EVALUATE THE EFFECT OF SOME FACTORS AFFECTING SOLUBILIZATION OF PHOSPHORUS IN RHIZOSPHERE

[84]

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ABSTRACT

The aim of this work was to conduct pot experiment to study the ability of plant roots on solubilizing various sources of phosphorus and factors that may facilitate or inhibit their activity. A split medium – split root technique experiment was conducted to study the effect of $CaCO_3$, pH, organic matter (humic acid) and bentonite on the pH, P solubility in the medium and P concentration in bean plants (*Vicia faba* var. balady). The changes in pH of the lower solution was recorded, also the root exudate was collected in 500 ml of $CaCl_2$ solution 0.5×10^{-4} *M* and pH 6.85. Results showed that the highest recorded total dry weight was found when both N forms were applied in the ratio of 1:4 NO^-_3: NH^+_4 or NO^-_3 alone. The mechanism of solubilizing rock phosphate by exudating protons or organic, amino and other organic compounds is possible at the root surface even in alkaline soil as long as the rock phosphate material was added near the root and organic matter was added to limit the fixing power of the inorganic components as $CaCO_3$ and excess soluble Ca. Also, the availability of P from rock phosphate sources depends on its reactivity value.

Keywords: Organic matter; Bentonite; $CaCO_3$; pH; Rock phosphate; Rhizosphere; Split medium technique; Faba bean plants.

INTRODUCTION

The mobility of phosphorus (P) in the shallow subsurface is a matter of critical importance and considerable complexity. Its importance stems from the fact that P an essential nutrient for all plants and animals life. Agricultural fertilizers and other soil amendments, such as mineral P fertilizers and animal manure, provide P that is readily available to plants. The short-term availability of P to crops is strongly influenced by biochemical processes that affect organic matter, while its long-term status is generally determined by geochemical transformations. Phosphorus is an essential nutrient for plants and is one of the most commonly applied as mineral fertilizer **(Jenkins and Jenkins, 2012).**

Afif et al (1993) found that at high application rates, P available to plants is negatively correlated to the amount of lime in soil, but not to Fe, clay content, or CEC. In contrast, other studies indicate that P retention increases with the ratio of Fe oxides to $CaCO_3$ **(Carreira and Lajtha,1997)** The preponderance of the evidence **(Zhou and Li, 2001)** suggests that non-carbonate clays provide most of the P adsorbing surfaces in many calcareous soils, especially at low P concentrations. It has even been reported that a 1.6% (w/w) coating of Fe_2O_3 on calcite increases the P sorption capacity 9-fold **(Tunesi et al 1999)**. As the P content of the soil increases, sorption by carbonates becomes more important. **Hopkins and Ellsworth (2005)** reported that Calcareous soil is defined as having the presence of significant quantities of free excess lime (calcium or magnesium carbonate). Lime dissolves in neutral to acid soil pH, but does not readily dissolve in alkaline soil and, instead, serves as a sink for surface adsorbed calcium phosphate precipitation. They found that the bioavailability of P is strongly tied to soil pH. The formation of iron and aluminum phosphate minerals results in the reduced solubility of P in strongly acidic soil, while P solubility improving as pH approaches nearly

neutral. This maximum solubility and plant availability of P at pH 6.5 declines again as the pH increases into the alkaline range.

Humic acid is a commercial product of organic fertilizers containing most elements that improve soil fertility and increase nutrients availability, thus enhances plant growth and yield as well as decreases the harmful effect of stresses **(Doran et al 2003**). The effect of HA on the availability of P and micronutrients has been given particular attention because of observed increases in uptake rates of these nutrients following application of humic acid **(Satisha and Devarajan, 2005).** Humic acid (HA) from wheat straw leachate can inhibit the formation of insoluble Ca phosphates and thus may enhance P bioavailability **(Grossl and Inskeep, 1991).**

Robbins et al 1999, has reported that large-scale manure applications to a silty clay loam soils results in a linear increase in available P, up to a soil dependent limit. Humic and fulvic acids comprise a wide variety of organic materials that are present in all agricultural soils. Their effects on plant growth and nutrition are well documented, **(Adani et al 1998)** and they can be applied to improve soil structure and increase crop yields. Reports on the influence of humic materials on P retention and release have largely focused on the mineral components of the soils studied. Recent work indicates that the occurrence of Al and Fe has a significant effect on the P sorption capacity, despite the presence of large amounts of organic matter **(Giesler et al 2005)**. Earlier, it had been shown that P decreases the sorption of organic C to acid mineral soils, suggesting a ligand exchange process at the surface **(Kaiser and Zech, 1997)**.

As regards the reverse, *i.e.* the release of P under the influence of dissolved humic materials; **(Delgado et al 2002)** have published one of the few accounts dealing with this issue. They found that application of humics to the soil increases the recovery of Olsen P in all soils tested, except in those with very high Na content. Humic acids preparation was reported to increase the uptake of mineral elements **(Mackowiak et al 2001),** to promote the root length **(Cenellas et al 2002**), and to

increase the fresh and dry weights of crop plants **(Chen et al 2004)**. Due to the positive effect of humic substances on the visible growth of plants. Thus, the aim of this work is to evaluate the effect of some factors such as $CaCO_3$, pH, bintonite, organic matter (humic acid) on P solubility in the rhizosphere as well as increasing the efficiency of such nutrient in the studied soils.

MATERIALS AND METHODS

The materials

Organic matter

Organic matter was used as humic acid (HA), potassium humate powder, supplied by Union for Agriculture Development CO. The chemical composition of potassium humate is given in **Table 1**.

Bentonite and rock phosphate

A sample was taken from the El-Ahram Mining Company. The crushed original minerals were ground and passed through 125-250µm diameter and were dried in an oven at 100 ± 5 °C for 24 h. The results of the some physical and chemical properties are shown in **Table 1**.

The experiment

Split Medium Technique experiments

The combined sand and solution culture technique previously designed and used by **(Elgala and Amberger 1988)** was used in this investigation, as shown in **Fig. 1**. The technique was used to study the role of plant roots in mobilizing P from rock phosphates or super phosphates. In this work broad bean plants (*Vicia faba* var. balady) was used as test plants. Seeds were first germinated between filter papers. After one week, five seedlings were transferred to each of the upper Neubauer pots to which 400 g of acid washed sand as pure media were placed. The seedlings were irrigated with various composition of a nutrient solution **(Elgala and Amberger, 1988).**

Table. 1. Some physical and chemical properties of the used humic acid (HA), bentonite and rock phosphate (RP)

| Characteristics	Humic acid	Bentonite	Rock phosphate
Silt, %	nd	30.2	nd
Clay, %	nd	57.3	nd
SP, %	nd	78.0	nd
EC_e, dS.m^{-1}	1.30	2.30	3.6
pH (1: 5)	4.70	8.13	7.3
OM, %	86.7	nd	nd
OC, %	50.2	nd	nd
CEC, c mol c/kg	174	83.0	4.69
Soluble ions (soil paste ext.), meq/l			
Ca^{2+}	1.80	12.0	30.0
Mg^{2+}	1.50	4.60	5.0
Na^+	0.70	5.00	4.57
K^+	8.20	1.27	0.29
Cl^-	3.70	5.40	1.20
HCO_3^-	2.80	3.70	20.0
CO_3^{2-}	nd	nd	nd
SO_4^{2-}	6.50	13.8	18.7
Chemically available nutrients, µg/g			
N	7.00	nd	nd*
P	22.0	0.20	3.42
K	210	80.0	nd
Fe	12.8	3.80	nd
Mn	10.2	12.5	nd
Zn	3.00	9.40	nd
Cu	1.60	0.20	nd
Total macronutrients, %			
N	4.80	nd	0.50
P	0.83	0.75	8.45
K	6.00	1.80	0.02
C:N ratio	10.4	nd	nd

*nd= not detected
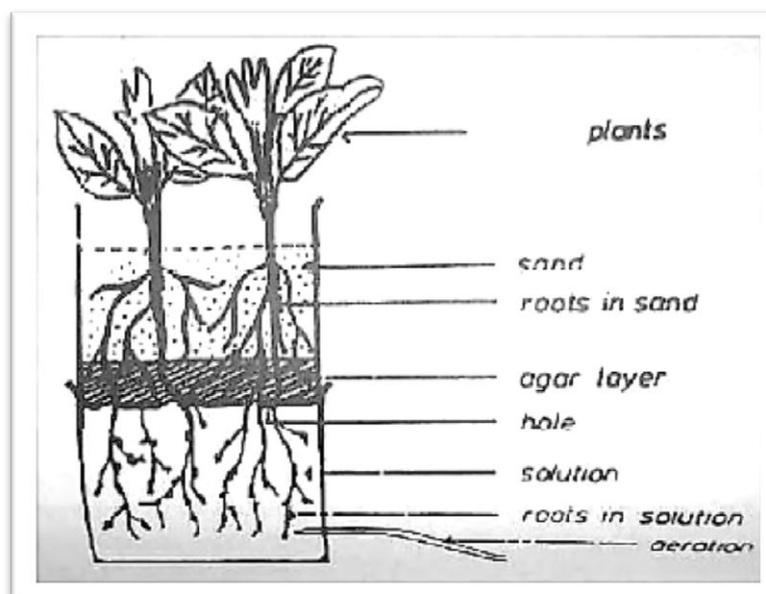
Fig. 1. Split medium technique

As follows: (a) Nutrient solution without P (modified Hoagland nutrient solution 0.5 M), (b) Complete nutrient solution with P (NO$_3^-$: NH$_4^+$ is 1:4) , (c) Solution B with N in the NH$_4^+$ form, (d) Solution B with N in the NO$_3^-$ form.

The pH of the nutrient solution was adjusted to pH 5.5. The experiment was conducted in green house, after 15- days from seedling each pot was placed over another one as shown in **Fig. 1** and the solution in the lower pot was the same as the irrigation solution of the upper pot. The roots penetrated the agar layer and the root systems grew normally in the lower pots. The changes in pH of the lower solution was recorded, also the root exudate was collected in 500 ml of CaCl$_2$ solution 0.5 × 10^{-4} M and pH 6.85.

After one week from placing, the lower pot containing the CaCl$_2$ solution, the following treatments were applied on the control without P (–P) growing plants:

1. Nutrient solution without P (Control – P)
2. Complete nutrient solution (Control)
3. Nutrient solution whereas N form was NH$_4^+$ (Hoagland (N=NH$_4^+$))
4. Nutrient solution whereas N forms was NO$_3^-$ (Hoagland (N=NO$_3^-$))
5. Nutrient solution without P + 1 g. *R.P (P=RP)
6. Nutrient solution without P + 1g.* S.P (P=SP)
7. Treatment No 5+ 1g. CaCO$_3$ (P=RP+CaCO$_3$)
8. Treatment No 5+ 1g. bentonite (*P = RP + bentonite)

9. Treatment No 5+ 1g. H.A(P=RP + HA)
10. Treatment No 5+ 1g. bentonite + 1 g. H.A(P=RP + bentonite + HA)
11. Treatment No 6 +1g. CaCO$_3$ (P=SP + CaCO$_3$)
12. Treatment No 6 +1g. bentonite (P=SP + bentonite)
13. Treatment No 6 +1g. H.A (P=SP + HA)
14. Treatment No 6 +1g. bentonite + 1g. H.A (P=SP + bentonite + HA)

* SP means super phosphate.
*RP means rock phosphate.
(P=RP) means the source of P was rock phosphate.

After one week from applying the treatments, plants were harvested and separated to shoots and roots, washed with distilled water, dried at 70°C, fresh and dry weight was recorded then kept for P analyses. The solution in the lower pots of all the treatments was filtered (using filter paper); completed to 500 ml solution, the pH was measured with the glass electrode and then analyzed for soluble P**(John, (1970)**.

Statistical Analyses

The experiment was designed in a completely randomized design and each treatment was replicated three times. Data were statistically analyzed by multiple factor analysis of variance with the Duncan's multiple range test (5% level), using MSTAT program.

RESULTS AND DISCUSSION

After 30 days results illustrated in **(Fig. 2, 3 and Table 2**) showed that , in general, the highest $NaHCO_3$ extractable P were given by the treatment of SP + bentonite + HA. The behavior of rock phosphates with the studied factors indicated slight solubilization as compared to the addition of HA or bentonite separately or together. The presence of relatively high soluble Ca^{2+} and low native humus beside the presence of relatively high pH value could be the reason for the limited solubility of the rock phosphate. However, the extractable P from the rock phosphate + organic matter treatment increased compared to the control may be due to increasing P mobilization.
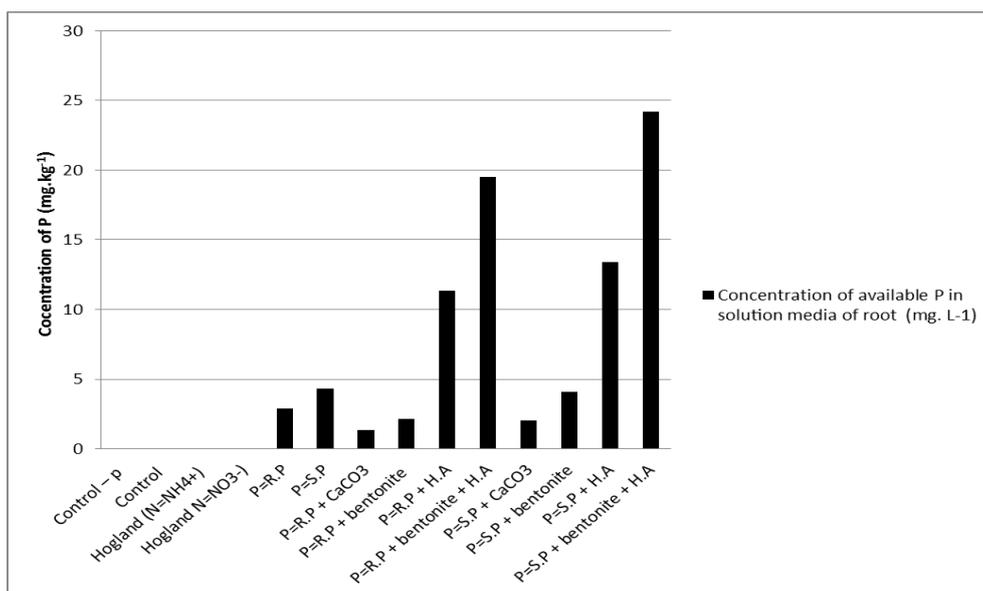

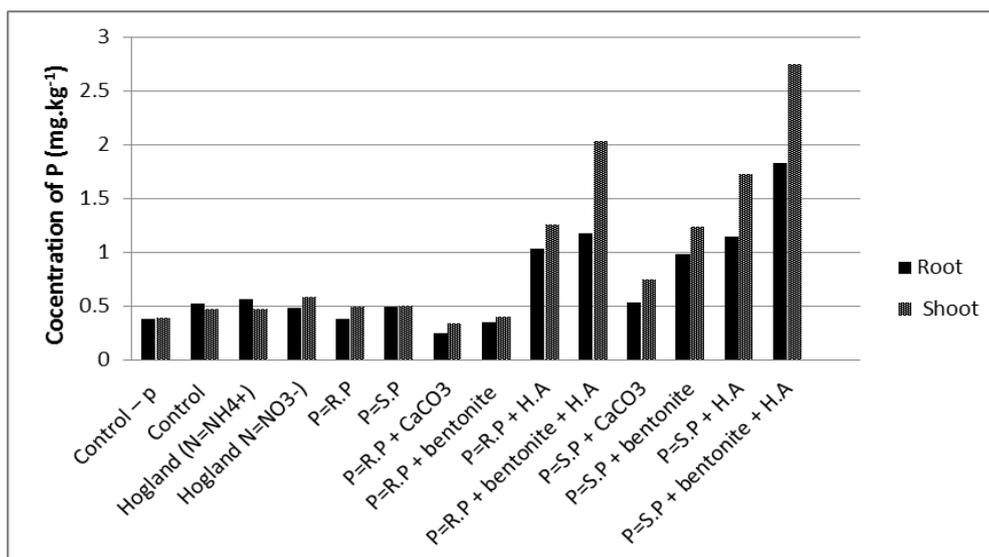**Fig. 2.** Effect of $CaCO_3$, pH, HA and bentonite on availability of P

Fig.3. Effect of CaCO3, pH, HA and bentonite on concentration of P in plant

Table. 2. Effect of CaCO$_3$,pH,HA and bentonite on availability of P

Treatments	Concentration of available P in solution media of root (mg. L^{-1})	Concentration of P in plant (mg . kg^{-1})	
		Root	Shoot
Control – p	0.00100 K	0.3760 L	0.3940 M
Control	0.001333 K	0.5260 H	0.4763 J
Hogland (N=NH$_4^+$)	0.0007333 K	0.5610 F	0.4700 K
Hogland N=NO$_3^-$)	0.001000 K	0.4803 J	0.5817 G
P=R.P	2.900 G	0.3800 K	0.4947 I
P=S.P	4.326 E	0.4890 I	0.5003 H
P=R.P + CaCO$_3$	1.376 J	0.2520 N	0.3360 N
P=R.P + bentonite	2.125 H	0.3500 M	0.4020 L
P=R.P + H.A	11.32 D	1.029 D	1.255 D
P=R.P + bentonite + H.A	19.50 B	1.176 B	2.033 B
P=S.P + CaCO$_3$	2.030 I	0.5313 G	0.7430 F
P=S.P + bentonite	4.080 F	0.9783 E	1.235 E
P=S.P + H.A	13.41 C	1.144 C	1.728 C
P=S.P + bentonite + H.A	24.21 A	1.831 A	2.75

. The same streak within factors are not different but A value>B>C….etc. at the 5% level according to Duncan's multiple range test.

* SP means Super phosphate.   * RP means Rock phosphate.   (P=RP) means the source of P was Rock Phosphate.

The composting rock phosphate materials with straw or other organic waste materials resulted in phosphate mobilization. They further indicated that the driving force for rock phosphate solubilization is the pH decrease and the chelating effect of simple organic acids as citric and malic, and complex acids as humic and fulvic acids on Ca^{2+} ions from insoluble phosphate forms. Also, increasing available P in soil by addition with these treatments reflected on increasing P concentration in bean roots and shoots, with high concentrations in shoots more than roots **(Amberger and Singh 1981**, **Amberger, 1991)**. **Sahin et al (2014)** reported that humic substances in interaction with P in the soil could decrease the P fixation and increase the P uptake of plants. Humic acid served as a buffer at a broad pH interval and several macro and micro elements could be taken by the plants since the soil was neutralized.

Results illustrated in **Fig. 4 and Table 3** indicated a decrease in the pH value of about 3-units in the solution when NH$_4^+$ was used as a source of N in the nutrient solution, while there was an increase of about 0.2-unit of the pH when NO$_3^-$ was the only N-form added. Such phenomenon was reported by several investigators for leguminous and non-leguminous plants **(Fisher et al 1989; Gahoonia and Nielsen, 1992** and **Gahoonia et al 1992)**. It was further postulated that with NH$_4^+$ as N source more cations than anions were taken up to maintain an electrical charge balance and conse

quently a net H$^+$ ion extrusion from the root occurred **(Hoffland et al 1987)**.

The results found for bean plants showed also a drop in pH of about 2.4 units for P- deficient plants; it was reported that under certain stress conditions, roots, in order to overcome such phenomena exudates organic molecules as citric and malic acids **(Hoffland et al 1989** and **Grieson, 1992)**, amino acids and reducing or chelating compounds **(Amberger and Singh, 1981; Elgala and Amberger, 1988** and **Grover, 2003)**.

On the other hand, results of pH value in the presence of plants show a pronounced increase as a result of root activity which reached to an increase of about 0.1 to 5 units in all P treatments **(Fisher et al 1989 and Gahoonia et al 1992b)**. It is worth to mention that more increase in pH was recorded with the low reactive source of RP + CaCO$_3$, followed by the same P source with addition of HA. **Bais et al (2006)** divided root exudates into two classes of compounds. Low-molecular weight compounds such as amino acids, organic acids, sugars, phenolics, and other secondary metabolites account for much of the diversity of root exudates, whereas high molecular weight exudates, such as mucilage (polysaccharides) and proteins, were less diverse but often composed a larger proportion of the root exudates by mass. **(El-Sherif et al 1976)** reported that from the factors affecting P-fixation in soils were pH, clay minerals, CaCO$_3$ content as well as soluble Ca in alkaline and soluble Al and Fe in acid soils.
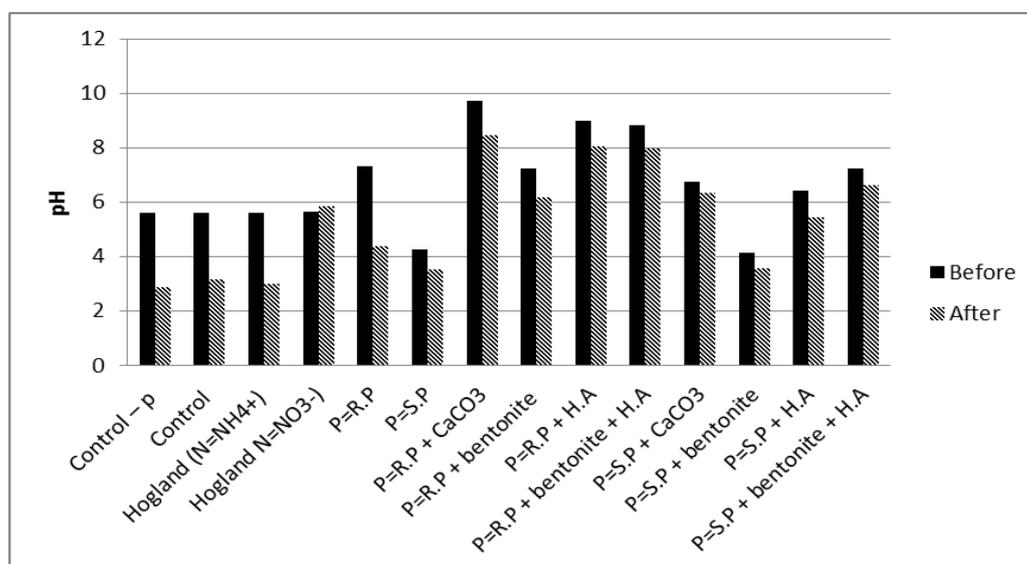
Fig. 4. Effect of CaCO$_3$, pH, HA and bentonite on pH of root's solution

Table .3. Effect of CaCO$_3$, pH, HA and bentonite on pH of media, fresh and dry weight (g.)

	pH of solution media of root		Fresh root	Dry root	Fresh shoot	Dry Shoot
	Before	**After**				
Control – p	5.613 I	2.867 M	1.113 C	0.2630 N	3.813 EF	0.5960 H
Control	5.597 I	3.167 K	4.317 A	0.4580 E	5.493 B	0.7010 D
Hogland (N=NH$_4^+$)	5.620 HI	2.980 L	1.403 C	0.3707 I	4.220 DE	0.5873 I
Hogland N=NO$_3^-$)	5.650 H	5.823 G	4.577 A	0.4500 F	6.400 A	0.8790 A
P=R.P	7.330 D	4.367 I	1.263 C	0.4000 H	4.583 CD	0.6960 DE
P=S.P	4.243 J	3.530 J	1.680 C	0.3103 M	3.697 F	0.5930 H
P=R.P + CaCO$_3$	9.737 A	8.467 A	4.873 A	0.3267 L	4.543 CD	0.5560 J
P=R.P + bentonite	7.237 E	6.167 F	4.223 A	0.3617 J	5.480 B	0.6330 G
P=R.P + H.A	8.970 B	8.027 B	3.140 B	0.4303 G	4.497 CD	0.7647 C
P=R.P + bentonite + H.A	8.837 C	7.960 C	4.017 AB	0.5003 B	5.360 B	0.8810 A
P=S.P + CaCO$_3$	6.753 F	6.337 E	3.940 AB	0.4937 C	4.730 C	0.6940 E
P=S.P + bentonite	4.120 K	3.557 J	3.297 B	0.5467 A	4.477 CD	0.8660 B
P=S.P + H.A	6.420 G	5.423 H	4.717 A	0.4780 D	5.697 B	0.6770 F
P=S.P + bentonite + H.A	7.240 E	6.610 D	3.193 B	0.3320 K	6.637 A	0.6813 F

- The same streak within factors are not different but A value> B>C…….etc. at the 5% level according to Duncan's multiple range test.

* SP means Super phosphate.

*RP means Rock phosphate.

(P=RP) means the source of P was Rock Phosphate.

The fresh and dry weights of bean shoots shows relatively higher values when NO_3^- the only form was supplied **(Table 3)**. It is interesting to note that the root/shoot ratio was the highest for P-deficient plants, **Foehse and Jungk (1983)** and **Hoffland et al (1989)** indicated that root growth and number of root hairs and length increased, also the amount of H^+ ion increased as plants became more P-deficient.

Data of fresh and dry bean weights illustrated in **Figs. 5 and 6 and Table 3** confirm the previous finding of the role of root exudates in mobilizing insoluble P sources and also the action of soil components as the fresh and dry weights of both shoots and roots remarkably increased with the lowest effect with RP when was applied alone. The highest fresh root weight was recorded by the treatment of SP + HA, the highest dry root weight was recorded by the treatment of SP + bentonite. While, the highest fresh shoot weight was recorded by the treatment of SP + bentonite + HA, the highest dry shoot weight was recorded by the treatment of RP + bentonite + HA.
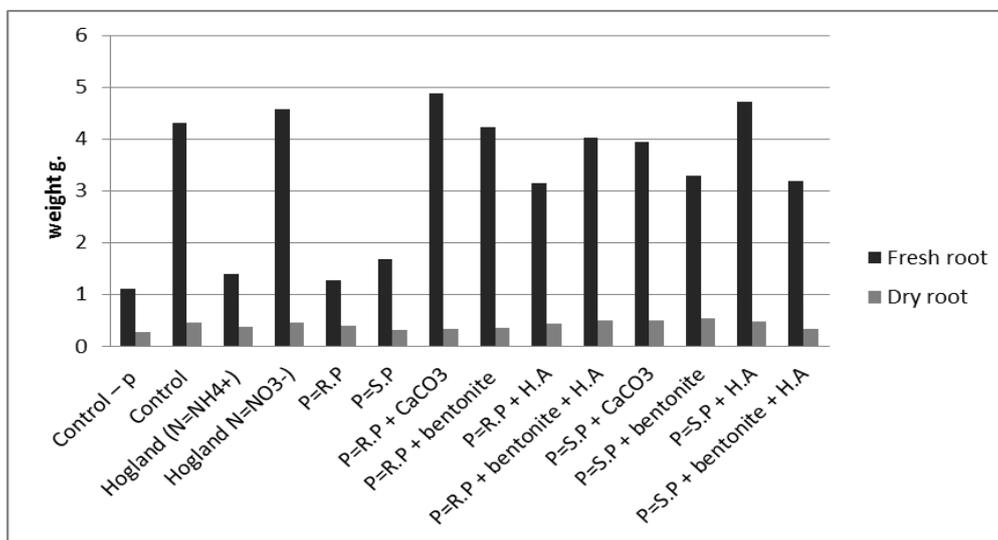

Fig. 5. Effect of $CaCO_3$, pH, HA and Bentonite on pH of fresh and dry weight for shoot (g.)
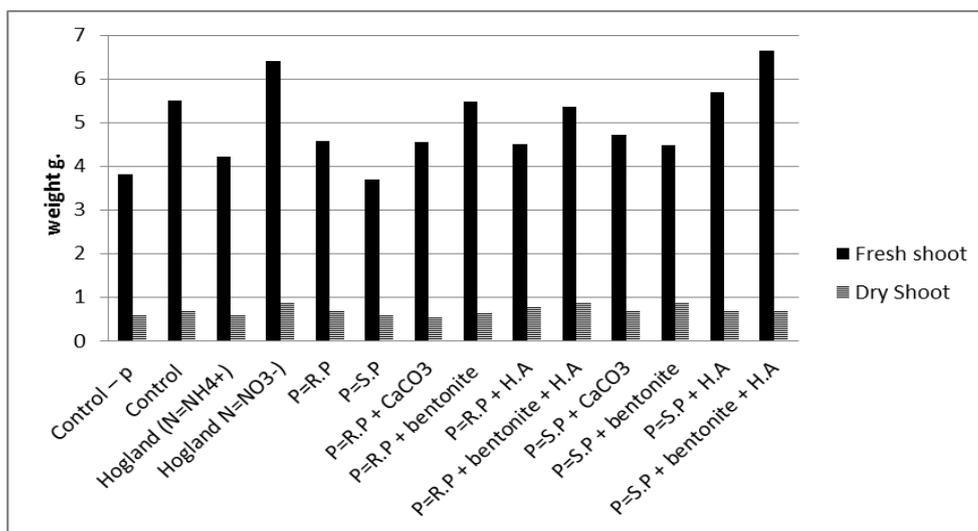

Fig. 6. Effect of $CaCO_3$, pH, HA and Bentonite on pH of fresh and dry weight for root (g.)

Thus, it is clear that roots of bean plants were able to utilize insoluble sources of rock phosphate placed in solution but soil components interacted with solubilized P and hinder it unavailable to plants especially when $CaCO_3$ was added. So the mechanism of solubilizing rock phosphate by exudating protons or organic, amino and other organic compounds is possible at the root surface even in alkaline soil as long as the rock phosphate material was added near the root and organic matter a combined with to limit the fixing power of the inorganic components as $CaCO_3$ and excess soluble Ca. Also, the availability of P from rock phosphate sources depends on its reactivity value. These results agreed with those reported by **Bais et al (2006)**.

CONCLUSIONS

The factors affecting P-fixation in soils were pH, clay minerals, $CaCO_3$ content as well as soluble Ca in alkaline and soluble Al and Fe in acid soils. Rock phosphate can be used as a source of P in alluvial and calcareous soils as long as organic matter is mixed close to the root system. the role of root exudates in mobilizing insoluble P sources and also the action of soil components as the fresh and dry weights of both shoots and roots remarkably increased with the lowest effect with RP when was applied alone. Moreover, application of rock phosphate + organic matter treatment increased compared to the control may be due to increasing P mobilization. The result indicated that SP + bentonite treatment was the most effective to increases dry root weight. while, the highest dry shoot weight was recorded by the treatment of RP + bentonite + HA.

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دراسة على بعض العوامل المؤثرة على تيسر الفوسفور فى منطقة الريزوسفير

[84]

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الموجـــــز

تهدف هذه الدراسة إلى دراسة تأثير بعض العوامل مثل كربونات الكالسيوم، المادة العضوية (هيومات بوتاسيوم)؛ درجة حموضه الوسط؛ وكذلك بعض المركبات الطبيعية مثل البنتونيت على زيادة تيسر الفوسفور للنبات بإستخدام صخر الفوسفات وسماد السوبر فوسفات الثلاثى وتقدير الكمية الميسرة منهما وتركيزهما فى النبات تحت الدراسة.

وعلى هذا تم إجراء تجربة الوسط المنقسم وكان الهدف منها دراسة تأثير المعاملات المختلفة على زيادة تيسر الفوسفور للنبات (فى مرحلة النمو الخضرى) تحت تأثير إفرازات الجذور وتأثير العوامل محل الدراسة.

تم تسجيل التغيرات في الرقم الهيدروجيني لمحلول الجذور السفلي، كما تم قياس تركيز الفوسفور الميسر

فى محلول الجذر وفى المجموع الجذرى والمجموع الخضرى و كذلك الوزن الطازج والجاف لكلاهما.

أوضحت النتائج أن أعلى وزنٍ جاف تم تسجيله تم العثور عليه عندما تم تطبيق كلاً من الأشكال N في نسبة 1: 4 NO^-_3: NH^+_4 أو NO^-_3 بمفردها. كما تظهر النتائج أن درجة تيسر الفوسفور كانت أعلى فى وجود المادة العضويه مع البنتونيت والسوبر فوسفات ثم المادة العضويه مع البنتونيت وصخر الفوسفات تليها المادة العضويه مع صخر الفوسفور بشرط وضع المعاملات فى الطبقة السطحيه جوار منطقة إنتشار الجذور حيث وجد أن إفرازات الجذور لها تأثير مؤثر على تيسر الفوسفور للنبات وزيادة معدلاته.

الكلمات الدالة: المادة العضوية،البنتونيت، كربونات الكالسيوم $CaCO_3$، درجة الحموضة، صخر الفوسفات، منطقة الريزوسفير، تقنية الوسط المنقسم، نباتات الفول