final pH, since the final pH was low with glucose-yeast extract medium (3.57) with high redox potential (172). This result in contrast with El Badry *et al*., [10] A. niger isolate its results reveal that Pikovskaya broth media gave maximum insoluble phosphate ore dissolution

Whereas, it was not significant change in pH for Czapek's Dox medium and glucose-yeast extract medium while, the amount of soluble phosphate was low with Czapek's Dox medium, this may be due to high growth which may consume phosphate, Figure (5), this accept with Hefnawy *et al*., [22].

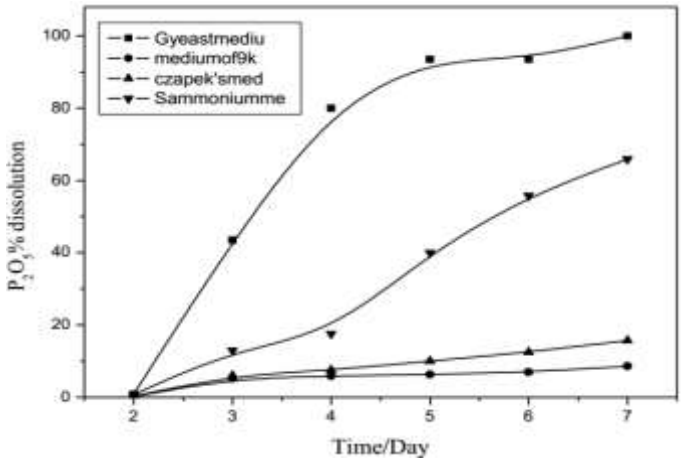

Fig-5: The Effect of Type of Medium on Dissolution of P_2O_5 Content of Ore.

Effect of different incubation temperatures:

It was studied by using three different temperature(20, 30, 40 °C) in presence 0.25 g Abu Tartur phosphate ore for 50 ml of glucose yeast extract medium and inoculated with $2x10^6$ SFU of *A. niger* and incubated at 30 °C and 160 rpm and measuring P_2O_5, pH and redox potential. The growth and rock phosphate solublization by *A. niger* were increased with increasing incubation temperature up to 30°C and then decreased above this temperature. Phosphate solublization by *A. niger* reaches to approximately 100% at 30°C after 7 days of incubation (Figure 6). The optimum growth of fungus occurred at 30ºC which refers to high production of organic acids lead to increase phosphate content solublization accomplishment the final pH is the lowest one at this temperature and increase redox potential. Working at higher temperature led the leaching efficiency to go down where the organic acid structure suffers partial thermal decomposition with the temperature raising [23]. Quite Similar results were obtained by Hefnawy *et al.*, [22] who reported that *A.terrus* was able to solublize 75% of uranium content of the ore at 30°C.

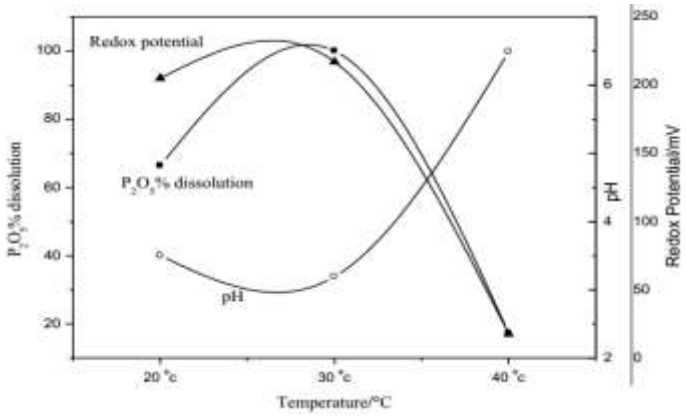

Fig-6: The effect of Temperature on Dissolution of P_2O_5 Content of Abu Tartur Phosphate Ore.

Effect of bulk density

Different weights of ore (0.25, 0.5, 1, 1.5, 2) gm were evaluated with previous optimum conditions then measuring P_2O_5, pH and redox potential after 7 days of incubation. *A. niger* grow well in the presence of different concentrations of phosphate ore in the growth medium up to 2 gm for 50 ml of medium (Figure (7)). The optimum growth and best phosphate solublization occurrs at weight of 0.25 gm of the Abu Tartur phosphate ore which reaches to 100 % and decreases above this weight, also accomplishment the final pH value at a weight of 0.25 gm ore is the lowest pH value and highest redox potential; this may be due to the production of high amounts of organic acids. At weight 0.25 gm of ore *A. niger* can solublize approximately 100% of phosphate content of the ore.

The dissolution of phosphate decreased with increasing phosphate ore concentration in the growth medium, that may be attributed to toxic effect of some metal ions which may be released into the culture medium such as Mn^{+2} and Na^{+1}, Ca^{+2} ions and these ions can react with soluble phosphate and form insoluble phosphate so decrease total soluble phosphate, these results found to be almost similar to that obtained by Hefnawy *et al*., [22]. Also, it may be due to inhibitory effect on further phosphate solubilization [24], the negative effect of soluble P on microbial acid productivity [25] might also be responsible for final soluble P concentration Another explanation for this might be formation of an organo-P compound induced by organic metabolites released, which in turn, reduces the amount of available P [26].

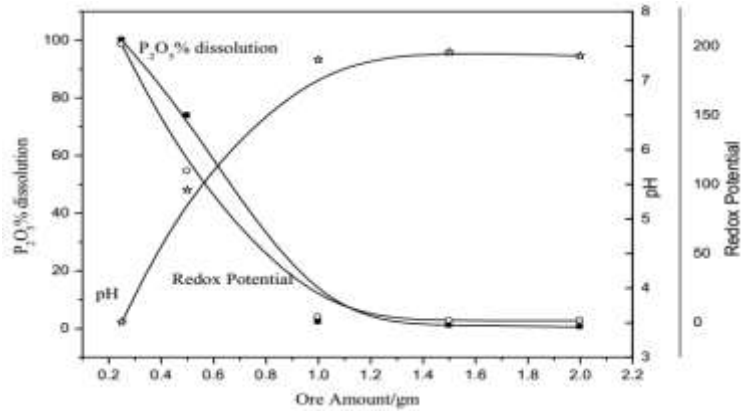

Fig-7: The Effect of Ore Amount on Dissolution of P2O5 Content of Ore.

Effect of *A.niger* inoculum size

It was studied by using four different inoculums size of *A. niger* ($2x10^6$, $4x10^6$, $6x10^6$, $8x10^6$) SFU of *A.niger* were evaluated with previous optimum conditions then measuring P_2O_5, pH and redox potential after 7 days of incubation**.**

Inoculum size of *A. niger* affected on dissolution of phosphate content of the ore. The most potent inoculums size was 2 $x10^6$ SFU of *A. niger* and decreases at high inoculums size of *A. niger* with no significant change in final pH value and this may be due to competition factor between spores themselves, decreases the aeration and also high growth which may consume phosphate. At inoculum size of $2x10^6$ spores, *A. niger* can solublize approximately 100% of phosphate content of the ore after 7 days of incubation, Figure (8).

This agrees with Laura Osorno [27] who found that maximum capacity of *Mortierella* sp. was detected with lowest population level ($1x10^7$ CFU) suggested that this fungus is effective using nutrients and producing the required acidity to dissolve rock phosphate and by increasing the population density of *Mortierella* sp. their effectiveness decreases, which may be due to intraspecific competition.

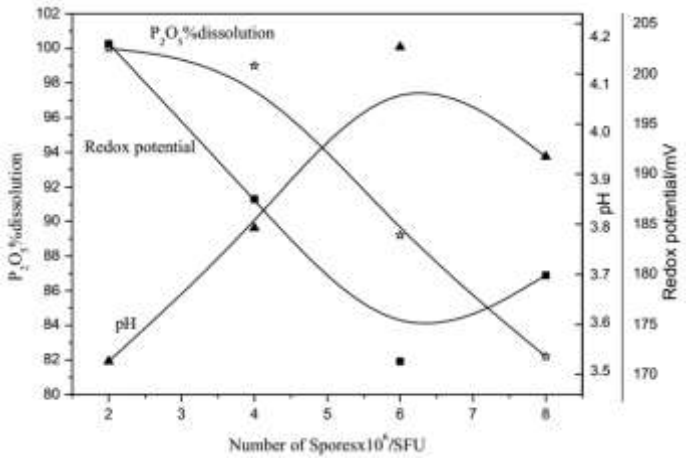

Fig-8: The Effect of *A.Niger* Amount on Dissolution of P_2O_5 Content of Ore.

Effect of different carbon sources

Different carbon sources of (glucose, starch, dextrose, lactose, sucrose) were evaluated with previous optimum conditions then measuring P_2O_5.

Nutritional constituent of the culture medium played an important role in phosphate solublization from its ores. The results revealed that *A. niger* grow well on yeast extract liquid medium containing different carbon sources. Whereas, the maximum amount of soluble phosphate was recovered only in the culture filtrate of *A. niger* with glucose and starch followed by sucrose and dextrose, while lactose exhibits low amount of soluble phosphate (Figure 9). Glucose is represented the best carbon source utilized by *A. niger* during phosphate solublization process which may be used in the production of certain organic acids that involved in solublization process, Whereas, A. niger isolate of El Badry *et al*., [10] the maximum amount of soluble phosphate is detected only in the culture filtrate of with sucrose and starch Figure (10). According to Cerezine *et al*. [28], glucose and fructose are the most frequent and abundant sugars detected in plant exudates that possibly affect the microbial population which solubilizes insoluble phosphates.

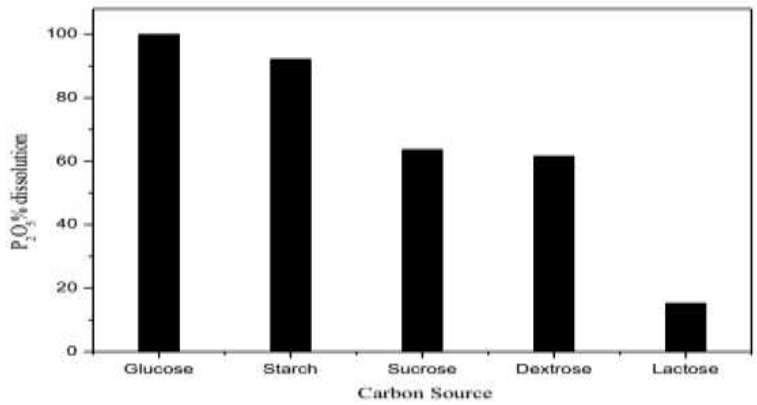

Fig-9: The Effect of Carbon Source on Dissolution of P_2O_5 Content of Ore.

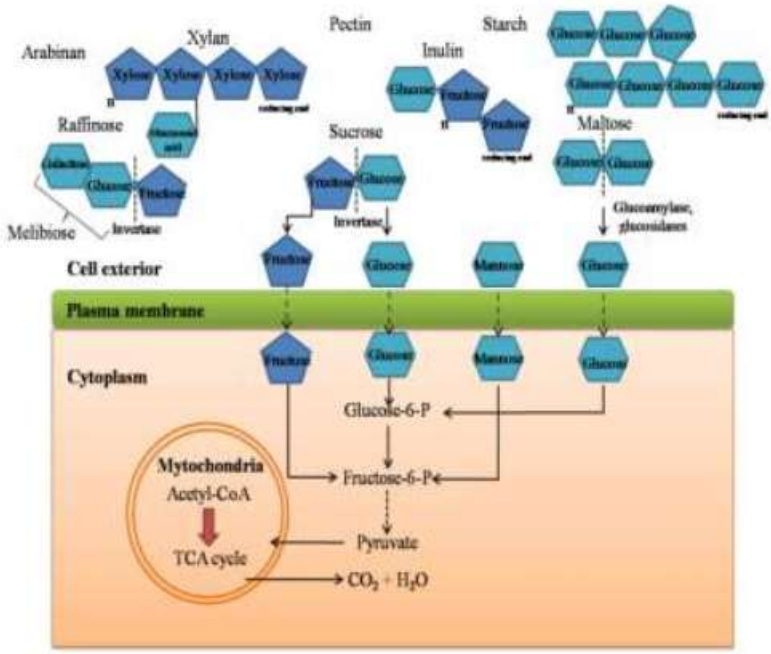

Fig-10: Assimilation of Carbon Sources in *Aspergillus Niger* and Production Organic Acids In TCA Cycle.

Effect of different nitrogen sources

Different nitrogen sources of (ammonium chloride, ammonium oxalate, tryptone, asparagine, peptone) were evaluated with previous optimum conditions then measuring P_2O_5.

A.niger solublized high amount of phosphorus from rock phosphate ore with all tested nitrogen sources. Peptone was found to be the best nitrogen source utilized by *A.niger* for maximum phosphate solublization which reaches to 100% after 7 days of incubation followed by ammonium chloride and the lowest dissolution of phosphate content of the ore at using ammonium oxalate as nitrogen source, Figure (11) and this accepts with Manish Kumar [29]. Variation of medium composition markedly altered the phosphate mobilization by phosphate solublizing microbes [26].

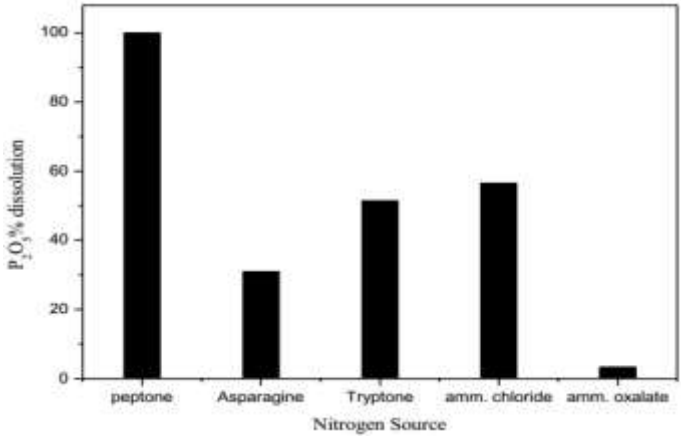

Fig-11: The Effect of Nitrogen Source on Dissolution of P_2O_5 Content of Ore.

Effect of Diameter of conical flask base

Different diameters of conical flask base (4.5, 5.5, 6, 8) cm were evaluated with previous optimum conditions then measuring P_2O_5, pH and redox potential.

The growth of *A.niger* affected with change of diameter of conical flask base from 4.5 cm up to 8 cm, Figure (12). Increasing the diameter of conical flask base up to 5.5cm, increasing the dissolution phosphate content of the ore and decreasing pH value and increasing redox potential reflect to high increase the dissolution phosphate content of the ore which reaches to 100% after of 7 days of incubation. Also the diameter of conical flask effected on dissolution of phosphate content of Abu Tartur phosphate ore which increase the diameter of conical flask lead to increase the aeration that necessary for growth of *A. niger* and

more increase for production of acids as in the equations (1, 2) [34]:

$$C_6H_{12}O_6+1.5O_2 \xrightarrow[\text{Citric acid}]{} C_6H_6O_6+ 2H_2O \quad (1)$$

$$C_6H_{12}O_6+4.5O_2 \xrightarrow[\text{Oxalic acid}]{} 3C_2H_2O_4+ 3H_2O \quad (2)$$

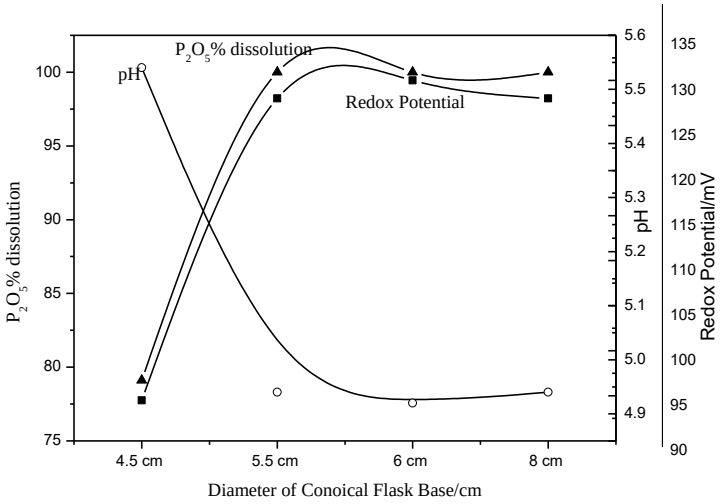

Fig-12: The Effect of Diameter of Conical Flask Base

Addition supplements media during incubation

It was studied by addition of medium, spores of *A.niger* and both of them during experiment is carried out which were evaluated with previous optimum conditions then measuring P_2O_5 each 2 days of incubation.

It was observed that the growth of *A.niger* at this conditions did not reflect the solublization activity and dissolution of phosphate content of the ore reaches to 100% at 7 day of incubation period, Figure (13), so the addition of spores of *A.niger* during experiment of bioleaching don't have significant effect in dissolution of phosphate content of ore and this may be due to consumption of nutrient by the first addition of spores of *A.niger*, also addition of medium during experiment that is carried out don't have significant effect on dissolution of phosphate and also addition of both of fungus and medium at the same time.

Under these optimum conditions lead to optimum the growth of *A.niger* and utilization of glucose and production of organic acids lead to maximum dissolution of phosphate content in Abu Tartur phosphate ore reaches to 100%.

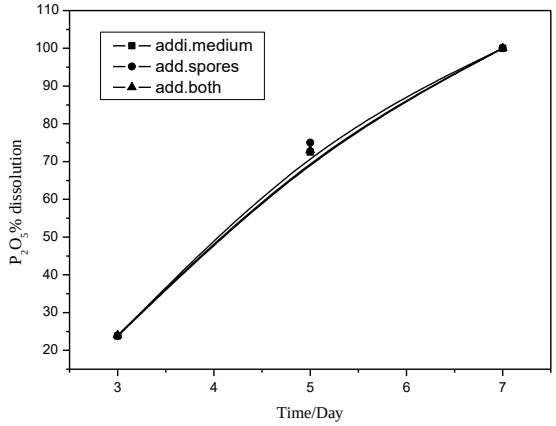

Fig-13: The Effect of Addition of Medium, Spores of Fungus and Both of Them during Experiment Carried Out on Dissolution of Phosphate Content of Abu Tartur Ore.

Effecting of initial pH

Different initial pH (4, 5, 6, 7, 8) of medium were evaluated with previous optimum conditions then measuring P_2O_5.

The growth of *A.niger* affected with the initial pH of the glucose-yeast extract liquid medium, Figure (14). The maximum growth of *A. niger* on a medium containing rock phosphate observed at initial pH 6 which at this pH value phosphate solublization exhibited high amounts it represented 100% after 7 days of incubation. It is also observed that pH at pH 6 is sharply decreased.

The best solublization activity of *A.niger* was recovered at initial pH 6 with consequent lowering in the final pH and this due to utilization of glucose and production of organic acids as second metabolites. This is in agreement with the result obtained by Achal *et al.*, [30] who suggested that tri-calcium phosphate and rock phosphate solubilization by phenotypic mutants of *Aspergillus tubingensis* was due to lowering of the final pH of the culture filtrate and also the activity of acid phosphatase and phytase.

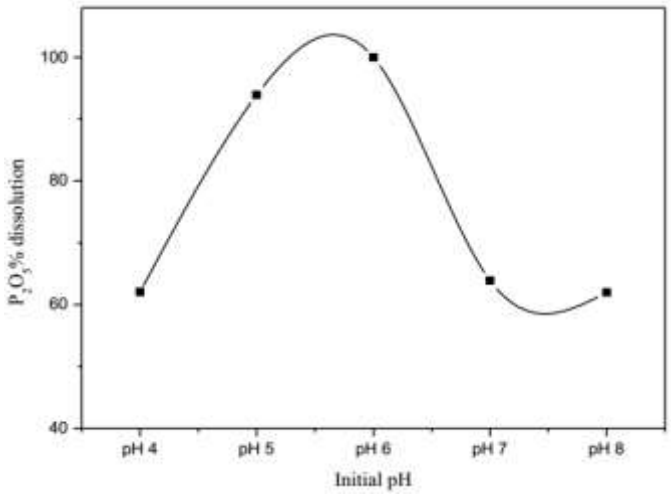

Fig-14: The Effect of Initial pH on Dissolution of Phosphate Content of Abu Tartur Phosphate Ore.

Effect of aeration on phosphate content dissolution from ore:

Different shaking speed of flask (50, 100, 150, 200) rpm were evaluated with previous optimum conditions then measuring P_2O_5.

The growth of *A.niger* varied with change of shaking of flask that studied from 50 to 200 rpm which found that amount of dissolution of phosphate content of this ore increased with increase of shaking speed from 50 to 150 rpm but began to decrease at high speed more than 150 rpm and this may be due to shear stress produced from strong shaking reduce the growth of *A.niger* and this is accepted with Xiao *et al*, [31] Figure (15).

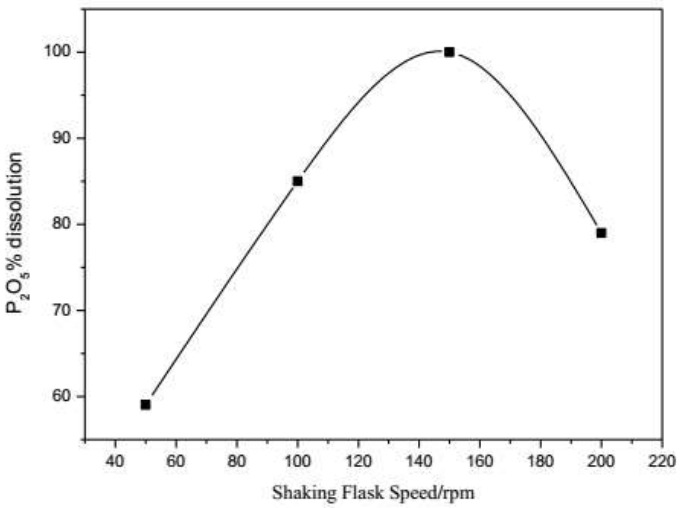

Fig-15: The Effect of Shaking Speed of Flask on Dissolution of Phosphate Content of Abu Tartur phosphate ore.

Growth curve of *A.niger*

The growth curve of *A. niger* was carried out by calculating its dry weight which began to grow from third day and this refered to log phase then reach to optimum growth at seventh day from growth then the growth remained constant to fourteenth and this refered to stationary phase of its growth and at this phase the fungus produced all the acids then its growth began to decrease and this is decline phase (Figure 16).

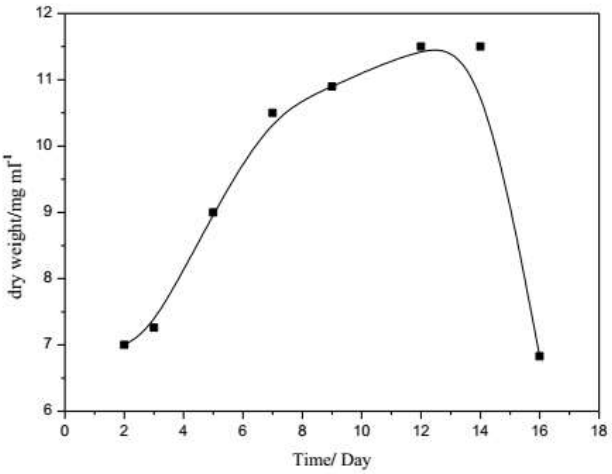

Fig-16: Growth Curve of *A. Niger*

Organic acids production

A.niger was able to produce organic acids such as oxalic and fumaric acids in the growth medium as secondary metabolites. Utilization of insoluble phosphate as main phosphorus source highly increased the production of some organic acids by organism. Detection of acids present in filterate of bioleaching experiment by HPLC which found that presence of higher amount of oxalic acid and fumeric acids in flasks containing of *A.niger* and 0.25gm Abu Tartur phosphate ore in 50 ml of glucose-yeast extract medium than control flask without ore and this lead to decrease of pH of medium from 6 to 3 and increase of redox potential and improve the dissolution of phosphate content in Abu Tartur ore to reach to 100% (Figure17), these organic acids are produced from utilization of glucose by *A.niger*, Figure(18), *A. niger* produced citric acid but it was not detect in HPLC because it utilized in dissolution of phosphate content of Abu Tartur ore and this agreeed with Hefnawy *et al*. [22], and also this accept with Hung and Ting [32] who reported that citric, oxalic and gluconic acids produced by *A. niger* were found to be an enhancing factor which improve fungal bioleaching and metal extraction from municipal solid waste incinerator fly ash.

To ensure production of citric acid by *A. niger* by using bromo cresol green as indicator (1%) supplemented in czapek's agar on plate at pH6 which the colour change from blue to yallow and the spore suspension of the isolate was spread on the surface of the medium plates from third day of cultivation, Figure (19) and this refered to production of citric acid and this agreed with Helen Shnada Auta *et al.* [33].

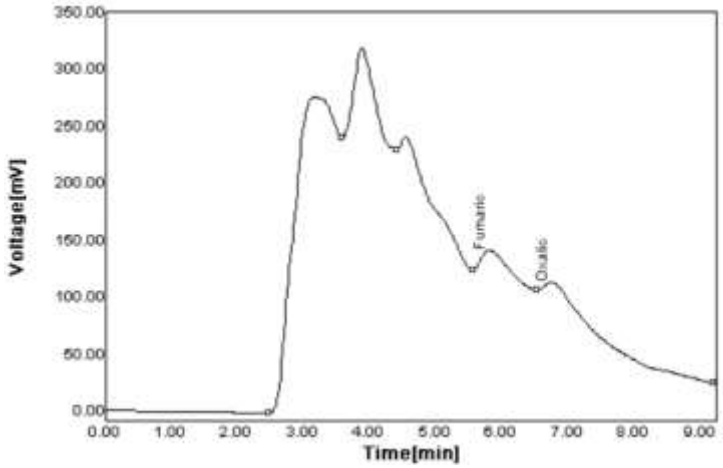

Fig-17: Detection of Fumaric and Oxalic Acids by Using HPLC (High Performance Liquid Chromatography) Analysis of Filtrate of Bioleaching of Abu Tartur Phosphate Ore by Using *Aspergillus Niger*.

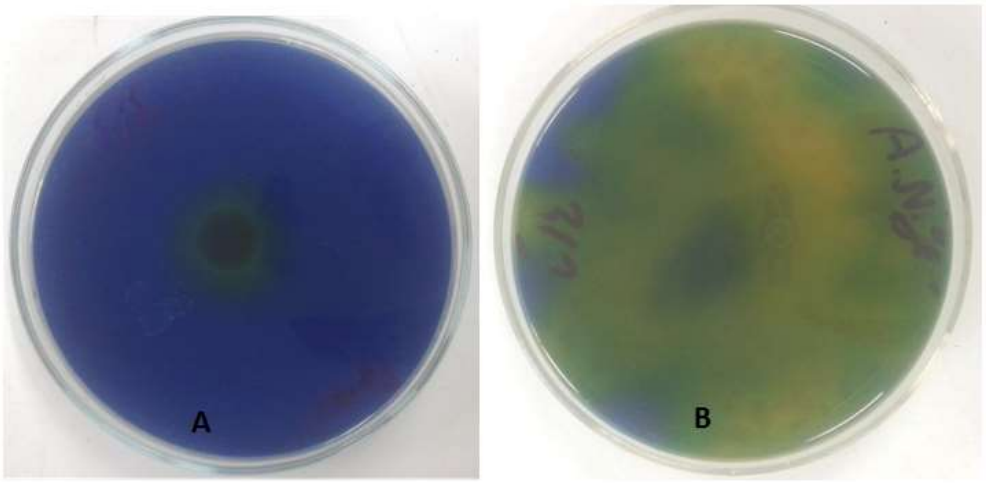

Fig-18A: Cultivation of *A. Niger* for 2 Days on Czapk's Dox Medium Supplemented with 1% Bromo Cresol Green as Indicator
Fig-B: Cultivation of *A.Niger* for 7 Days.

REFERENCE

1. Tilak, K. V. B. R., Ranganayaki, N., Pal, K. K., De, R., Saxena, A. K., Nautiyal, C. S., ... & Johri, B. N. (2005). Diversity of plant growth and soil health supporting bacteria. *Current science*, *89*(1), 136-150.
2. Vassilev, N., Medina, A., Azcon, R., & Vassileva, M. (2006). Microbial solubilization of rock phosphate on media containing agro-industrial wastes and effect of the resulting products on plant growth and P uptake. *Plant and Soil*, *287*(1-2), 77.
3. Sperber, J. I. (1957). Solution of mineral phosphates by soil bacteria. *Nature*, *180*(4593), 994-995.
4. Peix, A., Rivas-Boyero, A. A., Mateos, P. F., Rodriguez-Barrueco, C., Martınez-Molina, E., & Velazquez, E. (2001). Growth promotion of chickpea and barley by a phosphate solubilizing strain of Mesorhizobium mediterraneum under growth chamber conditions. *Soil Biology and Biochemistry*, *33*(1), 103-110.
5. Narsian, V., & Patel, H. H. (2000). Aspergillus aculeatus as a rock phosphate solubilizer. *Soil Biology and Biochemistry*, *32*(4), 559-565.
6. Reddy, M. S., Kumar, S., Babita, K., & Reddy, M. S. (2002). Biosolubilization of poorly soluble rock phosphates by Aspergillus tubingensis and Aspergillus niger. *Bioresource Technology*, *84*(2), 187-189.
7. Chuang, C. C., Kuo, Y. L., Chao, C. C., & Chao, W. L. (2007). Solubilization of inorganic phosphates and plant growth promotion by Aspergillus niger. *Biology and Fertility of Soils*, *43*(5), 575-584.
8. Richa, G., Khosla, B., & Reddy, M. S. (2007). Improvement of maize plant growth by phosphate solubilizing fungi in rock phosphate amended soils. *World Journal of Agricultural Sciences*, *3*(4), 481-484.
9. Pandey, A., Das, N., Kumar, B., Rinu, K., & Trivedi, P. (2008). Phosphate solubilization by Penicillium spp. isolated from soil samples of Indian Himalayan region. *World Journal of Microbiology and Biotechnology*, *24*(1), 97-102.
10. El-Badry, M. A., Elbarbary, T. A., Ibrahim, I. A., & Abdel-Fatah, Y. M. Azotobacter vinelandii Evaluation and Optimization of Abu Tartur Egyptian Phosphate Ore Dissolution.
11. Johnson, L. F., Curl, E. A., Bond, J. H., & Fribourg, H. A. (1960). Methods for studying soil microflora-plant disease relationships. *Methods for studying soil microflora-plant disease relationships.*
12. Thom, C., & Church, M. B. (1926). The Aspergilli. 272 pp. illust. *Williams and Wilkins Co., Baltimore*.
13. Wickerham, L. J. (1951). *Taxonomy of yeasts* (No. 1029). US Dept. of Agriculture.
14. Cunningham, J. E., & Kuiack, C. (1992). Production of citric and oxalic acids and solubilization of calcium phosphate by Penicillium bilaii. *Applied and Environmental Microbiology*, *58*(5), 1451-1458.
15. Sundara-Rao, W. V. B., & Sinha, M. K. (1963). Phosphate dissolving microorganisms in the soil and rhizosphere. *Indian J. Agric. Sci*, *33*(4), 272-278.
16. Olsen, S. R. (1954). *Estimation of available phosphorus in soils by extraction with sodium bicarbonate*. United States Department Of Agriculture; Washington.
17. Shaikh, Z., & Qureshi, P. (2013). Screening and isolation of organic acid producers from samples of diverse habitats. *Int J Curr Microbiol Appl Sci*, *2*(9), 39-44.
18. KANG, S. C., HAT, C. G., LEE, T. G., & Maheshwari, D. K. (2002). Solubilization of insoluble inorganic phosphates by a soil-inhabiting fungus Fomitopsis sp. PS 102. *Current science*, *82*(4), 439-442.

19. Gupta, N., Sabat, J., Parida, R., & Kerkatta, D. (2007). Solubilization of tricalcium phosphate and rock phosphate by microbes isolated from chromite, iron and manganese mines. *Acta Botanica Croatica*, *66*(2), 197-204.
20. Pradhan, N., & Sukla, L. B. (2006). Solubilization of inorganic phosphates by fungi isolated from agriculture soil. *African Journal of Biotechnology*, *5*(10).
21. Darmwal, N. S., Singh, R. B., & Rai, R. (1989). Isolation of phosphate solubilizers from different sources. *Current Science*, *58*(10), 570-571.
22. Hefnawy, M. A., Gharieb, M. M., Omima, A. E., & Asmaa, M. A. (2009). Evaluation and optimization of rock phosphate and tri-calcium phosphate solublization by some soil fungi. *Egypt J Exp Biol*, *5*, 75-84.
23. Mohapatra, S., Pradhan, N., Mohanty, S., & Sukla, L. B. (2009). Recovery of nickel from lateritic nickel ore using Aspergillus niger and optimization of parameters. *Minerals engineering*, *22*(3), 311-313.
24. Narsian, V., Thakkar, J., & Patel, H. H. (1995). Mineral phosphate solubilization by Aspergillus aculeatus. *Indian Journal of Experimental Biology*, *33*, 91-93.
25. Kubicek, C. P., Röhr, M., & Rehm, H. J. (1985). Citric acid fermentation. *Critical Reviews in Biotechnology*, *3*(4), 331-373.
26. Illmer, P., Barbato, A., & Schinner, F. (1995). Solubilization of hardly-soluble AlPO4 with P-solubilizing microorganisms. *Soil Biology and Biochemistry*, *27*(3), 265-270.
27. Osorno, L., & Osorio, N. W. (2014). Effect of carbon and nitrogen source and concentration on rock phosphate dissolution induced by fungi. *Journal of Applied Biotechnology*, *2*(2), 32.
28. Cerezine, P. C., Nahas, E., & Banzatto, D. A. (1988). Soluble phosphate accumulation by Aspergillus niger from fluorapatite. *Applied Microbiology and Biotechnology*, *29*(5), 501-505.
29. KUMAR, M. (2013). Oxalic acid production by Aspergillus Niger. *International Journal of Pharma & Bio*.
30. Achal, V., Savant, V. V., & Reddy, M. S. (2007). Phosphate solubilization by a wild type strain and UV-induced mutants of Aspergillus tubingensis. *Soil Biology and Biochemistry*, *39*(2), 695-699.
31. Xiao, C. Q., Chi, R. A., Huang, X. H., Zhang, W. X., Qiu, G. Z., & Wang, D. Z. (2008). Optimization for rock phosphate solubilization by phosphate-solubilizing fungi isolated from phosphate mines. *Ecological Engineering*, *33*(2), 187-193.
32. Wu, H. Y., & Ting, Y. P. (2006). Metal extraction from municipal solid waste (MSW) incinerator fly ash—Chemical leaching and fungal bioleaching. *Enzyme and microbial technology*, *38*(6), 839-847.
33. Auta, H. S., Abidoye, K. T., Tahir, H., Ibrahim, A. D., & Aransiola, S. A. (2014). Citric acid production by Aspergillus niger cultivated on Parkia biglobosa Fruit Pulp. *International Scholarly Research Notices*, *2014*.
34. Saeed, S., Bhatti, H. N., & Bhatti, T. M. (2002). Bioleaching studies of rock phosphate using Aspergillus niger. *OnLine Journal of Biological Sciences*, *2*(2), 76-78.