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Impact of fermented solution produced by rock phosphate solubilizing *Aspergillus tubingensis* RPf10 strain on bean plant growth

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ABSTRACT

In a pot experiment, *Aspergillus tubingensis* RPf10 fermented solution at different P concentrations (from 49.25 to 197.01µgP ml^{-1}) were applied as foliar spray during 45 day of bean plants cultivation at different treatments. The treatment T7 irrigated with Hoagland and foliar spray with non-filtered fermented solution (presence of fungus) at 197.01 µgP/ml could be more favorable than that irrigated with Hoagland and foliar spray with filtered fermented solution (T6) and then Hoagland solution only (T5) for plant growth, as increased the plant height, yield, P content and percentage fermented solution induced plant growth response about 1.05, 0.99 & 1.04, 0.98 fold and 2.53, 1.97, 2.87 & 4.33 fold after 45 days, respectively suggesting its potential use as fertilizer.

***Keywords*:** *Aspergillus tubingensis*, rock phosphate, phosphate solubilization, bean plant growth, foliar spray

Introduction

Phosphorus is major essential macronutrients for plant growth and development and hence they are commonly added as fertilizer to optimize yield. Phosphorus also play an indispensable biochemical role in photosynthesis, respiration, energy storage and transfer, cell division, cell enlargement and several other processes in the living plant. It helps plants to survive winter rigors and also contributes to disease resistance in some plants (Sagervanshi *et al.,* 2012). However, a greater part of soil phosphorous, approximately 95-99% is present in the form of insoluble phosphates and hence cannot be utilized by the plants (Kannapiran and Ramkumar, 2011). The use of phosphate-solubilizing microorganisms (PSM) is emerging as a biotechnological alternative for producing soluble P fertilizers from rock phosphate (RP) (Vassileva *et al*, 2010). Seed or soil inoculation with phosphate-solubilizing microorganisms (PSMs) was known to improve solubilization of fixed soil phosphorus and applied phosphates resulting in higher crop yields (Jones and Darrah, 1994; Vassilev *et al*, 1996; Vassilev *et al*, 2006 and Jain *et al*, 2010). PSMs were a low-cost solution that enriches the soil giving a thrust to economic development without disturbing ecological balance. Rock P materials are cheaper sources of P and K; however, most of them are not readily available to a plant because the minerals are released slowly and their use as fertilizer often causes insignificant yield increases of current crop (Zapata and Roy 2004).

The aim of this investigation was to study the effect of fermented solution produced by *Aspergillus tubingensis* RPf10 in raw material medium supplemented with rock phosphate on bean plant growth and P uptake efficiency.

Materials and Method

Microorganisms, media and seeds

Aspergillus tubingensis RPf10 strain was used as solubilizer of phosphate in this study. This strain was previously isolated from rock phosphate and identified using phenotypic and genotypic characteristics (Abou-Taleb *et al.*, 2018). It was maintained by transferring at regular intervals on malt agar slants ((EMCC, 1992) and incubated at 30°C for 24 h. Slants were kept at 4°C until used. *Rhizobium phaseoli* was obtained from field Crops Research Institute, Department of Leguminous

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Crops, Agricultural Research Centre (ARC), Giza, Egypt. The culture medium used for grow nodulation root bacteria was yeast extract mannitol medium (YEM) (Vincent, 1970) [(gL^{-1}): mannitol, 10.0; yeast extract, 1.0; K_2HPO_4, 0.5; $MgSO_4.7H_2O$, 0.2; NaCl, 0.1; $CaCO_3$, 3.0; and adjusted to pH 7.2].

Seeds of bean (*Phasealus nebraska*) obtained from field Crops Research Institute, Department of Leguminous Crops, Agricultural Research Centre (ARC), Giza, Egypt.

Fermentation process

It was carried out in 250 ml plugged Erlenmeyer flask, containing 100 ml sterile modified fermentation medium by Abou-Taleb *et al.* (2018) [(gL^{-1}): sugar beet waste, 30; rock phosphate, 10 and adjusted to pH 7.0] and inoculated with 3% spore suspensions (3.18×10^8 spores ml^{-1}) for *Aspergillus tubingensis* RPf10 strain which incubated at 30°C on rotary shaker at 150 rpm for 12 days. The fermented medium was filtrated using filter papar No.1, the supernatant was taken as the fermented solution.

Soils

A pot experiment was conducted in sandy soil. The basic properties of the soil presented in Table (1).

Table 1: Properties of the sandy soil**.**

Elements	N	P	K	Fe	Mn	Zn	Cu
Conc. of element mgL^{-1}	19.2	16.5	152	2.74	0.78	0.79	0.28

The analysis was carried out in the central Lab., Fac. of Agri., Ain Shams Univ., Qalubia, Egypt.

Nutrient (Hoagland) solution

It was used for surface irrigated plant. Its composition was shown in Table (2):

Table 2: Composition of a modified Hoagland nutrient solution.

Compound	Molecular weight g/ mol	Concentration of stock solution M	Concentration of stock solution g /1iter	Volume of stock solution per litre of final solution ml	Element	Final concentration of element µM	Final concentration of element ppm
Macronutrients							
KNO_3	101.1	1.00	101.10	6.0	N	16000	224
$Ca(NO_3).4H_2O$	236.2	1.00	236.16	4.0	K	6000	235
$NH_4H_2PO_4$	115.1	1.00	115.08	2.0	Ca	4000	160
$MgSO_4.7H_2O$	246.5	1.00	246.49	1.0	P	2000	62
					S	1000	32
					Mg	1000	24
Micronutrients							
KCl	74.6	25.0	1.9		Cl	50.0	1.77
H_3BO_3	61.8	12.5	0.8		B	25.0	0.27
$MnSO_4.H_2O$	169.0	1.0	0.2		Mn	2.0	0.11
$ZnSO_4.7H_2O$	287.5	1.0	0.3	1.0	Zn	2.0	0.131
$CuSO_4.5H_2O$	249.7	0.3	0.1		Cu	0.5	0.032
H_2MoO_4 (85% MoO_3)	162.0	0.3	0.0		Mo	0.5	0.05
Fe-EDTA3	558.5	53.7	30.0	1.0	Fe	20.0	1.12

Pot experiment

A pot experiment was conducted in sandy soil during summer season of 8 / 2014. The pot experiment was carried out in the greenhouse of bio-fertilizer unit, Fac. of Agric., Ain Shams Univ., Shoubra, El-Kheima, Cairo, Egypt, using polyethylene pots (30 cm in diameter and 30 cm deep) filled with 6 Kg soil previously soaked with 5% HCl and followed by twice washes with tap water. Five pots

were used as replicates for each treatment. Pots were arranged in greenhouse in a completely randomized design.

Seeds of were surface sterilized by rinsing in ethanol (90%) and soaking for 10 min in hydrogen peroxide (3% wv^{-1}) followed by two washes in sterile distilled water.

Seeds were germinated in sterilized dishes containing sterile cotton. Sterile distilled water was added at intervals to keep the cotton and germination seeds wet. Seeds were incubated at 30° C for 2:4 days or until radicals were 2 - 3 cm long and appearance of root hairs. Germination was daily observed and germination percentage was 100% which calculated by following formula:

$$= n / N * 100$$

Where, n = Total number of germinated seeds and N = Total number of initiated seeds.

Ten seeds were planted in each pot at a 2 cm depth. Immediately after planting, the seedlings were inoculated with 5 ml inoculum of *Rhizobium phaseoli* strain after 2, 9 and 16 days. Plants were thinned to 5 plants per pot.

The pots were divided into two main groups. The first group was treated with filtrated fermented solution and the second group was treated with non-filtrated fermented solution of *Aspergillus tubingensis* RPf10. Then each group was divided into two sub groups. The first sub-group was surface irrigated twice weekly once with a Hoagland (as nutrient) solution and once with water. The second subgroup was surface irrigated twice weekly (once with a Hoagland free P and once with water). Both groups containing 7 treatments, sprayed with different amounts of fermented solution produced by *Aspergillus tubingensis* RPf10 on sugar beet wastes medium containing soluble rock phosphate.

The seven treatments, namely:

1- Irrigation with tap water (control).
2- Irrigation with Hoagland only.
3- Irrigation with Hoagland free P only.
4- Spraying with 49.25 µg P ml^{-1} of fermented solution.
5- Spraying with 98.5 µg P ml^{-1} of fermented solution.
6- Spraying with 147.75 µg P ml^{-1} of fermented solution.
7- Spraying with 197.01 µg P ml^{-1} of fermented solution.

The plant samples were collected after 30 or 45 days. At harvest, the plant growth parameters were recorded being plant height, root & shoot dry weight and P content of plant.

Analytical methods

Plant (root + shoot) dry weight was determined after drying in an oven at 105°C until constant weight. Phosphorus content in bean plant was determined by using the colorimetric molybdenum blue method described by Jackson (1958).

The specific plant length and percentage fermented solution induced plant growth response (FGR %) were calculated using the following formula (Cloete *et al*, 2009).

SPL = Plant height (cm)/Plant dry weight (g/pot).

% FGR = ((Plant dry weight treatment - Plant dry weight control)/ Plant dry weight control) × 100.

Statistical analysis

Data were analyzed statistically using IBM SPSS® statistics software (2011).

Results and Discussion

Fermentation experimental

In the previous study, *Aspergillus tubingensis* RPf10 grown on modified fermentation medium as a low cost medium containing sugar beet waste and rock phosphate Abou-Taleb *et al.* (2018), the highest RPs content and RPSE% were recorded after 12 days at 30°C being 196.17 µgP ml^{-1} and 6.54%, respectively. Also it could be observed that the fermented solution produced by this fungal strain contained some inducible factors of plant growth such as IAA, organic acid (citric acid) and some micro- & macro-elements have been reported by Abou-Taleb *et al.* (2018).

Application of *Aspergillus tubingensis* RPf10 fermented solution as a nutrient solution for bean plant growth improvement

In this experiments, the *Aspergillus tubingensis* RPf10 fermented solution at different phosphorus concentrations ranged from 49.25 to 197.01 µgP/ml were used as nutrient solution or phosphate fertilizer for bean plant growth. During the plants growth period, the irrigation was carried out using the nutrient Hoagland solution free phosphorus or non-free phosphorus, in addition to foliar spray with filtrated or non-filtrated fermented solutions. The irrigation with Hoagland only (T2), Hoagland free phosphorus (T5), and water (T1) were served as controls.

Data present in Tables (3 & 4) and Figs. (1 & 2) showed that slight increase in the plant growth parameters expressed as plant height (cm), plant yield (g/pot) and phosphate content (µgP/ml) of bean plant growth irrigated by Hoagland free phosphorus (T2) than that irrigated with water (T1), whereas the irrigation with complete Hoagland (T5) increased these parameters about 1.2, 1.33 and 13.38 folds and about 1.5, 1.41 and 20.18 folds, respectively comparing to control (T1) after 30 and 45 day harvest period. The improvement of bean plant growth during 45 days was noticed after the foliar spray with Aspergillus tubingensis RPf10 fermented solution before and after filtrations. Where the values of plant growth parameters were increased during the harvest period with increasing the phosphate concentration of fermented solution to record the highest value after 45 days at 197.01 µgP/ml of fermented solution. At this treatment, the irrigation with Hoagland free P and foliar spray with filtrated (T3) and non-filtrated (T4) fermented solutions gave the plant height of 27 & 29 cm, plant yield of 1.97 & 2.01 g/ pot and phosphate content of 30.65 & 30.77 µgP/ml and percentage fermented solution induced plant growth response of 93.14 & 97.06%, respectively with slight improvement between the growth plants of T3 and T4 treatments. At the latter treatment (T4), the plant height, yield, specific plant length and P content increased to about 2.90, 1.97, 1.47 & 39.96 fold, 2.23, 1.65, 1.35 & 34.97 fold and 1.07, 1.02, 1.05 & 1.00 fold with foliar spray with non-filtrated fermented solution comparing to irrigation with water (control, T1), Hoagland solution free P (T2) and Hoagland solution free P + foliar spray with filtrated fermented solution (absence of fungal strain) (T3), respectively after 45 day harvest period. Whereas the percentage fermented solution induced plant growth response was increased about 4.95 and 1.04 fold at T4 treatment (foliar spray with 197.01 µgP/ml of non-filtrated fermented solution) comparing to irrigation with Hoagland solution free P (T2) and Hoagland solution free P + foliar spray with filtrated fermented solution (absence of fungal strain) (T3), respectively after 45 day harvest period. With regard to, the irrigation with Hoagland and foliar spray with filtrated (T6) or non-filtrated (T7) fermented solutions, the results in Table (45) also indicated that the plant growth parameters increased as the phosphorus concentrations increased to 197.01 µg $P.ml^{-1}$ of fermented solution after 30 and 45 days of snowing with foliar spray of filtered fermented solution (absence fungi) (T6) which achieved the highest values 2.05 & 2.87 g/pot of yield, 14 & 36 cm of plant height, 153.09 & 164.20 % of fermented solution induced plant growth response and 25.54 & 42.74 µgP/ml of P content after 30 and 45 days of plant snowing, respectively.

Whereas, the foliar spray of non-filtered fermented solution (presence fungi) (T7) at P concentration of 197.01 µgP ml^{-1} gave the highest yield of plant 2.14 & 2.84 g/pot, 16 & 38 cm of plant height, 181.37 & 178.43 % of fermented solution induced plant growth response and 24.84 & 44.72 µg P/ml of P content after 30 and 45 days of plant snowing, respectively.

The integrative effects of fermented products, rhizosphere microorganisms and soil plant systems appear to play a vital role in enhancement of the plant growth and soil quality (Vassilev and Vassileva, 2003). Vassileva *et al.* (1998) studied the various combination of olive cake and RP, previously treated and untreated by *A.niger* were introduced into a calcareous and phosphorus (P) - deficient soil to improve the growth of *Trifolium repens* in a greenhouse experiments. Greater growth and P uptake of mycorrhizal and non micorrhizal plants were achieved when microb-treated olive cake and rock P were applied to soil compared with other treatments.

Moreover, the P solubilizers *Bacillus* sp., solubilized phosphorus and increased growth and yield of cotton (Gyaneshwar *et al.,* 2002, El-Komy, 2005 and Qureshi *et al.,* 2012). Although combined PSB inoculation with application of rock P consistently increased shoot and root dry weight as compared to control, a treatment which joints together both bacteria and mineral rocks, further increased plant growth 26% in shoot and 29% in root dry weight for pepper and 22% in shoot and 27% in root dry weight for cucumber plant over the control during 30 days following planting (Han *et al.,* 2006). Also, Omer (1998) reported that the application of RP to nonsterilized soil with bacteria inoculation increased

P uptake, shoot and total dry mass of wheat plant. Ramachandran *et al.* (2007) found that the efficient PSB strain has efficiently solubilized and released P from insoluble RP and improved the shoot and root growth of the black pepper cuttings either alone or in combination with VAM (*Glomus fasiculatum*) culture. Vassilev *et al.* (2007) stated that shoot dry weight of plants grown in soil amended with pre-treated sugar beet waste and RP was increased more than five times and reached 330 mg per pot, compared with treatment supplemented with untreated (SB & RP).

Table 3: Effect of foliar spray with filtrated and non-filtrated *Aspergillus tubingensis* RPf10 solution on bean plant growth irrigated with Hoagland solution free phosphorus.

Harvest time (days)	Parameters	Treatments									
		T1	T2	T3 µgP ml^{-1}				T4 µgP ml^{-1}			
				49.25	98.5	147.75	197.01	49.25	98.5	147.75	197.01
30	Plant height (cm)	10	11	11	11	13	12	10	12	14	14
	Plant yield (g/pot)	0.81	0.9	1.01	1.09	1.18	1.33	0.99	1.05	1.11	1.19
	SPL (cm/g/pot)	12.3	12.2	10.9	10.1	11.0	9.0	10.1	11.4	12.6	11.8
45	Plant height (cm)	10	13	14	17	24	27	11	17	21	29
	Plant yield (g/pot)	1.02	1.22	1.35	1.63	1.74	1.97	1.38	1.49	1.96	2.01
	SPL (cm/g/pot)	9.80	10.66	10.37	10.43	13.79	13.71	7.97	11.41	10.71	14.43

T1= Bean plant irrigated with water (control), T2= Bean plant irrigated with Hoagland free phosphorus.
T3= Bean plant irrigated with Hoagland free P + Foliar spraying with filtrated fermented solution (Absence fungi).
T4= Bean plant irrigated with Hoagland free P + Foliar spraying with non-filtrated fermented solution (presence fungi).
SPL (cm/g/pot) = Specific plant length.
Values in the same column followed by the same letter do not significantly differ from each other, according to Duncan's (1955) at 5% level.

Table 4: Effect of foliar spray with filtrated and non-filtrated *Aspergillus tubingensis* RPf10 solution on bean plant growth irrigated with Hoagland solution.

Harvest time (days)	Parameters	Treatments									
		T1	T5	T6 µgP ml^{-1}				T7 µgP ml^{-1}			
				49.25	98.5	147.75	197.01	49.25	98.5	147.75	197.01
30	Plant height (cm)	10	12	12	14	14	14	13	13	15	16
	Plant yield (g/pot)	0.81	1.08	1.3	1.47	1.88	2.05	1.21	1.32	1.73	2.14
	SPL (cm/g/pot)	12.35	11.11	9.23	9.52	7.45	6.83	10.74	9.85	8.67	7.48
45	Plant height (cm)	10	15	18	22	29	36	16	24	27	38
	Plant yield (g/pot)	1.02	1.44	1.78	2.15	2.61	2.87	1.46	1.95	2.44	2.84
	SPL (cm/g/pot)	9.80	10.42	10.11	10.23	11.11	12.54	10.96	12.31	11.07	13.38

T1= Bean plant irrigated with water (control), T5= Bean plant irrigated Hoagland solution.
T6= Bean plant irrigated Hoagland solution+ Foliar spraying with filtrated fermented solution (Absence fungi).
T7= Bean plant irrigated Hoagland solution+ Foliar spraying with non-filtrated fermented solution (presence fungi).
SPL (cm/g/pot) = Specific plant length.
Values in the same column followed by the same letter do not significantly differ from each other, according to Duncan's (1955) at 5% level.

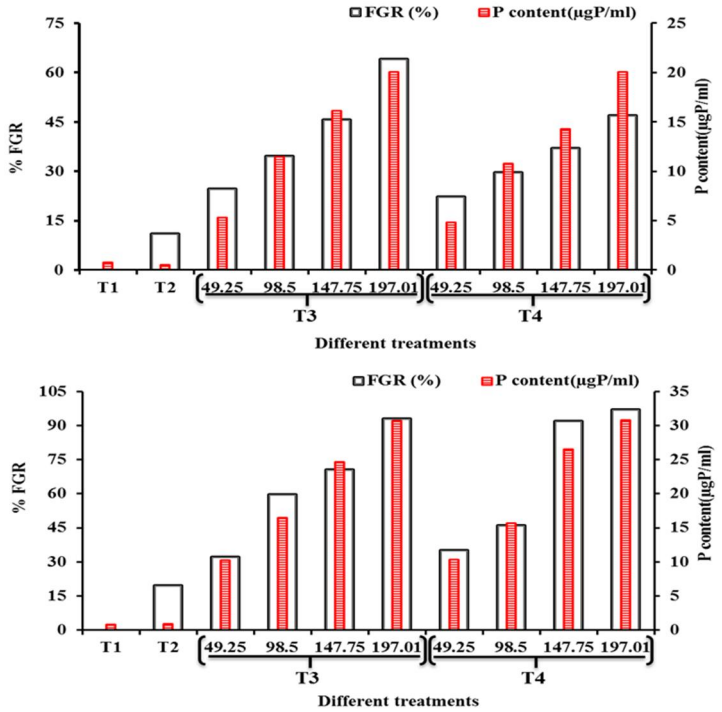

Fig. 1: The parameters of bean plant growth as affected by different foliar spray with filtrated and non-filtrated *Aspergillus tubingensis* RPf10 solution under irrigated with Hoagland solution free phosphorus conditions after 30 days (A) and 45 days (B) of growth.

T1= Bean plant irrigated with water (control), **T2**= Bean plant irrigated with Hoagland free phosphorus, **T3**= Bean plant irrigated with Hoagland free P + Foliar spraying with filtrated fermented solution (Absence fungi), **T4**= Bean plant irrigated with Hoagland free P + Foliar spraying with non-filtrated fermented solution (presence fungi). **% FGR =**percentage fermented solution induced plant growth response.

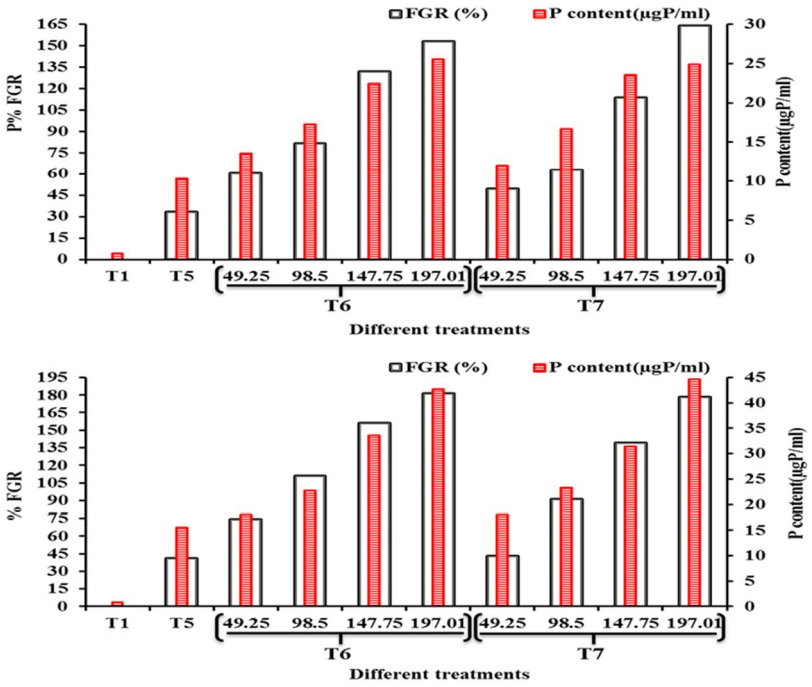

Fig. 2: The parameters of bean plant growth as affected by different foliar spray with filtrated and non-filtrated *Aspergillus tubingensis* RPf10 solution under irrigated with Hoagland solution conditions after 30 days (A) and 45 days (B) of growth.

T1= Bean plant irrigated with water (control), **T5=** Bean plant irrigated Hoagland solution, **T6=** Bean plant irrigated Hoagland solution+ Foliar spraying with filtrated fermented solution (Absence fungi), **T7=** Bean plant irrigated Hoagland solution+ Foliar spraying with non-filtrated fermented solution (presence fungi). **% FGR =** Percentage fermented solution induced plant growth response.

Conclusion

Foliar spray by un-filtrated fermented solution at 197.01 µgP/ml increased the plant height, yield and P content about 2.53, 1.97 and 2.87 fold after 45 days harvest period comparing to control treatment irrigated with Hoagland solution only, suggesting its potential use a P fertilizer.

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