Australian Journal of Basic and Applied Sciences, 6(10): 518-524, 2012
ISSN 1991-8178

Interaction Of Some Herbicides With Phosphate Solublization By *Aspergillus Niger* And *Aspergillus Fumigatus*

M.A. Hefnawy, Omima A. Essa and Asmaa A. Amar

Botany Department, Faculty of Science, Menoufia University, Egypt

Abstract: *Aspergillus niger* and *Aspergillus fumigatus* were the best isolated soil fungi to solublize rock phosphate and tri-Ca-phosphate, their optimum solublization conditions were investigated (Hefnawy *et al.*, 2009). The growth of both organisms and phosphate solublization was not affected at lower concentrations of tested herbicides (glyphosate and putraline) up to 200 mg L^{-1} whereas, at higher concentrations the growth as well as phosphate solublization markedly decreased. At 800 mg L^{-1} round up, rock phosphate solublization by *A. niger* and *A. fumigatus* decreased to approximately 25 and 75% respectively comparing with the control. While, for tri-Ca-phosphate it decreased to approximately 30 and 85% respectively. At 800 mg L^{-1} AMEX 48% rock phosphate solublization by *A. niger* and *A. fumigatus* decreased to approximately 30 and 56% respectively comparing with the control. While, for tri-Ca-phosphate it decreased to approximately 50 and 60% respectively. Utilization of insoluble phosphate as main phosphorus source increased the production of organic acids by both organisms. However, addition of 800 mg L^{-1} of both herbicides showed markedly reduction in total organic acids production, as well as phosphate solublization.

Key words: *Aspergillus niger*, *Aspergillus fumigatus*, phosphate solublization, herbicides, organic acids

INTRODUCTION

Phosphates are essential ingredients in the fertilizers used to supply food and feed for mankind and animals. There is no substitute for phosphate rock as a raw material in the production of phosphate fertilizers in the world at this time. As the world population continues to increase, so does the demand for phosphate.

Several authors have identified the ability of fungi, mainly of *Aspergillus* and *Penicillium* genus, to solubilize phosphates under in vitro conditions (Omar, 1998; Seshadri *et al*., 2004; Wakelin *et al*., 2004). That ability is generally associated to the release of organic acids, decreasing the pH (Seshadri *et al*., 2004). *Aspergillus* and *Penicillium* genera were the most well studied fungi used in bioleaching studies as they were found to be able to liberate considerable amounts of organic acids such as citric, oxalic and tartaric acid which were thought to be the main phosphate solubilizing tool (Aung and Ting , 2004).

Vyas *et al*., (2007) estimated phosphate solubilization by *Eupenicillium parvum* in Pikovskaya broth. The obtained results showed high solublization of tricalcium phosphate and aluminum phosphate by this fungus and exhibited high levels of tolerance against desiccation, acidity, salinity, aluminum, and iron. *Aspergillus niger* and *Aspergillus fumigates* were the best soil fungi for rock and tri-calcium phosphate solubilization, under optimum conditions *A. niger* solublized 78, 74%, respectively whereas, *A. fumigates* solublized 56 and 54% respectively (Hefnawy *et al*., 2009).

Much work has been done on phosphate solublization in vitro studies whereas, little work has been carried out on the efficiency of these organisms in the presence of herbicides. Glyphosate herbicide when applied at the recommended field rate to a clay loam and a sandy loam forest soil resulted in few changes in microbial community structure (Ratcliff *et al*., 2006). Application of herbicides under field conditions, in general, highly stimulated the population and activities of the target microorganisms, which resulted in a greater amount of atmospheric nitrogen fixation and phosphate solubilization in the rhizosphere soils of the tested crop(Das and Debnath, 2006). However, the response of fungi to herbicides in pure culture may not accurately reflect their response to the same chemicals in field conditions (Greaves, 1987; Wardle & Parkinson, 1990).

Review of the literature offers conflicting results regarding the impact of glyphosate on fungal growth. The number of colony-forming units (CFUs) of soilborne fungi from a forest soil sample was estimated at concentrations (1, 2, 5, 10, 50, 100, and 1000 µg mL^{-1} of round up (35.6% glyphosate). At a concentration 50 µg mL^{-1} and higher of glyphosate, significantly decreased the number of CFUs and exhibited colony diameters 50% less than that of their respective controls of 13 isolated fungal species (Tanney and Hutchison, 2010).

This work aims to investigate the efficiency of some soil fungi to solubilize phosphate in the presence of certain herbicides.

Corresponding Author: M.A.Hefnawy, Botany Department, Faculty of Science, Menoufia University, Egypt
E-mail: hef3000@yahoo.com

MATERIAL AND METHODS

Insoluble Phosphate Samples:

Rock phosphate samples were collected in sterile plastic bags from phosphate mines present in Safaga and Elkosir on the red sea cost in Egyptian eastern desert**.** Insoluble $Ca_3(PO_4)_2$ samples were obtained from Talkha fertilizers company, El-Dakahlia governorate Egypt. Both samples contain 30% and 33.75% phosphate respectively as determined by inductive coupled plasma emission spectroscopy (I C P E S) at the Egyptian Geological Survey laboratories, Cairo, Egypt.

Organisms:

Aspergillus niger and *Aspergillus fumigatus* used in this study were previously isolated, identified and evaluated as the best isolated soil fungi for solublization of rock phosphate and tri –Ca – phosphate and their optimum solublization conditions was also investigated (Hefnawy *et al*., 2009).

Herbicides:

Putraline and glyphosate are water miscible herbicides and marketed under trade names of AMEX 48% and Round up respectively

Culture Media:

Czpek's Dox medium contains (g\l): $NaNO_3$, 2; KH_2PO_4,1; $MgSO_4.7H_2O$, 0.5; KCl, 0.5; $FeSO_4.5H_2O$, traces; sucrose, 30 and 1 liter distilled water. During this study KH_2PO_4 was replaced with 1% of insoluble phosphate samples.

Phosphate Determination:

Aspergillus nigar and *Aspergillus fumigates* were grown on Czpek's Dox liquid medium supplemented with 1% phosphate ore and incubated at 28°C. After 10 days of incubation the culture filtrate was filtrated several times using Whatman No1 filter paper and centrifuged at 6000 rpm for 20 min. The amount of soluble phosphate in the culture filtrate was determined calorimetrically according to the method described by Olsen *et al.,* (1954).

Procedures:

25 ml of culture filtrate was transferred to 50 ml Pyrex graduate flask, 5 ml of molybdate reagent (12.5 g of sodium molybdate ($NaMO_4.2H_2O$) was dissolved in 5M sulphuric acid and was completed to 500 ml with 5M sulphuric acid) was added followed by 2 ml of hydrazinium sulphate reagent (1.5g of hydrazinium sulphate was dissolved in deionised distilled water and diluted to 1 liter) and completed to 50 ml with distilled water and then mix well. The flask was placed in boiling water bath for 10 min. After cooling the absorbance was measured at 830 nm against reagent blank. Phosphate content in each sample was calculated using a standard phosphate curve of potassium dihydrogen phosphate.

Growth of A. niger and A. Fumigates At Different Herbicide Concentrations:

Czpek's Dox liquid medium supplemented with different concentrations of Round up and AMEX-48% separately. The herbicides were added to the growth medium after autoclaving in concentrations of (5, 10, 20, 200, 400, 800, 2000 mg L^{-1}). Triplicate sets of flasks were prepared for each fungus and herbicide concentration. The flasks were inoculated with 0.6 mm fresh mycelial agar disk of *A. niger* or *A. fumigatus* separately. After incubation for 10 days at 28 °C, the fungal dry weight was determined.

Phosphate Solublization In Presence Of Different Concentrations Of Tested Herbicides:

A. niger and *A. fumigatus* was grown on Czpek's Dox liquid medium containing 1% w/v rock phosphate and supplemented with different concentrations of both tested herbicides, (5, 10, 20, 200, 400, 800 and 2000 mg L^{-1})separately. Triplicate sets of flasks were incubated at 28ºC for 10 days. The culture was filtrated several times using Whatman No.1 filter paper and the amount of soluble phosphate was determined in the culture filtrate as mentioned above.

Organic Acids Production:

Each of *A. niger* and *A. fumigatus* was grown on Czapek's Dox liquid medium (control), and supplemented with 1% w/v of rock phosphate alone or with 800 ppm of each tested herbicide separately. Cultures were filtrated after 10 days of incubation at 28ºC, and centrifuged at 6000 rpm for 20 min. Organic acids were estimated in culture filtrate by HPLC (GBC) LC. 1445, Agricultural research center, Cairo, Egypt.

Results

Growth and Phosphate Solublization by A. Niger and A. Fumigatus in The Presence of Different Concentrations of Herbicides:

It is obvious that the growth of both organisms was not affected at lower concentrations up to 200 mg $L^{-1}$of the tested herbicide. While ,it slightly decreased at 400 mg L^{-1} and sharply decreased at 2000 mg L^{-1}. It seems that phosphate solublization from Rock phosphate and tri-Ca-phosphate by both organisms at different concentrations of round up followed the same pattern of growth. Phosphate solublization at lower concentrations of round up is quite similar to that of the control up to concentration 200ppm while, it decreased above this concentration and exhibited a drastic decrease at 2000 mg L^{-1}. At 800 mg L^{-1}round up, rock phosphate solublization by *A. niger* and *A. fumigatus* decreased to approximately 25 and 75% respectively comparing with the control. While, for tri-Ca-phosphate it decreased to approximately 30 and 85% respectively (Table 1,2).

Same pattern was also observed with the herbicide AMEX 48% for growth and phosphate solublization from rock phosphate and tri- Ca-phosphate by both tested organisms. At 800 mg L^{-1} mg L^{-1}AMEX 48% rock phosphate solublization by *A. niger* and *A. fumigatus* decreased to approximately 30 and 56% respectively comparing with the control. While, for tri-Ca-phosphate it decreased to approximately 50 and 60% respectively (Table 3,4).

Table 1: Effect of different concentrations of herbicide (round up) on rock phosphate ore solublization by *Aspergillus niger* and *Aspergillus fumigatus* grown on Czapek,s Dox liquid medium containing 1% of the ore for 10 days at 28±2◦C. Data are represented as mg L^{-1} ± standard error of the mean value.

Fungal spp.		Herbicide concs. Mg L^{-1}							
		0	5	10	20	200	400	800	2000
A.niger	Sol. P Mg/ml	2.25±0.8 (75.11%)	2.26±0.36 (75.24%)	2.258±0.52 (75.25%)	2.26±0.4 (75.30%)	2.001±0.27 (66.71%)	2.00±0.51 (66.70%)	1.74±0.23 (58.14%)	0.20±0.12 (6.88%)
	D.Wt. Mg/ml	11.24±4.8	11.02±1.1	10.62±3.02	10.66±3.32	10.48±1.01	9.86±2.3	6.42±0.91	1.084±0.2
	F.pH.	3.5±0.2	3.57±0.2	3.39±0.01	3.7±0.1	3.66±0.2	3.1±0.14	3.23±0.02	3.97±0.31
A.fumigatus	Sol. P Mg/ml	1.65±0.5 (55.09%)	1.65±0.71 (55.87%)	1.65±0.8 (55.10%)	1.65±0.15 (54.88%)	1.60±0.62 (53.33%)	1.527±0.45 (50.89%)	0.42±0.1 (13.97%)	0.18±0.1 5.92%
	D.Wt. Mg/ml	6.124±1.6	6.44±0.82	6.64±0.4	7.1±2.1	6.16±0.62	5.98±0.61	5.266±0.1	1.28±0.1
	F.pH.	4.52±0.35	4.49±0.3	4.64±0.24	4.52±0.01	4.92±0.2	5.1±0.4	4.25±0.2	4.13±0.05

Table 2: Effect of different concentrations of herbicide (round up) on tri-Ca- phosphate solublization by *A. niger* and *A. fumigatus* grown on Czapek,s Dox liquid medium containing 1% of the ore for 10 days at 28±2◦C. Data are represented as mg L^{-1} ± standard error of the mean value.

Fungal spp.		Herbicide concs. Mg L^{-1}							
		0	5	10	20	200	400	800	2000
A.niger	Sol. P Mg/ml	2.65±0.62 (78.42%)	2.68±0.95 (79.42%)	2.67±0.2 (79.11%)	2.66±0.2 (78.86%)	2.56±0.52 (75.98%)	2.53±0.31 (74.93%)	1.86±0.5 (54.99%)	0.45±0.13 (13.41%)
	D.Wt. Mg/ml	10.70±0.26	11.04±0.1	11.26±2.06	11.24±3.1	9.98±0.9	8.84±2.07	6.5±0.91	1.88±0.2
	F.pH.	3.6±0.2	4.2±0.2	4.07±0.01	3.7±0.3	3.5±0.1	3.3±0.2	3.26±0.02	3.1±0.02
A.fumigatus	Sol. P Mg/ml	1.97±0.82 (58.5%)	2.00±0.2 (59.91%)	2.02±0.2 (59.85%)	2.02±0.3 (59.85%)	1.93±0.63 (57.24%)	1.91±0.71 (56.1%)	1.47±0.15 (43.5%)	0.31±0.13 (9.3%)
	D.Wt. Mg/ml	8.3±1.1	8.64±1.02	9.03±1.0	9.1±0.73	8.02±0.8	8.5±0.7	7.48±0.1	1.77±0.1
	F.pH.	4.22±0.11	4.73±0.3	4.32±0.3	4.33±0.1	5.15±0.2	4.1±0.2	4.26±0.1	4.85±0.3

Organic Acids production by A. Niger and A. Fumigatus in The Presence of Rock Phosphate and Herbicides:

A. ngier and *A. fumigatus* were able to produce high amounts of organic acids such as oxalic, citric and formic acids in the control medium as a secondary metabolites. Utilization of rock phosphate as a main phosphorus source highly increased the production of oxalic, tartaric,and acetic acids by *A. niger*. Whereas, formic and citric acids were decreased. On the other hand, at 1% rock phosphate oxalic and malic acids exhibited high increase while, formic and citric acids markedly decreased by *A. fumigates*. Addition of 800ppm of the tested herbicides to the growth medium containing 1% rock phosphate exhibited marked reduction in the production of all detected organic acids (Table 5, 6 and Fig. 1,2). When *A. niger* grown on Czapek,s Dox liquid medium supplemented with 1% RP, the amount of oxalic acid highly increased to proximally 3.7 times higher than in the control. Whereas, it decreased in the presence of RP with 800 ppm round up or AMEX-48%. However it still higher than in the control. The production of formic acid and citric acid was high in the control medium while, they highly decreased in the presence of RP and RP+ round up or AMEX-48%. This may be attributed to the consumption of these acids in the solublization process. Same observation to some extent was also found with *A. fumigatus*, oxalic and malic acids were the major detected organic acids, they highly increased in the presence of RP in the growth medium whereas, they decreased with addition of round up or AMEX-48%. Formic and citric acids were produced in high quantities in the control medium and detected in very little amount in the presence of RP and round up or AMEX-48%. Total organic acid production by both organisms highly increased at 1% rock phosphate while, it decreased by addition of 800 mg L^{-1} of both herbicides.

Table 3: Effect of different concentrations of herbicide (AMEX-48%) on rock phosphate ore solublization by *A.niger* and *A. fumigatus* grown on Czapek,s Dox liquid medium containing 1% of the ore for 10 days at 28±2◦C. Data are represented as mg L^{-1} ± standard error of the mean value.

Fungal spp.		Herbicide concs. Mg L^{-1}							
		0	5	10	20	200	400	800	2000
A.niger	Sol. P Mg/ml	2.22±0.5 (75.81%)	2.28±0.44 (75.90%)	2.34±0.63 (75.25%)	2.28±0.32 (78.10%)	2.13±1.1 (75.91%)	2.11±0.72 (70.30%)	1.57±0.5 (52.40%)	0.21±0.05 (6.87%)
	D.Wt. Mg/ml	11.06±0.7	11.57±2	11.4±1.01	10.59±2.01	9.48±4.2	9.8±1.01	6.64±1.02	0.92±0.11
	F.pH.	3.2±0.2	3.7±0.2	3.9±0.02	4. 5±0.06	4.9±0.02	4.6±0.02	4.6±0.02	4.8±0.31
A.fumigatus	Sol. P Mg/ml	1.81±0.2 (60.23%)	1.83±0.11 (61.70%)	1.92±0.6 (64%)	2.01±0.2 (67 %)	1. 99±0.5 (66.47%)	1.93±0.1 (64.38%)	0.79±0.01 (26.43%)	0.22±0.03 7.17%
	D.Wt. Mg/ml	7.9±1.6	9.24±1.01	8.72±1.5	9.3±1.1	8.1±3.05	7.9±1.01	5.9±1.01	0.52±0.01
	F.pH.	4.3±0.35	4.15±0.3	4.8±0.04	4.62±0.12	5.04±0.12	4.91±0.04	5.74±0.02	6.25±0.1

Table 4: Effect of different concentrations of the herbicide AMEX-48% on Tri-Ca-P solublization by *A. niger* and *A. fumigatus* grown on Czapek,s Dox liquid medium containing 1% Tri-Ca-P. at 28±2°C for 10 days. Data are represented as mg ml^{-1} ± standard error of the mean value.

Fungal spp.		Herbicide concs. Mg L^{-1}							
		0	5	10	20	200	400	800	2000
A.niger	Sol. P Mg/ml	2.59±0.55 (76.64%)	2.58±0.71 (76.50%)	2.64 ±0.33 (78.09%)	2.59±0.36 (76.60%)	2.57±0.15 (76.08%)	2.47±0. 2 (73.23%)	1.3±0. 15 (38.52%)	0.32±0.02 (9.36%)
	D.wt.(mg/ml)	11.19±3.1	11.71±1.06	11.74±3.007	11.02±5.002	8.62±1.15	7.84±2.05	4.7±0.23	1.23±0.01
	F.pH.	3.22±.01	4.77 ± 0.2	5.71 ± 0.14	4.32 ±0.16	3.91±0.04	4.4±0.06	4.59±0.2	4.87±0.04
A. *fumgatus*	Sol. P Mg/ml	2.06±0.703 (61.01%)	2.08±0.12 (61.78%)	2.07±0.35 (61.45%)	2.05±0.31 (60.80%)	1.87±0.604 (55.45%)	1.63±0.23 (48.15%)	0.79±0.01 (23.50%)	13.48±0.02 (7.99%)
	D.wt.(mg/ml)	9.68±1.06	9.75±5.03	9.59±2.004	9.43±2.007	7.71±1.04	6.06±0.905	4.11±0.65	0.02±0.001
	F.pH.	4.42±0.03	4.51±0.14	4.42±0.1	4.59±0.2	5.21±0.06	5.73±0.03	5.79±0.07	5.93±0.3

Table 5: Organic acids content in culture filtrate of *Aspergillus niger* grown on Czapek,s Dox liquid medium supplemented with1% rock phosphate in the presence or in the absence of 800 ppm round up or AMEX-48% , incubated for 10 days at 28±2°C.

organic acids	control	Rp.	Rp+round up	Rp+AMEX-48%
Oxalic acid	8.7	32.26	16.99	22.64
Tartaric acid	ND	3.33	10.70	ND
Formic acid	12.09	0.62	0.96	4.07
citric acid	17.3	0.07	ND	0.3
Acetic acid	ND	7.94	5.29	1.15
Total	38.09	44.22	33.94	28.16

*Data are expressed as mg ml^{-1} culture filtrate, ND, not detected.

Discussion:

As well known phosphate fertilizers are very important for crop production. Therefore, an alternative and native phosphate sources should be utilized without chemical processing such as rock phosphate ores.

Many soil fungal (e.g. *Pencillium billia, Aspergillus niger*) and bacterial (e.g. *Erwinia herbicola and some Bacillus spp)* isolates can solublize inorganic phosphates (Kucey, 1983). Several phosphate solublizing microorganisms (PSM) are able to solublize unavailable soil phosphate and increase soil phosphate contents (Richardson, 2001). 10 fungal species were isolated from agricultural soil. They were tested for their phosphate solublization activity, *A. niger* and *A. fumigatus* were found to be the most efficient organisms that are able to release high amounts of phosphate from insoluble phosphate ores.

Biohydrometallurgical approaches are generally a green technology with low-cost and low-energy requirement. Microbial bioleaching is based on the natural ability of microorganisms to transform solid compounds to a soluble and extractable form. This may involve enzymatic oxidation or reduction of the solid compound, or an attack on the solid compound by metabolic products (Hung- and Ting, 2005).

.

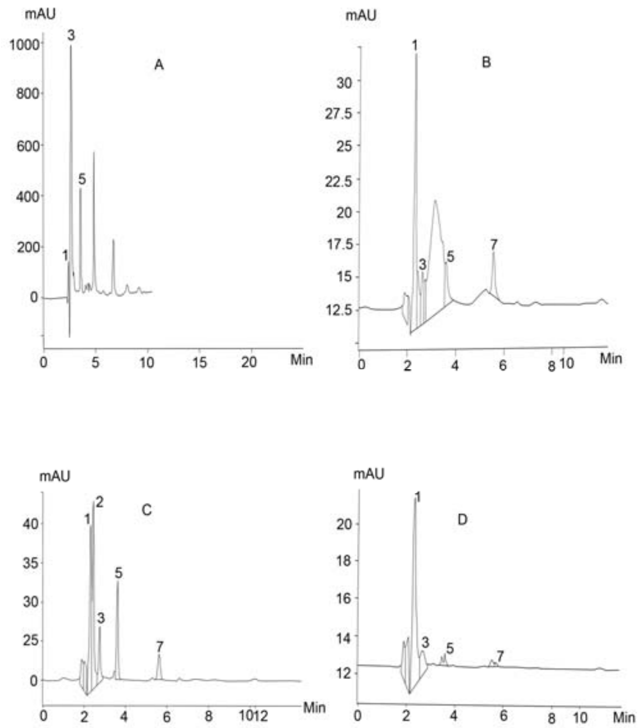
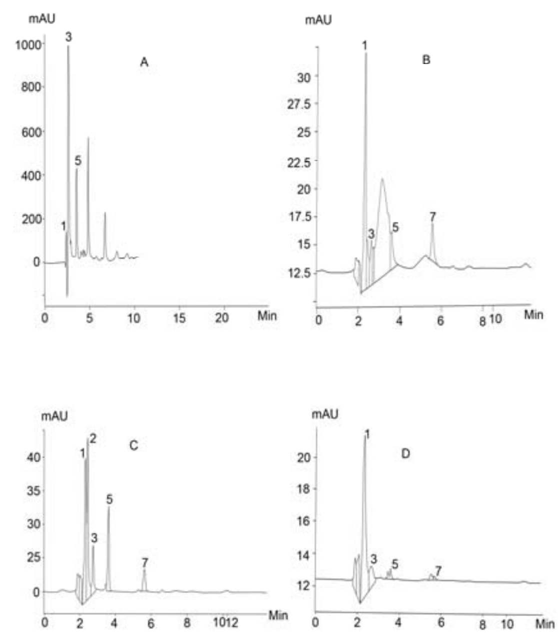


Fig. 1: HPLC chromatograms of organic acids produced by A. niger in the absence of RP(A), in the presence of RP (B) and in the presence of 800 mgL-1 of round up +RP (C), or AMEX 48%+RP(D). 1. Oxalic acid, 2. Tartaric acid, 3. Formic acid, 5. citric acid and 7. Acetic acid.



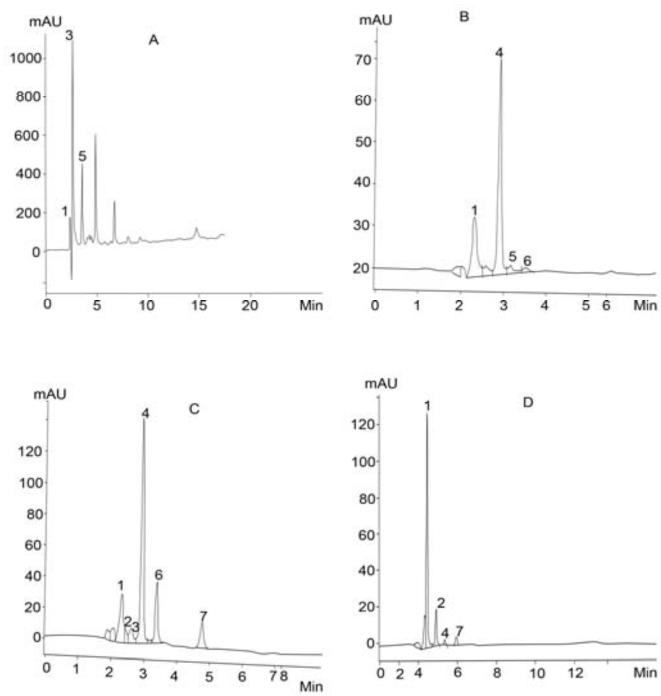


Fig. 2: HPLC chromatograms of organic acids produced by *A. fumigatus* in the absence of RP (A), in the presence of RP (B) and in the presence in 800 mgL^{-1} of round up +RP (C), or AMEX 48%+RP(D). 1. Oxalic acid, 2. Tartaric acid, 3. Formic acid, 4. Malic acid, 5. Citric acid, 6. lactic acid and 7.Acetic acid.

Much work has been done on the role of organic acids in metal solublization and mobilization. Organic acids released in the culture medium were found to be an important mechanism of $AlPO_4$ solublization. Organic acids alone are able to solublize $AlPO_4$ to a certain extent, although they are less effective compared to biotic leaching (Illmer *et al*., 1994).

Citric, malic, tartaric and acetic acids produced by *A. niger* were believed to have a great effect on the release of rare earth elements (Shan *et al*., 2002). Hung and Ting**,** (2005) 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

The obtained results in this study are in agreement with all previous literatures where, *A. niger* and *A. fumigatus* were able to produce oxalic, citric, malic and tartaric acids in the growth medium in the presence of 1% RP in the growth medium. Whereas, addition of 800 mg L^{-1}of herbicides round up or AMEX 48% exhibit a decrease in the production of total organic acids as well as phosphate solublizatiuon.

The growth of *A. niger* and *A. fumigatus* in this work was found to be slightly stimulated at low concentrations of the two tested herbicides (glyphosate and AMEX-48%) ranging from 5 up to 200 mg L^{-1} where, there was no significant effect on fungal growth and phosphate solublization. While, at higher concentrations of herbicides ranging from 400 up to 2000 mg L^{-1}, the fungal growth as well as phosphate solublization was obviously decreased. The reduction of phosphate solublization at higher concentrations of the tested herbicides may be attributed to the drop in fungal growth and organic acids production. This result is quite similar to that obtained by Das *et al*. (2003) where application of herbicides at the recommended dose 0.4 and 0.12 kg a.i. ha^{-1}, stimulated the population and activities of phosphate solublizing microorganisms and also the availability of phosphorus in the rhizosphere soil. In agreement with the results obtained in the present study It was proved that the application of glyphosate at normal field rate has no effect on free living microorganisms, in the soil or those associated with plants (Henriksen and Elen, 2005).

In contrast to the above results, it was reported that application of (atrazine and atrazine + metolachlor) on soil micro flora at recommended rates resulted in lower fungal counts compared to the control soil (Ayansina and Oso, 2006).

In conclusion: it was obvious that application of low doses of round up and AMEX-48% has no negative effect on fungal growth and phosphate solubilization by both tested fungi. However, at higher doses of herbicides phosphate solublization markedly decreased. Therefore, we recommend applying low doses of herbicides and to follow the instruction carefully when, applying rock phosphate or tricalcium phosphate as a fertilizer.

REFERENCES

Aung, K.M.M. and Y.P. Ting, 2004. Bioleaching of spent fluid catalytic cracking catalyst using *Aspergillus niger* . J. of Biotechnology, 116: 159-170.

Ayansina, A.D.V. and B.A. Oso, 2006. Effect of two commonly used herbicides on soil micro flora at two different concentrations . African Journal of Biotechnology, 5: 129-132.

Das, A.C., A. Debnath, 2006. Effect of systemic herbicides on N2-fixing and phosphate solublizing microorganisms in relation to availability of nitrogen and phosphorus in paddy soils of West Bengal. Chemosphere., 65(6): 1082-6.

Das, A.C., A. Debnath, D. Mukherjee, 2003. Effect of the herbicides oxadiazon and oxyfluorfen on phosphates solubilizing microorganisms and their persistence in rice fields. Chemosphere, 53(3): 217-21.

Greaves, M.P., 1987. Side effect testing: An alternative approach in pesticides effects on the soil micro flora. Taylor and Francis: London UK. pp: 183-190.

Hefnawy, M.A., M.M. Gharieb, A.E. Omima and M.A. Asmaa, 2009. Evaluation and optimization of rock phosphate and tri-calcium phosphate solublization by some soil fungi. Egypt J.Exp. Biol., 5: 75-84.

Henriksen, B. and O. Elen, 2005. Natural *Fusarium* grain infection level in wheat, barley and oat after earth application of fungicides and herbicides. J. Phytopathol., 53: 214-220.

Hung, Y.W. and Y.P. Ting, 2005. Metal extraction from municipal solid waste (MSW) incinerator fly ash—Chemical leaching and fungal bioleaching. J. Biotechnol., 116: 171-184.

Illmer, P., A. Barbato and F. Schinner, 1995. Solubilization of hardly-soluble $AlPO_4$ with P-solublizing microorganisms. J. Soil Biol. Biochem., 27: 265-270.

Kucey, R.M.N., 1983. Phoshate solublizing bacteria and fungi in various cultivated and virgin Albert soils. Canadian Journal of soil science, 63: 671-678.

Olsen, S.R., C.V. Cole, F.S. Watanabe and L.A. Dean, 1954. Estimation of available phosphorus in soils by extraction with sodium bicarbonate. J. Circular., 939: 19 -30.

Omar, S.A., 1998. The role of rock phosphate solublizing fungi and vesicular arbuscular mycorrhiza (VAM) in growth of wheat plants facilitated with rock phosphate, World Journal of Microbiology and Biotechnology, 14: 211-218.

Ratcliff, A.W., M.D. Busse and C.J. Shestak, 2006. Changes in microbial community structure following herbicide (glyphosate) additions to forest soils. J. Applied Soil Ecology, 34: 114-124.

Richardson, A.E., 2001. Prospects for using soil microorganisms to improve the acquisition of phosphorus by plants. Australian Journal of Plant Physiology, 28: 897-906.

Seshadri, S., S. Ignacimuthu and C. Lakshninarasimhan, 2004. Effect of nitrogen and carbon sources on the inorganic phosphate solubilization by different *Aspergillus niger* strains. J. Chemical Engineering Communications., 191: 1043-1052.

Shan, X., J. Lian and B. Wen, 2002. Effect of organic acids on adsorption and desorption of rare earth elements. J. Chemosphere., 47: 701-710.

Tanney, J.B., L.J. Hutchison, 2010. The effects of glyphosate on the in vitro linear growth of selected microfungi from a boreal forest soil. Can J Microbiol., 56(2): 138-44.

Vyas, P.A., P. Rahi, A. Chauhana and A. Gulati, 2007. Phosphate solubilization potential and stress tolerance of Eupenicillium parvum from tea soil. J. Mycological Research, 111: 931-938.

Wakelin, S.A., R.A. Warren, P.R. Harevy and M.H. Ryder, 2004. Phosphate solubilization by *Penicillium spp.* closely associated with wheat roots. Biology and Fertility of Soils., 40: 36-43.

Wardle, D.A. & D. Parkinson, 1990. Influence of the herbicide glyphosate on soil microbial community structure. J. Plant and soil., 122: 29-37.