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SYNERGISTIC EFFECT OF ARBUSCULAR MYCORRHIZA AND PHOSPHATE SOLUBILIZING BACTERIA ON WHEAT GROWTH AND PHOSPHORUS ACQUISIION UNDER FIELD CONDITIONS

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ABSTRACT

Two field experiments were conducted on distinct soil types, sandy and loamy, to assess the impact of phosphate-solubilizing bacteria (PSB) *Azospirillum brasilense* and *Bacillus subtilis*, applied individually or in combination with the arbuscular mycorrhizal (AM) fungus *Glomus* sp., on phosphorus (P) acquisition from low-bioavailability sources (rock phosphate, RP) and the yield of wheat (*Triticum aestivum* var. Sakha 94). The experimental design included two blocks: one inoculated with AM fungi and one without. Each block was subdivided into eight treatments, comprising four bacterial inoculation (non-inoculated control, individual strains, and their mixture) and two fertilizer treatments (control and RP application at 77.5 kg P_2O_5 ha^{-1}). Shoot dry weight was measured at the vegetative stage, while P uptake and yields of straw and grains were recorded at ripening. Soil available P and alkaline phosphatase activity were analysed after harvest. Results demonstrated that combined inoculation with bacterial strains and AM fungi significantly enhanced straw and grain yields compared to single inoculations or controls, in both soil types. However, plant responses varied with soil texture and nutrient status. In sandy soil treated with RP, the highest straw yields (7.03 and 7.00 ton ha^{-1}) were achieved with *A. brasilense* or *B. subtilis* combined with AM fungi, while the highest grain yield (3.97 ton ha^{-1}) was observed with *B. subtilis* plus AM fungi. In loamy soil, the greatest straw and grain yields (21.79 and 9.80 ton ha^{-1}) were recorded with *A. brasilense* plus AM fungi. Combined inoculations also improved P uptake in straw and grains relative to non-inoculated or singly inoculated treatments. Notably, the increase in alkaline phosphatase activity and available soil P was greater in sandy soil than in loamy soil, highlighting the stronger stimulatory effect of AM fungi under nutrient-deficient conditions.

Key Words: P-uptake, AM fungi, PSB, wheat yield, soil available P

INTRODUCTION

Developing counties use large amounts of agrochemicals to increase soil fertility and pest control. Specifically, most of reclaimed soils in Suez Canal region, Egypt are excessively treated with chemicals and pesticides. Plant growth promoting rhizobacteria (PGPR) and phosphate solubilizing bacteria (PSB) are both beneficial bacteria that can improve plant growth. PGPR incorporates a broader cluster of bacteria that stimulate plant growth via various mechanisms, including nutrient solubilization, hormone secretion, disease suppression. PSB particularly focuses on phosphate solubilizing in soil. Efficient PSB and/or arbuscular mycorrhizal (AM) fungi could be an important biotechnological tool for sustainable agriculture for their positive effects on soil fertility and crop productivity. They could reduce the required amounts and costs of agrochemicals.

The AM symbiosis is arguably the most important symbiosis on Earth and is formed by more than 60% of all known land plant species including important crop species, such as wheat, corn, soybean, and rice **(Said *et al.,* 2025)**. The extraradical mycelium (ERM) of the fungus acts as an extension of the root system and increases significantly the uptake of P, N, S, Mg, but also of trace elements, such as Cu and Zn. In addition to this positive effect on nutrient uptake, the AM symbiosis increases the resistance of plants against abiotic (drought, heavy metal, salinity) and biotic (pathogen) stresses. In exchange, the plant transfers between 5 to 15 % of its assimilated carbon to the AM fungus. An efficient use of the symbiosis can substitute P applications of up to 225 kg P_2O_5 ha^{-1} **(Sun *et al.,* 2024)**.

PGPR can e.g. increase nutrient availability through non-symbiotic nitrogen fixation, iron sequestration, solubilization of mineral phosphate and other nutrients and through the production of 1-aminocyclopropane 1-carboxylate (ACC) deaminase. This enzyme catalyzes the conversion of the ethylene precursor ACC to α- ketobutyrate and ammonia, thereby reducing the level of ethylene in developing or stressed plants **(Kumar *et al.,* 2022 and Sati *et al.,* 2023)**. Phytostimulators can directly promote plant growth by their effect on the concentration of plant hormones such as indole acetic acid (IAA), indole acetamide (IAM), gibberellic acid and cytokinins. Additionally, PGPR can stimulate the colonization of the root with AM fungi (mycorrhiza helper bacteria) and can thereby synergistically increase the effect of AM fungi on nutrient uptake and pathogen resistance. In soils with a low P bioavailability, free-living P-solubilizing bacteria could release P ions from sparingly soluble inorganic and organic P compounds and thereby contribute to an increase in the soil P pool that is available for the extraradical mycelium of the AM fungus and that can be transferred to the host. Several studies have shown that the presence of P-solubilizing bacteria in the soil increases the positive effect of mycorrhizal interactions on P nutrition **(Hnini *et al.*, 2024 and Ghanem *et al.*, 2024)**.

This study aims to investigate the potential of AM fungi and phosphate-solubilizing bacteria (PSB) to enhance wheat productivity and elucidate the interactions between them, leveraging their synergistic effects. Ultimately, we seek to produce innovative biotechnological approaches utilizing PSB and AMF as biofertilizers to promote sustainable wheat cultivation in Egypt and worldwide**.**

MATERIALS AND METHODS

1. Description of the experimental site and soils analysis

Two field experiments were conducted on two different sites in the Suez Canal region in winter season 2023. These locations represent soils of Ismailia Governorate and North Sinai (El-Salam Canal Project area). On one hand, the first study area was in the experimental farm of Faculty of Agriculture, Suez Canal University, Ismailia, between 30º 37' 3.09"N and 32º 15' 18.46"E (Experiment I). The soil of Ismailia site has sandy textural grade and very little variations in its chemical properties as well no great differences between the various soils in the morphological features. On the other hand, the second site was located at the East of Suez Canal, El-Salam Canal project, Sinai between 30° 58' 30" N and 32° 24' 15.6" E (Experiment II). Soils of El-Salam Canal Project differ in their properties according to the mode of formation, parent material and geographic location. Soils, which are adjacent to El-Salam Canal Project, are described as heavy saline alkali low-lying clay, which is fluvial, Aeolian, and lacustrine deposits.

Composite soil samples were taken from each site and analyzed for major soil properties. Selected physicochemical characteristics of air-dried, crushed, and sieved (<2 mm) soils were determined according to **(Sparks *et al.,* 1996)** as shown in **Table (1).** In addition, some chemical properties of Ismailia Canal and El-Salam Canal water that was used to irrigate experiment I and II, respectively, are illustrated in **Table 2.**

2. Bio-inoculant characterization and inoculation procedure

Two phosphate solubilizing bacterial (PSB) strains were used in both experiments, *Azospirillum brasilense* and *Bacillus subtilis*. These strains were attained from Microbiological Resource Center, Ain Shams University, Cairo and examined for some plant growth promoting (PGP) traits as shown in **Table 3**. The efficiency of *A. brasilense* and *B. subtilis* for solubilization of tricalcium phosphate was measured using Pikovskaya's broth after 10-day incubation period as described by **Mayadunna *et al.,* (2023)**. The qualitative assessment of siderophore production was inspected by Chromo Azurol S agar following the methodology described by **Alexander and Zuberer (1991)**. IAA examination was performed using Luria-Bertani medium with or without 0.10 % L-tryptophan as detailed by **Patten and Glick (2002)**.

Table 1. Some physical and chemical properties of soils and cattle manure (CM) were used in the current study.

Property	Unit	Soil I (Experiment I)	Soil II (Experiment II)	CM I	CM II
Sand	%	93.0	47.20	-	-
Silt	%	2.52	30.45	-	-
Clay	%	4.56	22.35	-	-
Textural class	-	Sand	Loam	-	-
$CaCO_3$	g kg^{-1}	2.70	34.9	-	-
pH	-	7.92†	7.65†	7.48‡	7.62‡
EC_e§	dSm^{-1}	0.340	4.19	10.2	11.4
Soluble cations§					
Ca^{2+}	meq l^{-1}	1.50	24.2	25.4	27.9
Mg^{2+}	meq l^{-1}	0.60	12.1	12.6	11.7
Na^{+}	meq l^{-1}	0.50	5.20	34.4	39.3
K^{+}	meq l^{-1}	0.80	0.402	29.6	35.1
Soluble anions§					
HCO_3^-	meq l^{-1}	0.400	2.48	21.4	25.6
Cl^-	meq l^{-1}	1.00	13.4	67.5	72.5
SO_4^{-2}	meq l^{-1}	2.00	26.0	13.1	15.9
Available P	mg kg^{-1}	7.30	9.22	134	98.9
Available N	mg kg^{-1}	9.51	12.3	157	129
Total P	g kg^{-1}	0. 081	0.162	9.02	7.11
Total N	g kg^{-1}	0.182	0.471	14.3	10.8
Organic C	g kg^{-1}	2.54	5.37	172	143

†In soil-water suspension (1:2.5).
‡In CM-water suspension (1:5).
§In CM and soil saturated extracts.

Table 2. Some chemical properties of irrigation waters used in this study

Irrigation source	EC dSm^{-1}	pH	Cations, meq l^{-1}				Anions, meq l^{-1}			SAR†
			Ca^{+2}	Mg^{+2}	Na^{+}	K^{+}	HCO_3^-	Cl^-	SO_4^{-2}	
Ismailia canal	0.36	7.98	0.97	0.50	1.73	0.4	1.30	1.50	0.80	2.02
El-Salam canal	1.25	7.30	2.60	3.80	5.60	0.6	0.40	6.6	5.60	3.13

†SAR: Sodium adsorption ratio

Table 3. Some plant growth promoting (PGP) traits of the bacterial strains used in the current study.

PGP trait	Siderophore production	P-solubilization mg P l^{-1}	IAA production, mg l^{-1}	
			Without L- TRP	With L-TRP
Azospirillum brasilense	ND	362.1	4.65	17.8
Bacillus subtilis	+	191.2	2.81	9.12

IAA: Indole acetic acid; L-TRP: L-tryptophan; ND = not detected.

The mycorrhizal inoculum was composed of an indigenous AM fungi (*Glomus* sp.) isolated from the rhizosphere of alfalfa (*Medicago sative*) and onion (*Allium cepa*) plants using the procedure of decanting and wet sieving **(Boyno *et al.*, 2023)**. For the PSB inoculations, the bacterial strains were culture in tryptic soy broth medium and incubated at 30 °C for 3 days. The viable cell count reached 10^7-10^8 colony forming unit ml^{-1} for two strains. The wheat seeds were soaked in the cell suspension and arabic gum (10%) to enable bacteria to stick to the seeds for 1h before sowing. The AM inoculum included a mixture of AM fungal spores, extraradical hyphae of *Glomus* sp. and colonized root fragments.

3. Rock phosphate ore

Rock phosphate (RP) used in this study was obtained from Al Ahram Co. for mining and fertilizers in Egypt. The rock phosphate is a sedimentary rock materials deposit supplied as raw mining and as apatite powder. The main chemical compositions of the RP sample are 23.80 % total P_2O_5, 0.99 mg kg^{-1} water soluble P, 12.82% CaO and 9.4% Fe_2O_3.

4. Experimental design and planting

Two field experiments were carried out in two different locations to evaluate the effects of inoculation with PSB and AM fungi on P acquisition and yield of wheat (*Triticum aestivum var.* Sakha 94). The experiments were laid out in randomized complete block design (RCBD) with three replications. Each experiment consisted of two blocks, one with and the other one without AM fungi inoculation. Each block divided into eight different sections, four bacterial treatments (non-inoculated control or inoculation with one of the two P-solubilizing bacterial strains and mixture from them), and two fertilizer treatments (control without P fertilization and rock phosphate application at rate of 77.5 kg P_2O_5 ha^{-1}. Plot dimensions were 3 x 3 m with twelve wheat rows in each plot. Cattle manures CM1 and CM2 were added to sandy and loamy soils, respectively, following the recommendations of the Egyptian Ministry of Agriculture. The chemical properties of the CM samples were determined as detailed by **Sparks et al. (1996)** and shown in **Table 1**. Ammonium sulphate (21.6% N) and potassium sulphate (50% K_2O) were used as sources of nitrogen and potassium fertilizers, respectively. The application rates for experiment I were 285 kg N ha^{-1} and 120 kg K_2O ha^{-1}, whereas Experiment II received 180 kg N ha^{-1} and 100 kg K_2O ha^{-1}.

5. Soil and plant samples

Rhizospheric soil samples were collected after 90 days from planting and assessed for alkaline phosphatase activity (µg p-nitrophenol released/g soil/h) following the method described by **Tabatabai, (1994).** After the harvest, soil samples were analyzed for the availability of inorganic P in 0.5 M $NaHCO_3$-soil extract (pH 8.5) by spectrophotometer according to Olsen method **(Sims, 2000).** Plant samples were collected at vegetative stage (60 day-old plants) for recording shoot dry weights at 70°C. After ripening (130 days from planting), straw and grain yields were recorded. The total P in straw and grains were determined by wet digestion using nitric (HNO_3)-perchloric ($HClO_4$) acid mixture (4:1 v/v) using the molybdenum-blue method **(Jackson, 1973).** The total P uptake was calculated by multiplying the biomass dry weight (straw or grains) with its P concentration.

6. Statistical analysis

All results were subjected to the analysis of variance (ANOVA). Statistical differences between means values were compared with Duncan's Multiple Range Test by SPSS software version 24. Differences at the confidence level of 0.95 were considered significant.

RESULTS

1. Plant growth parameters

Plant response to inoculation with the tested phosphate solubilizing bacteria (PSB) and AM fungi with or without phosphate rock was evaluated by measuring shoot dry weights after 60 days from sowing (vegetative stage). Results presented in **Fig. 1a** show that the bacterial inoculants (*A. brasilense*, *B. subtilis* and their mixture) without AM fungi increased the shoot dry weight (21-35%) in sandy soil as compared to control. The same result was observed in AM fungi inoculated soil, where the shoot dry weight increased by 62% compared to the control. The highest shoot dry weight (4.09 g $plant^{-1}$) was recorded in the soil treated with RP + *A. brasilense* + AM fungi (**Fig. 1a**). In loamy soil (Experiment II), the bacterial strains alone or in combination with AM fungi also stimulated shoot dry weight as displayed in **Fig. 1b**. *Azospirillum brasilense* was most effective strain in shoot growth stimulation, where the shoot dry weight increased to 30% under the treatment of *A. brasilense* + AM fungi + RP (**Fig. 1b**).

2. Wheat yield components

Tables 4 and 5 indicate that the straw and grain yields significantly increased due to inoculation with phosphate solubilizing bacteria (PSB) and AM fungi. However, the response of wheat plants to bacterial and fungal inoculations was affected by soil texture.

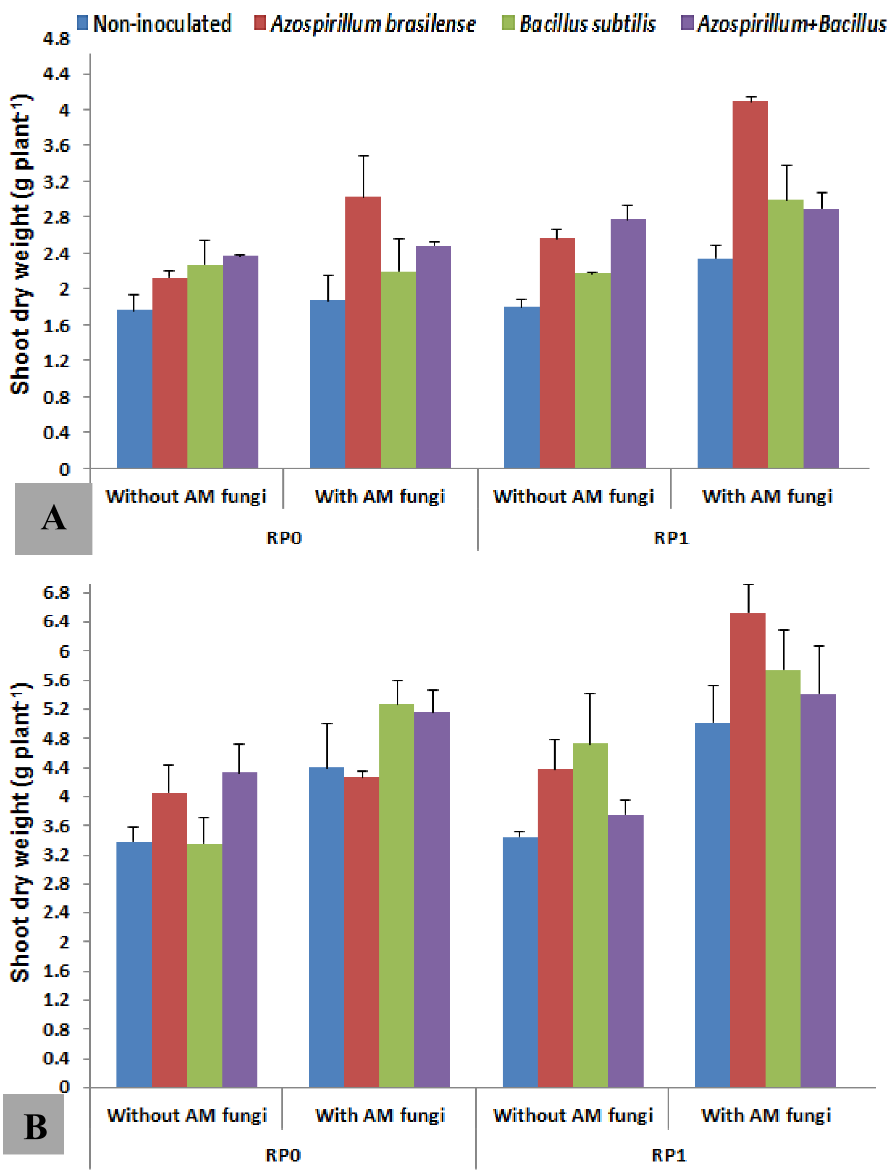

Fig 1. Effect of inoculation with phosphate solubilizing bacterial strains alone or in combination with AM fungi on shoot dry weight (g plant^{-1}) of 60 day-old plants grown in sandy (Experiment I, Fig. 1a). and loamy soil (Experiment II, Fig. 1b). RP_0: without rock phosphate, RP_1: addition of rock phosphate (at rate of 77.5 kg P_2O_5 ha^{-1}).

Table 4. Effect of rock phosphate, phosphate solubilizing bacteria and AM fungi on straw and grain yields (ton ha^{-1}) of wheat grown in sandy soil under field conditions (Experiment I)

Microbial treatment	Rock phosphate (RP) treatment			
	Without RP	With RP	Without RP	With RP
	Straw yield		Grain yield	
Without AM fungi				
Non-inoculated	4.48	4.99	2.13	2.35
Azospirillum brasilense	4.82	4.95	2.25	2.69
Bacillus subtilis	4.98	5.01	2.32	2.77
A. brasilense + B. subtilis	4.90	5.03	2.29	2.80
With AM fungi				
Non-inoculated	5.26	5.38	2.47	2.51
Azospirillum brasilense	5.98	7.03	3.37	3.60
Bacillus subtilis	5.81	7.00	2.89	3.97
A. brasilense + B. subtilis	5.90	6.53	3.13	3.43
$LSD_{0.05}$	1.15		0.638	

Rock phosphate was applied at rate of 77.5 kg P_2O_5 ha^{-1}

Table 5. Effect of rock phosphate, phosphate solubilizing bacteria and AM fungi on straw and grain yields (ton ha^{-1}) of wheat grown in loamy soil under field conditions. (Experiment II)

Microbial treatment	Rock phosphate (RP) treatment			
	Without RP	With RP	Without RP	With RP
	Straw yield (ton ha^{-1})		Grain yield (ton ha^{-1})	
Without AM fungi				
Non-inoculated	10.86	11.19	5.65	5.68
Azospirillum brasilense	12.27	13.64	6.20	6.80
Bacillus subtilis	12.33	14.33	5.99	6.96
A. brasilense + B. subtilis	12.22	13.03	6.13	7.50
With AM fungi				
Non-inoculated	12.11	12.86	5.80	6.72
Azospirillum brasilense	14.42	21.79	7.67	9.80
Bacillus subtilis	15.31	17.41	6.61	9.32
A. brasilense + B. subtilis	15.61	17.50	7.76	9.28
$LSD_{0.05}$	3.05		0.950	

Rock phosphate was applied at rate of 77.5 kg P_2O_5 ha^{-1}

Both straw and grain yields improved significantly in sandy and loamy soils that was treated with RP. The obtained results indicated that the combined inoculation with tested bacterial strains and AM fungi resulted in higher straw and grain yields than when these microorganisms were used individually in both soils. Additionally, the effect of dual inoculation in both soils treated with RP was found to be significant regarding both straw and grain yields. The highest straw yield (7.03 and 7.0 ton ha^{-1}) were observed when plants inoculated with *Azospirillum brasilense* or *Bacillus subtilis* in combination with AM fungi in sandy soil treated with RP, whereas the highest grain yield (3.97 ton ha^{-1}) were observed when plants inoculated with *Bacillus subtilis* and AM fungi (**Table 4**).

In loamy soil, the highest straw and grain yields (21.79 and 9.80 ton ha^{-1}, respectively) were observed when plant inoculated with *Azospirillum brasilense* in combination with AM fungi (**Table 5**). The co-inoculation of AM fungi and PSB showed the strongest positive response compared to AM fungi alone in soil treated with RP. Specifically, straw yield significantly increased by 42.02% and 39.72% due to co-inoculation of *Azospirillum brasilense* or *Bacillus subtilis* and AM fungi in sandy soil compared to single inoculation (**Table 4**).

3. Relative yield

Relative yield (RY) was calculated as a ratio to compare the wheat grain yield with the tested microbial inoculants to the maximum possible yield without inoculation. It was calculated by: $RY = ((Y_a \div Y_m) * 100)$ where: Y_a is the actual grain yield for a treatment and Y_m is the maximum grain yield under common practices without microbial inoculation (**Pearce *et al.*, 2022**).

The relative yield results displayed in **Fig. 2** clearly showed that the highest grain yield of wheat in both soils was obtained through the co-inoculation of mycorrhizae and phosphate solubilizing bacteria (PSB) under rock phosphate fertilization. This indicates the ability of these microorganisms to solubilize rock phosphate into an available form to plants, resulting in a significant increase in yield compared to the corresponding treatments without microbial inoculants which may suffer from P limitation.

4. P uptake by wheat plants

Tables 6 and 7 indicate that the P-uptake in straw and grains significantly increased due to inoculation with PSB strains or AM fungi and these increases were pronounced with RP fertilizer treatments. In non-mycorrhizal plants, inoculation with *Azospirillum brasilense* and/or *Bacillus subtilis* led to a significant increase in the P uptake of the straw in sandy and loamy soils as compared to control.

Data displayed in **Tables 6 and 7** clearly show that the combined inoculation with *Azospirillum brasilense* or *Bacillus subtilis* and AM fungi improved the uptake of P in straw and grains when compared with single inoculation treatment in sandy and loamy soils. The highest P uptake in straw was observed when plants inoculated with *Azospirillum brasilense* in combination with AM fungi in RP-fertilized sandy and loamy soils. The maximum P uptake in grains (26.3 and 65.7 kg ha^{-1}) was observed in the treatment of *A. brasilense* + AM fungi + RP fertilizer in sandy and loamy soils, respectively (**Tables 6 and 7**).

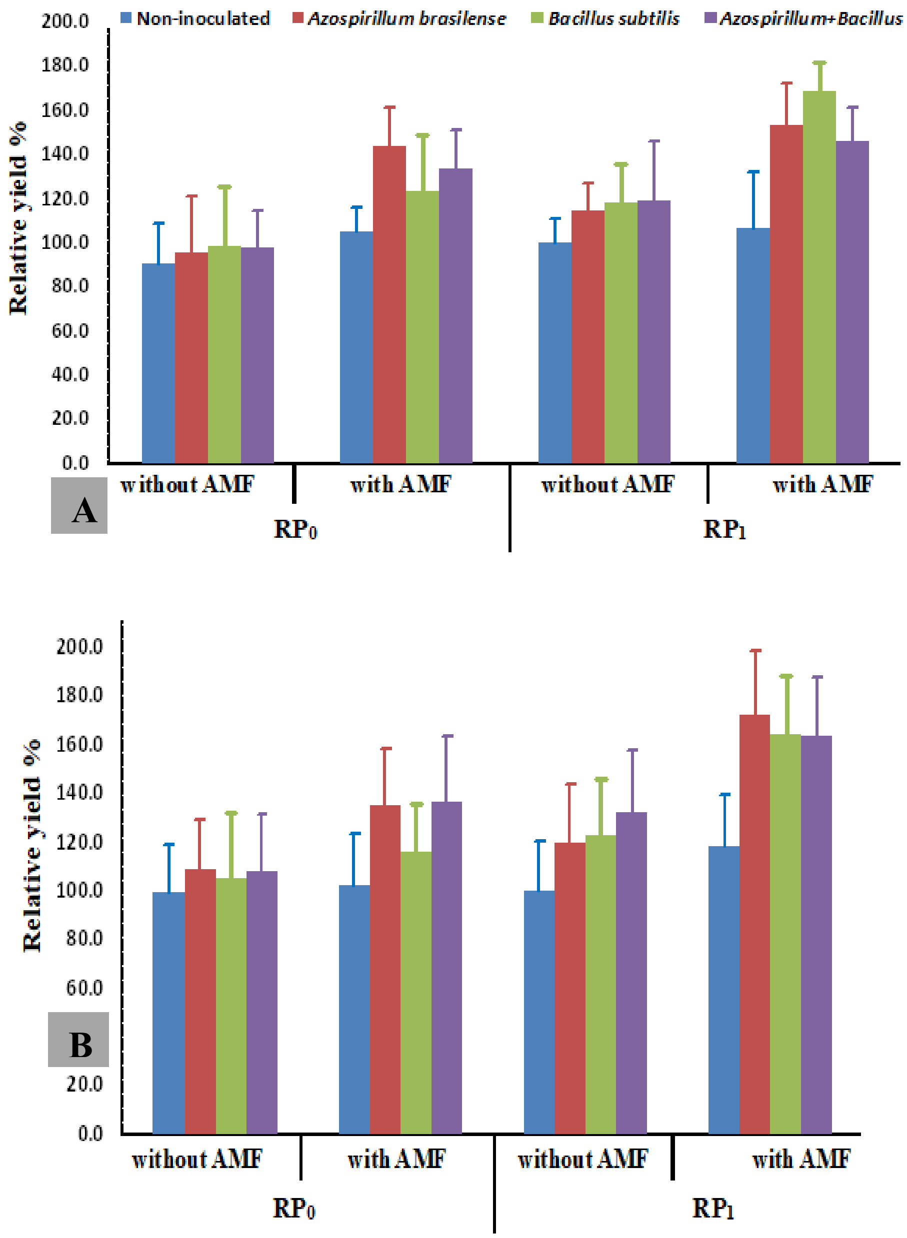

Fig 2. Effect of inoculation with phosphate solubilizing bacteria alone or in combination with AM fungi on relative grain yield (%) of wheat grown in sandy soil (Experiment I, Fig. 2a) and loamy soil (Experiment II, Fig. 2b). RP_0: without rock phosphate, RP_1: addition of rock phosphate (at rate of 77.5 kg P_2O_5 ha^{-1}).

Table 6. Effect of rock phosphate, phosphate solubilizing bacteria and AM fungi on P uptake (kg ha^{-1}) in straw and grains of wheat grown in sandy soil under field conditions. (Experiment I)

Microbial treatment	Rock phosphate (RP) treatment			
	Without RP	With RP	Without RP	With RP
	P-uptake in straw (kg P ha^{-1})		P-uptake in grains (kg P ha^{-1})	
Without AM fungi				
Non-inoculated	9.56	11.6	13.3	13.9
Azospirillum brasilense	14.5	16.8	12.8	17.9
Bacillus subtilis	15.6	13.1	12.7	17.2
A. brasilense + B. subtilis	15.1	15.2	12.7	17.9
With AM fungi				
Non-inoculated	12.1	13.1	15.8	16.8
Azospirillum brasilense	19.6	20.2	20.5	26.3
Bacillus subtilis	16.6	19.6	18.8	24.1
A. brasilense + B. subtilis	18.1	20.4	19.7	20.7
$LSD_{0.05}$	4.12		4.74	

Rock phosphate was applied at rate of 77.5 kg P_2O_5 ha^{-1}

Table 7. Effect of rock phosphate, phosphate solubilizing bacteria and AM fungi on P uptake (kg ha^{-1}) in straw and grains of wheat grown in loamy soil under field conditions (Experiment II)

Microbial treatment	Rock phosphate (RP) treatment			
	Without RP	With RP	Without RP	With RP
	P-uptake in straw (kg P ha^{-1})		P-uptake in grains (kg P ha^{-1})	
Without AM fungi				
Non-inoculated	42.9	49.7	32.0	37.2
Azospirillum brasilense	50.1	70.9	40.2	49.3
Bacillus subtilis	53.0	63.4	42.3	46.2
A. brasilense + B. subtilis	55.9	60.4	38.7	47.7
With AM fungi				
Non-inoculated	44.4	53.9	33.40	46.6
Azospirillum brasilense	51.4	106	45.60	65.7
Bacillus subtilis	67.6	96.0	53.50	63.1
A. brasilense + B. subtilis	61.9	85.8	46.00	62.8
$LSD_{0.05}$	4.96		4.06	

Rock phosphate was applied at rate of 77.5 kg P_2O_5 ha^{-1}

5. Available P in soil

Results presented in **Table 8** show that the co-inoculation with PSB and AM fungi increased available P in soil compared to individual inoculation in both trials. Specifically, the maximum available P values were observed when plants treated with *B. subtilis* + AM fungi (16.64 mg

kg^{-1} soil) in sandy soil treated with RP. Whereas the highest available P value in loamy soil was found under the treatment of *A. brasilense* + AM fungi + RP fertilizer (18.32 mg kg^{-1} soil). The obtained results indicated that the effect of phosphate solubilizing bacteria or AM fungi was greatest when RP applied. In non-mycorrhizal plants, the bacterial strains without PR fertilizer increased available P status in sandy and loamy soils; however, a much greater effect was observed when bacterial strains were used in conjunction with RP fertilizer (**Table 8**).

Table 8. Effect of rock phosphate, phosphate solubilizing bacteria and AM fungi on soil available P (mg kg^{-1}) in sandy (Experiment I) and loamy soils (Experiment II) after wheat harvest under field conditions.

Microbial treatment	Soil available P (mg kg^{-1})			
	Sandy soil (Experiment I)		Loamy soil (Experiment II)	
	Without RP	With RP	Without RP	With RP
Without AM fungi				
Non-inoculated	6.72	8.40	8.24	10.09
Azospirillum brasilense	8.07	11.68	9.83	13.20
Bacillus subtilis	9.41	11.60	10.42	12.61
A. brasilense + B. subtilis	8.83	10.59	10.13	13.61
With AM fungi				
Non-inoculated	8.41	11.09	8.66	10.84
Azospirillum brasilense	11.01	15.63	10.09	18.32
Bacillus subtilis	11.26	16.64	9.58	16.39
A. brasilense + B. subtilis	11.09	13.62	9.92	15.13
$LSD_{0.05}$	1.55		1.62	

Rock phosphate was applied at rate of 77.5 kg P_2O_5 ha^{-1}

6. Alkaline phosphatase activity in soil

Alkaline phosphatase activity was determined in soil samples at the anthesis stage (90 days post-planting). Alkaline phosphatase is considered an indicator of soil biological health, reflecting the synergistic effects between AM fungi and phosphate solubilizing bacteria (PSB), which can enhance plant growth by increasing phosphorus availability in soil. The data presented in **Fig. 3** show that co-inoculation with PSB and AM fungi significantly increased alkaline phosphatase activity in both soils compared to single inoculations. Notably, alkaline phosphatase activity reached its maximum value in both trials when the soil was fertilized with rock phosphate (RP) and inoculated with a combination of *B. subtilis, A. brasilense* and AM fungi. Furthermore, the results in **Fig. 3** indicate that the impact of microbial inoculants on alkaline phosphatase activity was more pronounced when RP was added to both soils, with a greater effect observed in sandy soil than in loamy soil.

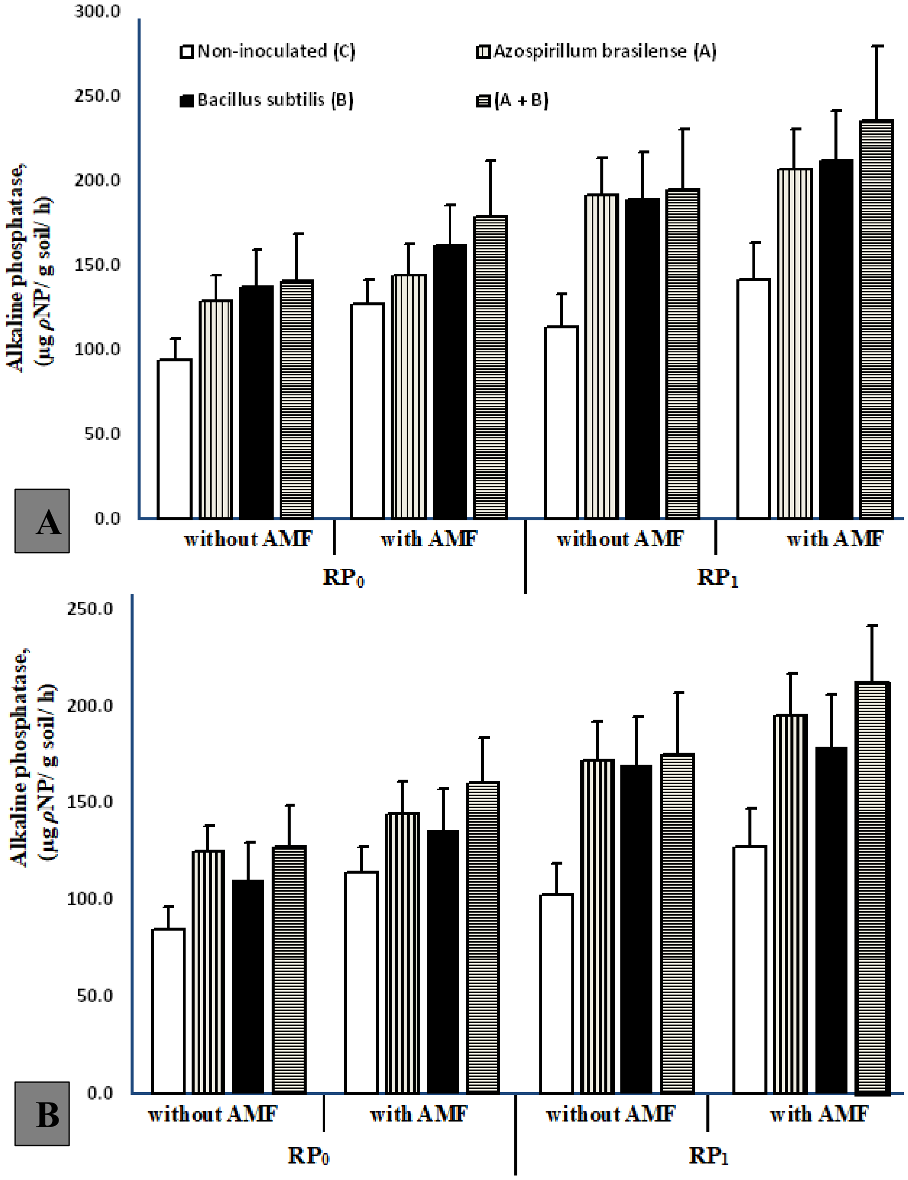

Fig. 3. Effect of inoculation with phosphate solubilizing bacterial strains alone or in combination with AM fungi on alkaline phosphatase activity (µg p-nitrophenol released/ g soil/ h) in sandy soil (Experiment I, Fig. 3a) and loamy soil (Experiment II, Fig. 3b) after 90 days from cultivation. RP_0: without rock phosphate, RP_1: with rock phosphate (at rate of 77.5 kg P_2O_5 ha^{-1}).

DISCUSSION

Glomus species were considered the predominant AM fungi in neutral to alkaline soils which play a critical role in diverse ecologies by enhancing plant growth and yield through enriching nutrient uptake **(Fall *et al.,* 2022; Alrajhi *et al.,* 2023)**. The significance of *Bacillus subtilis* and *Azospirillum brasilense* as phosphate solubilizing bacteria (PSB) has been well-documented in numerous studies **(El-Kharbotly and Ghanem 2020; Sati *et al*., 2023; Zhang *et al*., 2025)**. The current research evaluated the synergistic impacts of AM fungi and PSB strains on wheat plants, emphasizing their potential to mobilize phosphorus from sparingly soluble sources like rock phosphate. The results clearly displayed the prospect of AM fungi to interact with P solubilizing bacteria and enhance shoot, grain and straw yields of the wheat plants in both sandy and loamy soils. Thus, suggesting better plant growth after microbial inoculation compared to the uninoculated plants. Moreover, the co-application of rock phosphate (RP) and the microbial inoculants improved wheat growth in both soils compared to RP-uninoculated soils **(Hnini *et al*., 2024)**. However, wheat growth and yield were rigorously affected by soil texture. That is, soil texture limits nutrients status and consequently, the efficiency of microbial inoculants and plant growth **(Egamberdiyeva, 2007; Wang *et al*.,2025)**.

The significant enhancement of wheat growth and yield qualities due to microbial inoculants could be attributed to their plant growth promoting traits. For example, the tested strains were previously verified to have the ability to produce indole acetic acid (IAA) and siderophores as well to their capacity to colonize the root system and solubilize inorganic insoluble phosphate **(Abd El-Azeem *et al*., 2007; Kumar *et al*., 2022)**. Our results corresponded to the study of **Wahid *et al*., (2020)** who showed that the inoculation of wheat plants with phosphate solubilizing microorganisms and AM fungi enhanced wheat growth through increasing root colonization by AM fungi. The maximum root colonization was observed in the treatment containing *Bacillus* sp. and *Glomus* sp. with RP amendment.

The application of *Bacillus subtilis* or *Azospirillum brasilense* alone or in combination with AM fungi was effective in improving P content in straw and grains of wheat in both soils. Notably, the association with AM fungi and PSB seems to be more effective in acquisition of phosphorus especially under P-deficient soil conditions.

Abd El-Azeem and Bucking (2023) revealed that the combined inoculation with AM fungi and PSB significantly increased P use efficiency of wheat grains. In this regard, **Babana and Antoun (2006)**

observed that the positive interaction between AM fungi, P-solubilizing bacteria and RP increased P content in straw and grains of wheat significantly.

Phosphatase activity was evaluated due to its crucial role in the phosphorus cycle in soil, reflecting the potential of microbial inoculants to enhance soil phosphorus availability and plant uptake. Numerous studies have demonstrated that acid phosphatase enzyme is prevalent in acidic soils, whereas alkaline phosphatase dominates neutral to alkaline soils (**Dlugosz, *et al*., 2022; Daunoras *et al.,* 2024).** In our current study, alkaline phosphatase activity was measured due to the neutral to alkaline reaction of the soils under investigation. Accordingly, phosphatase activity has been reported to peak at the flowering stage and decline thereafter until harvest (**Jiang *et al.,* 2025**). Thus, we assessed the enzyme at 90 days post-planting. Our results revealed that microbial inoculants, particularly when combined, increased enzyme activity, highlighting the role of these microorganisms as a primary source of phosphatase enzymes in soil (**Ughamba *et al.,* 2025**). Notably, prior studies have emphasized phosphatase enzymes as a key mechanism employed by PSB and mycorrhizal fungi to solubilize phosphate from sparingly soluble sources **(Brito *et al.,* 2020; Chen*, et al*., 2024).**

These findings explained that free-living phosphate-solubilizing bacteria could release phosphate ions from sparingly soluble inorganic P compounds through their ability to produce organic acids and phosphatase enzymes, thereby increasing soil available phosphate pool for the extraradical AM fungal hyphae to pass on to the host plant. Our results proved that the tested bacterial strains, *Azospirillum brasilense* and *Bacillus subtilis* are promising biofertilizers and mycorrhizal-helper bacteria, which could synergistically interact with AM fungi to be more effective for improving sustainable nutrient supply to wheat plants from sparingly soluble sources like rock phosphate.

CONCLUSION

In P-deficient soils, it is probable to achieve better wheat yield by improving P bioavailability, and consequent plant uptake, by the direct co-application of RP fertilizer with AM fungi and P-solubilizing bacteria inoculants. Our results demonstrated that microbial inoculants could synergistically interact with AM fungi to be more effective for improving sustainable nutrient supply to wheat plants from sparingly soluble sources. Additional field experiments are required to explore the synergistic impacts of AM fungi and phosphate-solubilizing bacteria on various native soil microorganisms under different crops and agroecological conditions.

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تأثير التداخل بين فطريات الميكورهيزا والبكتيريا المذيبة للفوسفات علي تحسين نمو القمح وامتصاص الفوسفور تحت الظروف الحقلية

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أجريت تجربتان حقليتان على نوعين مختلفين من التربة وهما التربة الرملية والطينية، لتقييم تأثير سلالات من البكتيريا المذيبة للفوسفات *Azospirillum brasilense* و*Bacillus subtilis* سواء كلقاح فردي أو في خليط مع فطريات الميكورهيزا على امتصاص الفوسفور من مصادره غير الذائبة (صخر الفوسفات) وإنتاجية القمح صنف سخا 94. كانت المعاملات مرتبة في تصميم قطاعات كاملة العشوائية. القطاع الأول تم فيه التلقيح بالميكورهيزا ، أما القطاع الثاني فكان بدون تلقيح الميكورهيزا. وتم تقسيم كلاً من القطاعين إلى ثمان معاملات، تشمل أربعة معاملات تلقيح بكتيري وهي الكنترول بدون تلقيح، التلقيح بـ *Azospirillum brasilense* أو *Bacillus subtilis* وخليط منهما، وكانت هذه المعاملات الأربعه تطبق تحت اضافة صخر الفوسفات بمعدل 77.5 كجم P_2O_5 هكتار$^{-1}$ أو بدون اضافته. تم قياس الوزن الجاف للمجموع الخضري بعد 90 يوم من الزراعة وتم تقدير إنتاج القش والحبوب وامتصاصهما من الفوسفور بعد الحصاد. وتم قياس نشاط انزيم الفوسفاتيز القلوي في التربة بعد 90 يوم من الزراعة وأجري تحليل الفوسفور الميسر في عينات التربة بعد الحصاد.

أظهرت النتائج أن التلقيح المشترك بالسلالات البكتيرية والميكورهيزا أدى إلى تحسن كبير في إنتاجية القش والحبوب مقارنة بالتلقيح الفردي أو الكنترول في كلا النوعين من التربة. إلا أنه قد اختلفت استجابة النباتات حسب قوام التربة وحالة التسميد الفوسفاتي. وقد تحقق في كلا من التربة الرملية واللومية المسمدة بصخر الفوسفات أعلى إنتاجية للقش والحبوب مع معاملات التلقيح البكتيري بالاشتراك مع الميكورهيزا. كما أدت هذه التلقيحات المشتركة إلى تحسين امتصاص الفوسفور في القش والحبوب مقارنة بالمعاملات غير الملقحة أو الملقحة بلقاح فردي. ومن الجدير بالذكر أن الزيادة في نشاط انزيم الفوسفاتيز القلوي وكذلك الفوسفور الميسر في التربة كانت أكبر في التربة الرملية منها في التربة الطينية مما يسلط الضوء على التأثير التحفيزي الأقوى للميكورهيزا تحت ظروف نقص الفوسفور.

خلاصة هذه الدراسة أن اللقاحات الميكروبية يمكن أن تتداخل معاً لتصبح أكثر فعالية في تحسين إمدادات المغذيات لنبات القمح من المصادر قليلة الذوبان. ونوصي بإجراء تجارب حقلية إضافية لاستكشاف التأثيرات والتداخلات الأخري للميكورهيزا والبكتيريا المذيبة للفوسفات على مختلف الكائنات الحية الدقيقة الأصلية في التربة تحت ظروف محاصيل أخري وظروف بيئية زراعية مختلفة.