# Magnesium Foliar Application and Phosphorien Soil Inoculation Positively Affect *Pisum sativum* L. Plants Grown on Sandy Calcareous Soil

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***Abstract*—**The effects of soil inoculation with phosphorien-containing phosphate-dissolving bacteria (PDB) and/or magnesium (Mg) foliar application at the rates of 0, 0.5 and 1mM on growth, green pod and seed yields, and chemical constituents of *Pisum sativum* L. grown on a sandy calcareous soil were investigated. Results indicated that PDB and/or Mg significantly increased shoot length, number of branches plant$^{-1}$, total leaf area plant$^{-1}$ and canopy dry weight plant$^{-1}$, leaf contents of pigments, soluble sugars, free proline, nitrogen, phosphorus, potassium, magnesium, and calcium, and Ca/Na ratio, while leaf Na content was reduced. PDB and/or Mg also increased green pod and seed yields. We concluded that PDB and Mg have pronounced positive effects on *Pisum sativum* L. plants grown on sandy calcareous soil. PDB and Mg, therefore, have the potential to be applied for various crops to overcome the adverse effects of the newly-reclaimed sandy calcareous soils.

***Keywords*—**Bio-P-fertilizer, Mg foliar application, Newly-reclaimed soils, *Pisum sativum* L.

## I. Introduction

PEA (*Pisum sativum* L.) is one of the most popular vegetable crops grown in many of the Middle Eastern countries. Pea considers one of the main leguminous crops that are an important component of the agricultural sector in developing countries due to its ability to produce significant quantities of protein, carbohydrates, and nutrient-rich seed. It is widely cultivated on the newly-reclaimed sandy calcareous soils, which show a great deficiency in macronutrients especially, magnesium (Mg) and phosphorus (P).

Mg plays a vital role in stepping up the growth and quantitative as well as qualitative features of the plant. Mg deficiency is most prevalent in sandy textured soils because it is subject to oxidation to become in a form un-available for plant and loss by leaching [1]. In reclaimed sandy soils, foliar application of macro- and/or micro-nutrients are widely used and preferable [2], [3] and leads to significant increases in growth and productivity of some crops [4]–[6].

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P precipitation and immobilization is the most important problem under calcareous soil having high pH and calcium carbonate. Phosphorien is a bio-fertilizer product-containing PDB which hydrolyzes the insoluble phosphate into soluble one under the previous mentioned adverse conditions.

Adding bio-phosphorus-fertilizers lead to significant increments in vegetative growth and productivity of some crops [6]–[9].

Therefore, the present work aims to evaluate the influence of soil inoculation with PDB (phosphorien) and/or foliar application of Mg on vegetative features, green pod and green seed yields and some chemical constituents of pea (*Pisum sativum* L.) grown under sandy calcareous soil conditions.

## II. Materials and Methods

Seeds of pea cv. Master-B were provided by the Agricultural Research Center, Giza, Egypt. Phosphorien (*Bacillus megatherium*; phosphate-dissolving bacteria) was provided by the Ministry of Agriculture and mixing with Nile water to obtain the concentration of about $0.25\times10^7$ cfu ml$^{-1}$, and Mg concentrations used were 0, 0.5 and 1mM combined with or without phosphorien (6 treatments in total). The rates of Mg (0.5 and 1mM) were selected based on our preliminary studies, since these rates were generated the best responses. A two-field experiment was undertaken at the Experimental Farm, Faculty of Agriculture, Fayoum University, Egypt in 2012 and 2013 seasons. Preceding the initiation of experiment, soil samples to 25cm depth were collected and analyzed. Some of the chemical and physical properties according to the standard procedures of Wilde et al. [10] are presented in Table I.

During preparation of the experimental site, farmyard manure at the rate of 10 m$^3$, 150 kg calcium superphosphate (15.5% $P_2O_5$) and 100 kg elemental sulphur fed$^{-1}$ were broadcasted and incorporated in the soil. Pea seeds were sown on 3 and 6 October in 2012 and 2013, respectively on rows 60 cm apart, 20cm spacing between holes (five seeds were sown in each hole, after full seeds emergence were thinned to three seedlings in each hole).

Soil inoculation with phosphorien was performed by injecting 35 ± 5ml hole$^{-1}$ two times; 15 and 30 days after sowing at the rhizosphere area. The respective source of Mg was Librel 5.5% Mg in chelated form (EDTA, Ciba Specialty chemicals, United Kingdom). Mg solutions were foliar sprayed to run off two times; 25 and 40 days after sowing. Few drops of Tween-20 were added to the spraying solution as a wetting agent. The experimental layout was a randomized complete blocks design with 4 replications. Each experimental unit contained 5 rows, 4 m long and 60 cm wide.

After complete earthing, a seasonal total of 66 and 48 kg N and $K_2O$ in the form of ammonium nitrate (33.5% N) and potassium sulphate (48% $K_2O$), respectively were applied in two equal applications; 20 and 40 days after sowing, respectively. All other agro-management practices for commercial production of pea were followed whenever it was necessary.

TABLE I
PHYSICAL AND CHEMICAL PROPERTIES OF THE EXPERIMENTAL SOIL BEFORE PLANTING IN 2012 AND 2013 SEASONS

| Parameter | 2012 | 2013 |
|---|---|---|
| Clay [% (w/w)] | 25.7 | 28.5 |
| Silt [% (w/w)] | 21.1 | 20.2 |
| Sand [% (w/w)] | 53.2 | 51.3 |
| Soil texture | sandy | sandy |
| pH (1: 2.5) | 8.41 | 8.36 |
| ECe (dS $m^{-1}$) | 5.20 | 5.15 |
| Organic matter [% (w/w)] | 1.17 | 1.24 |
| $CaCO_3$ [% (w/w)] | 14.45 | 14.22 |
| Available N (mg kg–1 DW) | 46.34 | 49.20 |
| Available P (mg kg–1 DW) | 8.01 | 9.45 |
| Available K (mg kg–1 DW) | 75.16 | 80.41 |
| Available Fe (mg kg–1 DW) | 7.13 | 8.20 |
| Available Mn (mg kg–1 DW) | 4.59 | 5.64 |
| Available Zn (mg kg–1 DW) | 2.22 | 2.63 |
| Available Cu (mg kg–1 DW) | 0.48 | 0.49 |

For vegetative growth attributes, 10 plants from the two outer rows of each experimental unit were randomly chosen, cut off at the ground level to measure the shoot length (cm), number of branches $plant^{-1}$, total leaf area $plant^{-1}$ ($dm^2$) and canopy dry weight $plant^{-1}$ (g).

For green pod and green seed yields and their components, marketable green pod samples were harvested from twenty random selected plants of the two outer rows in each experimental unit to determine the number of pods $plant^{-1}$, average pod weight, number of seeds $pod^{-1}$ and 100-seed weight. All marketable green pods of the three inner rows, throughout the entire harvesting period, were picked and weighed to calculate the total green pods yield $fed^{-1}$. Green seeds were extracted and weighed to determine the total green seeds yield $fed^{-1}$.

For chemical constituent determinations, leaf sample of 10 randomly selected plants after 50 days of sowing were chosen from each experimental unit. Leaf chlorophyll and carotenoid contents in the $4^{th}$ leaf were colorimetrically determined as outlined by Arnon [11]. Leaf samples were dried in a forced air oven at 70°C till a constant weight and then ground. For leaf mineral determinations, samples of fine dry ground material each of 0.1g was digested with a mixture of sulphuric and perchloric acids as mentioned by Piper [12]. Total soluble sugars in dried leaves were extracted and determined according to Irigoyen et al. [13]. Free proline (μg $g^{-1}$ leaf dry matter) was extracted by 5-sulphosalicylic acid (3%) then, determined colorimetrically using acid ninhydrin reagent as outlined by Bates et al. [14]. Leaf N content was colorimetrically determined using Orange G dye as suggested by Hafez and Mikkelsen [15]. Leaf P content was colorimetrically estimated using the method of chloro-stannus molybdo-phosphoric blue color in sulphuric acid system according to the procedure of Jackson [16]. Leaf K and Na contents were determined using a Flame-photometer as documented by Page et al. [17]. Leaf Ca and Mg contents were measured using a Perkin-Elmer, Model 3300, Atomic Absorption Spectrophotometer as mentioned by Chapman and Pratt [18].

Data of the two seasons were subjected to the statistical analysis according to the design used [19]. The least significant difference test (LSD) at $p \leq 0.05$ level was utilized to verify the significant difference between treatments.

## III. RESULTS AND DISCUSSION

Data in Table II show that shoot length, number of branches per plant, total leaf area per plant and canopy dry weight significantly increased in plants that foliar sprayed with Mg at the 2 rates (0.5 and 1mM) compared to plants that not applied with Mg. Plants received Mg at the rate of 1mM gave higher growth traits than plants received Mg at 0.5mM Mg. Soil inoculation with phosphorien further increased growth traits. The best results were obtained from the combined treatment of soil inoculation with bio-phosphorus fertilizer and foliar application with 1mM Mg. The same trend was observed over both the 2012 and 2013 growing seasons.

These results can be explained on the basis that, phosphate-solubilizing bacteria produces organic and inorganic acids and/or $CO_2$ which dissolve the precipitated form of phosphate to an available one. Thereby, offered adequate quantity of phosphorus in root media, promotes roots growth to go forward and keep roots healthy [6], [20]. Phosphate-solubilizing bacteria also secret many growth-promoting substances such as auxins, gibberellins and cytokinins [21], these substances improve plant growth and stimulate beneficial microbial development in the rhizosphere zone [22]. Many investigators reported similar findings on broad bean [7], on onion [9], [23], on garlic [24] and on bean [6].

Application of Mg as foliar application is ready to be absorbed through leaves and not to be lost through fixation, decomposition or leaching under unfavorable soil conditions. Mg is essential component of chlorophyll molecule and plays a vital role in carbohydrate synthesis due to activation of many enzymes [25]. He also stated that Mg acts as an osmotic material in the cells against adverse conditions and consequently the metabolic activities are completely achieved due to the cell turgor. Hao and Papadoulos [26] on tomato and Rady and Osman [6] on bean, stated that plant dry matter production increased as Mg concentration increased to a certain level. These results are in accordance with [4], [5].

TABLE II
EFFECT OF SOIL INOCULATION WITH PHOSPHORIEN (BIO-P) AND FOLIAR APPLICATION WITH MAGNESIUM (MG) ON VEGETATIVE GROWTH TRAITS OF *PISUM SATIVUM* L. PLANTS GROWN IN 2012 AND 2013 SEASONS

| Treatment | | Shoot length | No. of branches palnt$^{-1}$ | Leaf area palnt$^{-1}$ | Canopy DW palnt$^{-1}$ |
|---|---|---|---|---|---|
| Bio-P | Mg (mM) | | | | |
| 2012 season | | | | | |
| | 0 | 25.9d | 1.5e | 2.06d | 17.1e |
| without | 0.5 | 31.1c | 1.7d | 2.39c | 18.9de |
| | 1 | 35.6b | 2.0b | 2.75b | 22.5bc |
| | 0 | 28.1cd | 1.8c | 2.35c | 21.3cd |
| with | 0.5 | 37.0b | 2.1b | 2.90b | 24.2b |
| | 1 | 45.0a | 2.3a | 3.32a | 27.9a |
| 2013 season | | | | | |
| | 0 | 27.2d | 1.5d | 2.17d | 18.0e |
| without | 0.5 | 32.6c | 1.8c | 2.51c | 20.5de |
| | 1 | 37.4b | 2.0bc | 2.89b | 23.7bc |
| | 0 | 31.5c | 1.8c | 2.47c | 22.3cd |
| with | 0.5 | 38.8b | 2.2b | 3.00b | 25.4b |
| | 1 | 47.2a | 2.5a | 3.48a | 29.2a |

[*]Values are means, and mean values in each column followed by a different lower-case-letter are significantly different by Fisher's least-significant difference test (LSD) at $P \leq 0.05$.

Likewise, the comparisons within soil inoculation with and without phosphorien reflect the valuable effect of soil inoculation with phosphorien and spraying Mg, particularly at 1mM, on all various studied vegetative parameters compared to the untreated control. Therefore, the best valuable combination was soil inoculation with phosphrien and foliar application of Mg at 1mM. The superiority of the combined treatment having the highest results might have come from improving the nutritional status of plants of this treatment (Table IV), the abundant values of leaf pigments (Table III), the obvious shortage in $Na^+$ (Table IV) saving more osmotic solutes which enable plant cells to maintain more water against the adverse conditions of the soil under study.

Data in Tables III and IV reveal that the contents of total chlorophyll, total carotenoids, total soluble sugars, free proline, N, P, K, Mg and Ca, and the ratio of Ca/Na significantly increased in plants, which received 0.5 or 1mM Mg as foliar application compared to those in plants, which not received any rates of Mg. Plants applied with Mg at the rate of 1mM had higher total chlorophyll, total carotenoids, total soluble sugars, free proline and nutrient contents and Ca/Na ratio than plants applied with Mg at 0.5mM Mg. Further increased contents of these attributes were observed with soil inoculation with phosphorien. The content of Na showed the reverse trend to other measurements. The best results were obtained from the combined treatment of soil inoculation with phosphorien and foliar application with 1 mM Mg. Similar trends were observed in both the 2012 and 2013 growing seasons.

TABLE III
EFFECT OF SOIL INOCULATION WITH PHOSPHORIEN (BIO-P) AND FOLIAR APPLICATION WITH MAGNESIUM (MG) ON LEAF CONTENTS OF TOTAL CHLOROPHYLL (MG G–1 FW), TOTAL CAROTENOIDS (MG G–1 FW), TOTAL SOLUBLE SUGARS (MG G–1 DW) AND FREE PROLINE (MG G–1 DW) OF *PISUM SATIVUM* L. PLANTS GROWN IN 2012 AND 2013 SEASONS

| Treatment | | Total chlorophyll | Total carotenoid | Soluble sugars | Free proline |
|---|---|---|---|---|---|
| Bio-P | Mg (mM) | | | | |
| 2012 season | | | | | |
| | 0 | 1.03d | 0.37d | 18.3d | 23.9c |
| without | 0.5 | 1.25c | 0.45c | 23.4c | 25.3bc |
| | 1 | 1.46b | 0.55b | 26.3c | 27.0b |
| | 0 | 1.25c | 0.49c | 25.8c | 26.3b |
| with | 0.5 | 1.46b | 0.58b | 30.2b | 29.2a |
| | 1 | 1.69a | 0.63a | 35.0a | 30.7a |
| 2013 season | | | | | |
| | 0 | 1.09d | 0.39d | 19.2d | 25.2c |
| without | 0.5 | 1.32c | 0.48c | 24.7c | 26.7bc |
| | 1 | 1.54b | 0.58b | 27.7c | 28.5b |
| | 0 | 1.32c | 0.51c | 27.2c | 27.7b |
| with | 0.5 | 1.54b | 0.61b | 31.9b | 30.8a |
| | 1 | 1.78a | 0.67a | 36.9a | 32.3a |

[*]Values are means, and mean values in each column followed by a different lower-case-letter are significantly different by Fisher's least-significant difference test (LSD) at $P \leq 0.05$.

The enhancing effects of phosphorien on the contents of leaf nutrients and photosynthetic pigments can be owe to the efficiency of phosphorien in dissolving immobilized P and producing appropriate amounts of phytohormones, which increased surface area per unit area of root with an eventual increase in the uptake of nutrients from the soil. Therefore, more storage of energy in the form of ADP and ATP which grant transportation of nutrient across the cell wall and the synthesis of nucleic acid and proteins as well as other photosynthates. These results are in accordance with some reports [6], [24], [27], [28].

The positive linear relationship between foliar application of Mg and leaf Mg content and interrelationship between leaf Mg and P contents due to that Mg acts as a carrier of P in plants [5], [6]. Marschner [25] stated that Mg is essential element involving in the biosynthesis of chlorophyll and consequently the higher leaf Mg content, and consequently the higher leaf chlorophyll content. The distinguished results of Mg foliar applicaton on total soluble sugars content may be attributed to the importance of Mg in biosynthesis of chlorophyll and in turn enhanced photosynthesis to go forward and more photosynthates formation including total soluble sugars and protein to be achieved. The combined treatment of soil inoculation with phosphorien plus foliar application of Mg at 1 mM significantly attained the highest leaf chlorophyll, carotenoid, N, P, K, Mg and Ca contents.

Data in Tables V and VI show that green pod yield and its components (number of pods per plant, pod weight and total pod yield per fed) and green seed yield and its components (number of seeds per pod, 100-seed weight and total seed yield per fed) significantly increased with plants when sprayed with Mg at the 2 rates (0.5 and 1mM) compared to plants that not applied with Mg. Plants received Mg at the rate of 1mM gave higher pod and seed yields than plants received Mg at 0.5

mM Mg. Soil inoculation with phosphorien further increased yields of pods and seeds. The best results were obtained when the combined treatment of soil inoculation with bio-phosphorus fertilizer and foliar application with 1 mM Mg was applied. The same trends were seen in both the 2012 and 2013 growing seasons.

The enhancing effects of soil inoculation with phosphorien on green yield of pods and seeds can be attributed to the positive effects of phosphorien on plant growth traits (Table II) and leaf photosynthetic pigment contents (Table III, which may coubled togther to enhance photosynthesis to go forward. Also, phosphorien-treated soil improved plant nutritional status (Table IV) and probably reflected better partitioning of photosynthates to reproductive organs with an eventual result increasing green pod and seed yields. Many investigators reported similar trends on onion [8], [9], on garlic [24] and on bean [6].

The desirable effect of Mg application on green yields was mainly due to the increment in number of pods/plant and partially to average pod weight while, the beneficial effect of Mg application on total seed yield seemed to be mainly due to the increasing in 100-seed weight and partially to number of seeds per pod.

TABLE IV
EFFECT OF SOIL INOCULATION WITH PHOSPHORIEN (BIO-P) AND FOLIAR APPLICATION WITH MAGNESIUM (MG) ON LEAF MINERAL CONTENTS (MG G–1 DW) AND CA/NA RATIO OF *PISUM SATIVUM* L. PLANTS GROWN IN 2012 AND 2013 SEASONS

| Treatment | | N | P | K | Mg | Na | Ca | Ca/Na ratio |
|---|---|---|---|---|---|---|---|---|
| Bio-P | Mg (mM) | | | | | | | |
| 2012 season | | | | | | | | |
| without | 0 | 21.0d | 2.76e | 18.5c | 1.40e | 9.98a | 2.17c | 0.22f |
| | 0.5 | 24.2c | 3.18d | 20.1bc | 1.70d | 9.07b | 2.43b | 0.27e |
| | 1 | 28.2b | 3.68c | 21.4b | 1.97c | 8.85b | 2.58b | 0.29d |
| with | 0 | 24.5c | 3.41d | 21.9b | 1.90c | 7.54c | 2.43b | 0.32c |
| | 0.5 | 29.3b | 4.21b | 24.3a | 2.37b | 7.43c | 2.93a | 0.39b |
| | 1 | 33.2a | 4.75a | 26.0a | 2.80a | 6.30d | 3.11a | 0.49a |
| 2013 season | | | | | | | | |
| without | 0 | 21.8d | 2.88e | 19.2c | 1.44e | 9.88a | 2.25c | 0.23d |
| | 0.5 | 25.2c | 3.31d | 20.9bc | 1.75d | 8.87b | 2.54b | 0.29c |
| | 1 | 29.3b | 3.83c | 22.2b | 2.03c | 7.76c | 2.69b | 0.35b |
| with | 0 | 25.5c | 3.55d | 22.7b | 1.96c | 7.73c | 2.79b | 0.36b |
| | 0.5 | 30.5b | 4.37b | 25.3a | 2.44b | 6.42d | 3.05a | 0.48a |
| | 1 | 34.5a | 4.94a | 27.0a | 2.88a | 6.24d | 3.18a | 0.51a |

*Values are means, and mean values in each column followed by a different lower-case-letter are significantly different by Fisher's least-significant difference test (LSD) at $P \leq 0.05$.

The improving effects of Mg on studied yield was mainly attributed to its positive action on enhancing vegetative growth traits (Table II), leaf photosynthetic pigments (Table III) and plant nutritional status (Table IV) for sustenance of cells turgor leading to maintenance of metabolic activities in plants at their highest levels. In this respect, on different crops [4]-[6], [26], it has been found a positive relationship between yield and Mg level which may attributed to the important role of Mg in increasing the activity of plant metabolism, which in turn reflected on the yield under study. At any concentration of Mg, soil application of phophorien was pioneer and recorded higher mean values of all studied parameters of green pod and seed yields than the un-inoculated ones.

Likely, soil inoculation with phosphorien reflects the desirable effect of Mg on all studied parameters of green pod and seed yields, particularly at 1 mM. The superiority of the best combined treatment on green pod and seed yields may be arised as a result of positive combined effects of soil inoculation with phosphate-solubilizing bacteria and foliar application with 1 mM Mg on growth traits (Table II), leaf photosynthetic pigments (Table III) and nutritional status of plants (Tables IV).

We concluded that, under the conditions of sandy calcareous soils, phosphorien (phosphate-solubilizing bacteria) was capable to hydrolyze the insoluble phosphate into soluble one, and the role of Mg foliar application in overcoming the great deficiency of Mg in such soils. Therefore, the positive reflection of these applications on growth, yield and chemical composition of pea (cv. Master-B) was expected.

TABLE V
EFFECT OF SOIL INOCULATION WITH PHOSPHORIEN (BIO-P) AND FOLIAR APPLICATION WITH MAGNESIUM (MG) ON GREEN POD YIELD AND ITS COMPONENTS OF *PISUM SATIVUM* L. PLANTS GROWN IN 2012 AND 2013 SEASONS

| Treatment | | No. pods plant$^{-1}$ | Pod weight (g) | Green pod yield (ton fed$^{-1}$) |
|---|---|---|---|---|
| Bio-P | Mg (mM) | | | |
| | | 2012 season | | |
| without | 0 | 4.6e | 1.8d | 0.87e |
| | 0.5 | 5.5d | 2.2c | 1.24d |
| | 1 | 6.5c | 2.5b | 1.60c |
| with | 0 | 5.9d | 2.1c | 1.25d |
| | 0.5 | 7.3b | 2.6b | 1.86b |
| | 1 | 8.8a | 3.0a | 2.38a |
| | | 2013 season | | |
| without | 0 | 4.8e | 1.9d | 0.91e |
| | 0.5 | 5.7d | 2.3c | 1.30d |
| | 1 | 6.9c | 2.6b | 1.69c |
| with | 0 | 6.2d | 2.2c | 1.32d |
| | 0.5 | 7.6b | 2.7b | 1.96b |
| | 1 | 9.2a | 3.2a | 2.43a |

*Values are means, and mean values in each column followed by a different lower-case-letter are significantly different by Fisher's least-significant difference test (LSD) at $P \leq 0.05$.

TABLE VI
EFFECT OF SOIL INOCULATION WITH PHOSPHORIEN (BIO-P) AND FOLIAR APPLICATION WITH MAGNESIUM (MG) ON GREEN SEED YIELD AND ITS COMPONENTS OF *PISUM SATIVUM* L. PLANTS GROWN IN 2012 AND 2013 SEASONS

| Treatment | | No. seeds pod$^{-1}$ | 100-seed weight (g) | Green seed yield (kg fed$^{-1}$) |
|---|---|---|---|---|
| Bio-P | Mg (mM) | | | |
| | | 2012 season | | |
| without | 0 | 4.8d | 13.0c | 635d |
| | 0.5 | 5.0cd | 15.3b | 767c |
| | 1 | 5.1cd | 16.4a | 844bc |
| with | 0 | 5.2bc | 14.3b | 747c |
| | 0.5 | 5.5b | 16.4a | 907b |
| | 1 | 5.9a | 18.5a | 1025a |
| | | 2013 season | | |
| without | 0 | 5.1c | 13.7c | 668e |
| | 0.5 | 5.2c | 16.1b | 807cd |
| | 1 | 5.4bc | 17.3ab | 876bc |
| with | 0 | 5.4bc | 15.1bc | 787d |
| | 0.5 | 5.8b | 17.3ab | 956b |
| | 1 | 6.3a | 19.0a | 1073a |

*Values are means, and mean values in each column followed by a different lower-case-letter are significantly different by Fisher's least-significant difference test (LSD) at $P \leq 0.05$.

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