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Effect of Inoculation with Phosphate Solubilizing Actinomycete Isolates on Phosphorous Uptake and Growth of Wheat Grown in Calcareous Sandy Soil

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Abstract

Two pot experiments were conducted in this study during the period of 2015 to 2017. The first pot experiment was conducted, during the winter, of 2015/2016 to test the effect of inoculation with nine actinomycete isolates on phosphorous uptake and growth of wheat (*Triticum Sativa*) plants in sandy calcareous soil and choose the best of two actinomycete isolates. All actinomycete isolates caused significant increase (p = 0.05) in plant height and shoot fresh weight except for strain A3, but non-significant increase in root fresh weight. It should be noticed that the isolates A4 and A10 induced the highest stimulative effects on growth parameters of wheat. The second pot experiment was conducted in 2016/2017 to test the effect of inoculation with actinomycete isolates (the best stains A4 and A10) on P-uptake and growth of wheat plants grown in sandy calcareous soil under different chemical phosphorous levels (P1=25%, P2=50% and P3=100% from recommended doses). The A4 actinomycete isolate showed higher promotive effects on plant height, root and shoot dry weights and P- uptake by the shoots than those shown by the A10 actinomycete isolate. Inoculation with the A4 actinomycete isolate resulted in an increase in p- uptake by plant shoots up to 297.97 %, of the uninoculated control. In other words, the best promotion effect from inoculation with A4 actinomycetes isolate was obtained at p- fertilization level 50 % of RDP. These results indicate the importance of plant inoculation with phosphate solubilizing microbes, such as A4 actinomycete isolate to decrease the application of p-fertilizers in agriculture.

***Keywords**: Inoculation, Streptomyces, inorganic phosphate, phosphate solubilization, chemical fertilizers.*

Introduction

Phosphorus (P) is an essential macroelement for plants, yet the total concentration of P in soils ranges from 0.02% to 0.5%; an average approximately 0.05%. The variation being largely due to differences in the weathering intensity and parent material composition (Stevenson, 1986). Thus, to increase the availability of phosphorus for plants, large amount of fertilizers are used on a regular basis, yet after application, a large proportion of the fertilizer phosphorus is quickly transferred to an insoluble form (Omer, 1998). In calcareous soils, phosphorus fertilizers are fixed by calcium carbonate through adsorption and precipitation, resulting in an efficiency of less than 20% (Tisdale *et al*. 1993). The solubilization of phosphate-bearing inorganic materials by microorganisms would seem to be an attractive solution that has been actively studied during the last decade. Several mechanisms, such as

lowering the pH by acid production, ion chelation and exchange reaction in the growth environment, have been reported to play a role in P-solubilization by phosphate-solubilizing microorganisms (PSM) (Halder *et al*. 1991; Rajankar *et al*. 2007).

Among these PSM, actinomycetes are of special interest since these filamentous sporulating bacteria are able to develop in extremely different soils (Jiang *et al.,* 2005; Pathom-Aree *et al.,* 2006) and produce various substances (anti-fungi, insecticides, anthelminthics, phytohormone-like compounds etc.) that could benefit plant growth (Fabre *et al.,* 1988; Manulis *et al.,* 1994; Ikeda, 2003; Jain and Jain, 2007).

Hamdali *et al.* (2008) showed that the abundance of actinomycetes solublizing Moroccan rock phosphate, from a Togolese phosphate mine, was approximately 19%. Reports in the literature suggested that microbial solubilization of mineral phosphate might be either due to the excretion of organic acids causing acidification of the external medium (Whitelaw, 2000), or to the excretion of chelating substances (such as sidero-phores) that form stable complexes with phosphorus adsorbents (aluminium, iron and calcium) (Welch *et al.,* 2002), and thus increase phosphate solubilization. The present investigation was carried out to study the effect of inoculation with phosphate solubilizing actinomycete strains on phosphorous uptake and growth of wheat plants grown in sandy calcareous soil under different chemical phosphorous levels.

Materials and Methods

Actinomycete isolates used:

Nine phosphate solubilizing actinomycetes isolate identified as *Streptomyces sp.* were previously isolated from rhizosphere soil by Farid (2019), were used in this study. The strains were maintained on starch casein agar (SCA) slants at 4 ºC in a refrigerator.

Effect of inoculation with the isolated actinomycete strains on promoting plant growth:

Two pot experiments were conducted during the period of 2015 to 2017 to test the response of wheat (*Triticum Sativa*) plants to inoculation with actinomycete isolates. Experiments were conducted in pots (25 cm in diameter) containing 5 kg air-dried sandy calcareous soil collected from El-Ghoriab farm. Some physical and chemical characteristics of representative soil sample of the pot experiments are shown in Table (1).

Table 1. Some Physical and chemical characteristics of representative soil sample of the pot experiments.

Soil property	Depth(cm) 0-30
Partical size distribution	
Sand %	92.01
Silt %	4.72
Clay %	3.27
Texture	Sandy
EC (1:1) dms^{-1} at 25 ºC	1.12
pH (1:1)	8.50
Total Calcium carbonate %	16.95
Organic matter %	0.12
Soluble ions (1:1) meq/100 g soil	
Ca^{++}	0.26
Na^{++}	0.54
K^{+}	0.05
Cl^{-}	0.52
$CO_3^{--} + HCO_3^{-}$	0.46
Total nitrogen %	0.02
$NaHCO_3$-extractable p (ppm)	4.86
DTPA-extractable micronutrients (ppm)	
Fe	2.19
Mn	1.76
Zn	0.33
Cu	0.76

The first pot experiment:

Wheat (cv. seds 1) was used as the test plant. The pot experiment of the first season was conducted, during the winter, of 2015/2016 to test the effect of inoculation with the actinomycete isolates on wheat plants in sandy calcareous soil and choose the best two actinomycete isolates. The seeds were soaked in concentrated H_2SO_4 for 30 seconds and washed with sterile distilled water seven times to remove H_2SO_4. Actinomycete isolates were grown each on 100 ml aliquot of starch casein broth medium in 250 ml Erlenmeyer flasks. The flasks were incubated at 28 ºC for 7 days. The cultures contained about 10^9 viable cells of actinomycetes/ml. Inoculation was made by soaking the grains in the broth cultures for one hour before planting and 5 ml from the remaining culture was added to each pot. Fifteen wheat grains uninoculated or inoculated with one of each of the actinomycete isolates were planted in plastic pot. Four replicate pots were assigned to each inoculation treatment as well as the uninoculated control. The pots were irrigated every 2-4 days by tap water. After germination the plants were thinned to 10 plants/pot.

The second pot experiment:

The second season pot experiment was conducted, during the winter of 2016/2017 to test the effect of inoculation with actinomycete isolates (the best two isolates) on P-uptake and growth of wheat plants in

sandy calcareous soil under different P-levels. The experimental treatments were arranged as spilt plots on the basis of a Randomized Complete Block Design (RCBD) with four replications. The main plot was devoted to different levels of the phosphorus chemical fertilizer (super phosphate 15.5% P_2O_5), consisting of P1=25%, P2=50% and P3=100% from recommended doses. Whereas the sub plots were assigned for the microbial inoculation: 1) uninoculated; 2) and 3) inoculated with the best two isolates of actinomycete.

Collection of Plant Samples:

Plant samples were collected in December 2016 (the first season), December 2017 (the second season) at 45 days after sowing (DAS), the wheat plants were uprooted. Immediately after sampling, the plants were transferred to the laboratory and the roots were washed to remove soil particles and organic debris. Plant height cm, Shoot and root fresh weights g/plant, shoot and root dry weights and P-uptake mg/plant were measured in samples.

Plant tissue analysis:

The plant samples of two pot experiments were taken after 45 days from planting and dried at 70°C in a forced draft oven for 24 hours and their dry weights were determined. All data on dry matter yields were used as inputs for P uptake determination. Dry weights of shoots digested using a mixture of conc. $HClO_4$ and H_2SO_4 for determining phosphorus content (Jackson, 1973).

Statistical analysis:

All data were organized into tables in accordance with the MS Excel Spreadsheet and then the data were analyzed by analysis of variance (ANOVA) and significant differences between means were compared using the least significant difference (L.S.D) test at P = 0.05 (Steel and Torrie, 1980).

Results and Discussion

The first pot experiment

The pot experiment was carried out in season of 2016/2017 to test the efficiency of the nine actinomycete isolates on growth of wheat in sandy calcareous soil. Data in Table (3) show the results obtained for inoculated and uninoculated treatments.

All actinomycete isolates caused significant increase (p = 0.05) in plant height and shoot fresh weight except for isolate A3, but non-significant increase in root fresh weight was noticed. Significant increase in shoot dry weight was recorded by inoculation with isolate A3, A4 and A10. Inoculation with isolates A1, A4, A5 and A8 caused significant increases in root dry weight compared with uninoculated control. It should be noticed that the isolates A4 and A10 induced the highest stimulative effects on growth parameters of wheat. These promotions of root and shoot lengths and weights of wheat are probably due to the hormonal effects and the increases in uptake of nutrients by the proliferated roots. Many investigators reported that the actinomycetes are capable of producing growth promoting substances; as hormones (IAA and GA_3), and siderophores in their cultures (Manulis *et al.* 1994; Ikeda 2003; Jain and Jain 2007).

The obtained results show that in general the inoculation with actinomycete strains increase the phospho-

rus uptake by wheat plant. Significant increase in phosphorus uptake in case inoculation with actinomycete isolates A1, A4, A8 and A10, compared with control. Actinomycetes are known to produce acidic metabolites which by change of soil pH or by direct chelation of metal cations, release fixed or insoluble phosphorus in available form (Storkanova *et al*., 1999, Narsian and Patel 2000, Reyes *et al.,* 2002). Many species of actinomycetes are able to solubilize phosphates in vitro and most of them live in the plant rhizosphere. The results of this pot experiment are in accordance with those obtained by Aldesuquy *et al*. (1998) who reported that inoculation of wheat plants with *streptomycete olivaceoviridis* culture significantly increased shoot fresh mass, dry mass, length, and root dry mass.

Table 3. Effect of inoculation with actinomycete isolates on p-uptake and growth of wheat plants grown in sandy calcareous soil.

Treatments	Plant height (cm)	Fresh weight (g/plant)		Dry weight (g/plant)		p-uptake (mg/plant)
		Shoot	Root	Shoot	Root	
Control (uninoculated)	27.60	1.98	1.39	1.20	0.80	13.12
Strain A1	32.40	2.96	1.75	1.21	1.05	19.67
Strain A2	31.00	2.54	1.65	1.19	0.90	16.04
Strain A3	30.00	2.42	1.40	1.45	0.97	16.57
Strain A4	34.00	3.09	1.72	1.38	1.19	21.20
Strain A5	31.20	2.45	1.57	1.08	1.10	15.20
Strain A7	31.80	2.60	1.44	1.20	0.83	18.21
Strain A8	32.20	2.85	1.56	1.29	1.02	23.09
Strain A10	31.80	3.05	1.64	1.38	1.00	21.50
Strain A18	31.00	2.76	1.79	1.25	0.95	19.30
L.S.D 0.05	**3.31**	**0,51**	**0.42**	**0.16**	**0.19**	**6.25**

Effects of inoculation with actinomycete isolates A4 and A10 on growth and p-uptake by wheat under different levels of p-fertilization:

The data obtained on the main effects of inoculation with actinomycete isolates A4 and A10 and different levels of P-fertilization (25, 50 and 100 % from recommended dose of phosphorous, RDP) are shown in Table 4. Inoculating the wheat grain with A4 and A10 actinomycete isolates caused significant increases (p = 0.05) in the plant height, root and shoot dry weights and P-uptake by the shoots of wheat plant compared to the uninoculated control plants. The A4 actinomycete isolates showed higher promotive effects on plant height, root and shoot dry weights and P- uptake by the shoots than those shown by A10 actinomycete isolate. Inoculation with the A4 actinomycete isolate resulted in an increase in p- uptake by plant shoots of 297.97 %, of the uninoculated control. Growth responses and P-uptake

of wheat plants to P fertilization are shown in Table (4). Only, the P fertilization at a level of 100 % of the recommended dose (RDP) had a significant influence on plant height, root and shoot dry weights and P uptake by the shoots and roots.

The interaction effects of the inoculation with the A4 and A10 actinomycete isolates and the P fertilization are given in Table (5). A promotive effect of inoculating the A4 actinomycete isolate on the plant height, root and shoot dry weights and P uptake by plant shoots was more obvious at the P level 50 % of RDP. However, at the higher level of p fertilization (100 % RDP), the promotive effect of inoculation decreased. In other words, the best promotion effect from inoculation with A4 actinomycetes isolate was obtained at P- fertilization level 50 % of RDP. The increases in the plant height, root and shoot dry weights and P uptake by plant shoots were: 37.39, 465.45, 289.65, and 233.6 %; respectively, of the uninoculated control fertilized with 100 % of RDP. These results indicate the importance of plant inoculation with phosphate solubilizing microbes, such as A4 actinomycete isolate for decreasing the application of P-fertilizers in agriculture.

Recent advances on the application of actinomycetes in cereal crop such as wheat was conducted. Aldesuquy *et al*. (1998) studied the effect of *streptomycete* culture filtrates on the growth of wheat plants. Shoot fresh mass, dry mass, length, and diameter, significantly increased with certain strains at varying sample times. *S. olivaceoviridis* had a pronounced effect on yield components (spikelet number, spike length, and fresh and dry masses of the developing grains) of wheat plants. The culture filtrates of all three strains appeared to enhance the growth and crop yield of wheat plants.

Table 4. Main effects of P-fertilization levels and inoculation with actinomycete isolate A4 and A10 on growth and p-uptake by wheat grown in sandy calcareous soil.

Treatments	Plant height	Fresh weights g/plant		Dry weights g/plant		P-uptake
	Cm	Shoot	Root	Shoot	Root	mg/plant
Ino. Treatment						
Control(uninoculated)	27.83	1.6	0.71	0.56	0.25	18.28
Isolate A4	40.50	6.95	2.92	2.82	1.18	72.75
Isolate A10	33.66	5.63	2.85	2.56	0.93	63.90
L.S.D 0.05	3.95	0.65	0.43	0.2	0.15	5.73
Level of P-fertilizer						
25 %	33.12	3.24	1.57	1.49	0.61	35.94
50 %	35.00	4.38	2.13	1.93	0.81	46.12
100 %	37.75	4.70	2.21	2.00	0.88	50.95
L.S.D 0.05	3.95	0.65	0.43	0.2	0.15	5.73

Table 5. Interaction effects of P-fertilization levels and inoculation with actinomycete isolates A4 and A10 on growth and p-uptake of wheat palnts.

Incoculation X P-fertilizer		Plant height (cm)	Fresh weights (g/plant)		Dry weights (g/plant)		P-uptake (mg/plant)
			Shoot	Root	Shoot	Root	
Control (uninoculated)	**25 %**	27.50	0.62	0.37	0.33	0.19	13.40
	50 %	27.25	2.04	0.75	0.79	0.27	18.65
	100 %	28.75	2.13	1.01	0.55	0.29	22.78
Isolate A4	**25 %**	40.75	5.31	2.21	2.49	0.87	55.46
	50 %	39.50	7.96	3.14	3.11	1.13	76.00
	100 %	41.25	7.59	3.19	2.86	1.08	72.59
Isolate A10	**25 %**	30.75	4.89	2.65	2.33	1.06	53.00
	50 %	31.75	5.70	2.97	2.29	0.85	63.65
	100 %	38.50	6.28	2.15	3.05	1.34	89.25
L.S.D 0.05		**3.95**	**0.65**	**0.43**	**0.20**	**0.15**	**5.73**

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تاثير التلقيح بسلالات الأكتينوميسيتات المذيبة للفوسفات على امتصاص الفوسفور و نمو نبات القمح النامى فى تربة رملية جيرية

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الملخص

فى هذه الدراسة تم عمل تجربتين أصص على نبات القمح باستخدام تربة رملية جيرية (ذات محتوى منخفض من عنصر الفوسفور). فى التجربة الأولى تم استخدام ٩ عزلات اكتينوميسيتات تنتمى الى جنس الاستربتوميسيس *Streptomyces* (A1, A2, A3, A4, A5, A7, A8, A10 , A18) لها القدرة على اذابة الفوسفات لدراسة تاثيرها على امتصاص الفوسفور و نمو نبات القمح خلال الموسم ٢٠١٥/٢٠١٦ حيث لقحت حبوب القمح بسلالات الأكتينوميسيتات التسعه منفردة مقارنة بمعاملة غير ملقحة. وقد أظهرت النتائج ان التلقيح بعزلات الأكتينوميسيتات سجلت زيادة معنوية في طول النبات والوزن الرطب للمجموع الخضري باستثناء العزلة A3 ، بينما سجلت كل العزلات زيادة غير معنوية في الوزن الرطب للمجموع الجذري مقارنه بالمعامله الكنترول (الغير ملقحة). كما يتضح ان العزلتين A4 وA10 سجلت افضل نتائج علي نمو نباتات القمح وعلي الفوسفورالممتص عند التلقيح بها مقارنه بالعزلات الآخري من التلقيح.

وفى تجربة الأصص الثانية خلال موسم ٢٠١٦/٢٠١٧ تم دراسة التلقيح بسلالات الأكتينوميسيتات (العزلتين A4 وA10) على امتصاص الفوسفور و نمو نبات القمح تحت مستويات مختلفة من التسميد الفوسفاتى(P1=25%, P2=50% and P3=100%) من الموصى به. و قد أظهرت النتائج أن العزلة A4 لها تأثير افضل من العزلة A10 حيث سجلت زيادة معنوية في طول النبات والوزن الجاف للمجموع الخضري والمجموع الجذري وكذلك في الفوسفور الممتمص مقارنه بالعزلة A10. واظهرت نتائج تأثير التداخل (التلقيح بالعزلتين A4وA10 مع التسميد الفوسفاتي) أن التلقيح بالعزلة A4 ادي الي زيادة معنوية واضحه في طول النبات والوزن الجاف للمجموع الخضري والمجموع الجذري وكذلك في الفوسفور الممتص بواسطه المجوع الخضري عند مستوي ٥٠% من التسميد الفوسفاتي مقارنه بالمعاملة غير الملحقة عند مستوي ١٠٠%من التسميد الفوسفاتي. هذه النتائج توضح اهمية تلقيح النبات بالميكروبات المذيبة للفوسفات (التلقيح الحيوي) مثل عزلة الأكتينوميسيتات A4 خصوصا في الأراضي الجيرية للتغلب علي مشاكل تثبيت الفوسفور فيها وذلك لتقليل استخدام الاسمدة الفوسفاتيه الكيماوية في الزراعة.