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Magnesium and phosphorien applications improve the efficiency of growth and productivity of squash (*Cucurbita pepo* L.) plants grown on a sandy calcareous soil

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ABSTRACT

Two field experiments were carried out to study 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 1 mM on the growth, some chemical constituents and fruit yield and its quality of squash (*Cucurbita pepo* L) grown on a sandy calcareous soil. The experiment were performed in a completely randomized blocks design with six treatments, each one with four replicates. The results indicated that PDB and/or Mg significantly increased stem length, canopy dry weight plant^{-1}, number of leaves plant $^{-1}$, stem length, canopy dry weight plant^{-1}, number of leaves plant^{-1}, leaf area leaf^{-1}, leaf area plant^{-1}, leaf contents of pigments, free proline, nitrogen (N), phosphorus (P), potassium (K), magnesium (Mg), and calcium (Ca) and the ratio of Ca/Na, while leaf Na content was reduced. The application of PDB and/or Mg also increased fruit yield and its components (i.e., number of fruits plant^{-1}, fruit weight plant^{-1} and fruit yield fed^{-1}). It has been concluded that the applications of Mg and PDB have pronounced a positive reflection on the growth, fruit yield and its components of squash plants grown on sandy calcareous soil. PDB and Mg, therefore, have the potential to be used as a soil inoculation and foliar application, respectively for squash and may for various crops to overcome the adverse effects of the newly-reclaimed sandy calcareous soils.

Key words: Squash, (Cucurbita pepo L), Bio-fertilizer, Magnesium foliar application, growth, yield, chemical constituents

INTRODUCTION

Squash is considered as one of the most variable genera within the Cucurbitaceae. It has various medicinal effects such as antidiabetic, antihypertensive, antitumor, antimutagenic, immunomodulation, antibacterial, antihypercholesterolemic, intestinal antiparasitic, antalgic, and antiinflammation effects. The utilization possibilities of various Cucurbitaceae species have been reported [1]. Squash is widely cultivated on newly-reclaimed soils in the Middle Eastern countries, including Egypt. Most of such soils are sandy calcareous, which show a great deficiency in macronutrients especially, magnesium (Mg) and phosphorus (P).

Magnesium plays a vital role in stepping up the growth and quantitative as well as qualitative features of the plant. Its deficiency is most prevalent in sandy textured soils because it oxidizes to become in an unavailable form for plant and it losses by leaching [2]. In reclaimed sandy soils, foliar application of macro- and/or micro-nutrients are widely used [3, 4]. These applications and lead to significant increases in vegetative growth and productivity of some crops [5, 6, 7, 8]. Phosphorus precipitation and immobilization is the most important problem in calcareous soil that characterized by high pH and calcium carbonate content. Phosphorien is a bio-fertilizer product that is phosphorien-containing phosphate-dissolving bacteria (PDB). It hydrolyzes the insoluble phosphate into soluble one under the adverse conditions of the sandy calcareous soil. Addition of the PDB to such soils lead to significant increments in vegetative growth and productivity of crops [7, 8, 9, 10, 11].

Therefore, the present work was designed with objective to evaluate the influence of soil inoculation with PDB and/or application of Mg to plant foliage on vegetative features, fruit yield and its quality in addition to some chemical constituents of squash (*Cucurbita pepo* L) plants grown under the adverse conditions of a sandy calcareous soil.

MATERIALS AND METHODS

Seed of squash (cv. Amjed hybrid) was purchased from Seminis-Petoseed Company, USA. Phosphorien (*Bacillus megatherium*; phosphate-dissolving bacteria) was provided by the Ministry of Agriculture and mixing with Nile water to obtain the concentration of about 0.25×10^{7} cfu ml^{-1}. Magnesium concentrations were 0, 0.5 and 1 mM, which used singly or in combination with phosphorien (6 treatments in total). The rates of Mg (0.5 and 1 mM) were generated the best responses in our preliminary studies (data not shown), therefore, these rates were selected for this study. A two-field experiment was undertaken in the two seasons of 2013 and 2014 at the Experimental Farm, Faculty of Agriculture, Fayoum University, Egypt. Soil samples to 25 cm depth were collected during soil preparation for sowing to analysis. Some of the chemical and physical properties according to the standard procedures of [12] are analyzed and presented in Table 1.

During preparation of the experimental site, farmyard manure at the rate of 15 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. Squash seeds were sown on 14 March 2013 and on 17 March 2014.

Table1: Physical and chemical properties of the experimental soil before planting in 2013 and 2014 seasons

I. Parameter	2013	2014
Clay [% (w/w)]	21.3	24.6
Silt [% (w/w)]	24.1	22.8
Sand [% (w/w)]	54.6	52.6
Soil texture	sandy	sandy
pH (1: 2.5)	8.07	7.96
ECe (dS m^{-1})	5.05	4.91
Organic matter [% (w/w)]	1.16	1.26
$CaCO_3$ [% (w/w)]	13.91	14.51
Available N (mg kg^{-1} DW)	42.88	42.55
Available P (mg kg^{-1} DW)	8.03	9.27
Available K (mg kg^{-1} DW)	72.43	75.40
Available Fe (mg kg^{-1} DW)	6.96	8.11
Available Mn (mg kg^{-1} DW)	4.47	5.39
Available Zn (mg kg^{-1} DW)	2.22	2.46
Available Cu (mg kg^{-1} DW)	0.45	0.44

Soil inoculation with phosphorien was performed by injection of 40 ml $hole^{-1}$ two times; 20 and 40 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 design was a completely randomized blocks with 6 treatments, each one with 4 replicates. Each experimental unit measured 14 m^2; 5 rows with 4 m long and 0.7 m width, with row spacing averaged 40 cm apart. Each two adjacent experimental unites were separated by 1 m alley.

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. All other agro-management practices for commercial production of squash were followed whenever it was necessary.

Measurements of growth traits

Forty-five days after sowing, five plants were randomly chosen from the two outer rows of each experimental unit and cut off at ground level to measure the stem length, canopy dry weight $plant^{-1}$, number of leaves $plant^{-1}$, leaf area $leaf^{-1}$ and leaf area $plant^{-1}$. Leaf area $leaf^{-1}$ was calculated using the following formula:

$$\text{Leaf area leaf}^{-1} = \frac{\text{Leaves area plant}^{-1}}{\text{Number of leaves plant}^{-1}}$$

Measurements of fruit yield and its quality

In each experimental unit, plants of the three middle rows were left to grow until the fruits approach the marketable stage. Then, fruits were picked from 20 plants to determine the number of fruits $plant^{-1}$, average fruit weight and fruits weight $plant^{-1}$. The total yield of fruit fed^{-1} was calculated from all plants of the three middle rows.

Nutrients and sodium determinations

Forty five days after sowing, leaf samples were collected from the fourth upper leaf of five randomly selected plants from each experimental unit to determine the concentrations of some nutrients and sodium. Leaf chlorophyll and carotenoid contents in the 4^{th} leaf were colorimetrically determined as outlined by [13]. 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 0.1 g was digested with a mixture of sulphuric and perchloric acids as mentioned by [14]. 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 [15]. Leaf N content was colorimetrically determined using Orange G dye as suggested by [16]. 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 [17]. Leaf K and Na contents were determined using a Flame-photometer as documented by [18]. Leaf Ca and Mg contents were measured using a Perkin-Elmer, Model 3300, Atomic Absorption Spectrophotometer as mentioned by [19].

Statistical analysis

Data of the two seasons were subjected to the statistical analysis according to the design used by [20].

The least significant difference test (LSD) at $p \leq 0.05$ level was utilized to verify the significant difference between treatments.

RESULTS AND DISCUSSION

Vegetative Growth Traits

Data in Table 2 show that stem length, canopy dry weight $plant^{-1}$, number of leaves $plant^{-1}$, leaf area $leaf^{-1}$ and leaf area $plant^{-1}$ significantly increased in plants that sprayed with Mg at the 2 rates (0.5 and 1 mM) compared to the control plants. Plants received Mg at the rate of 1 mM gave higher growth traits than plants received Mg at 0.5 mM 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 1 mM Mg. The same trend was observed over both 2013 and 2014 growing seasons.

These results can be explained on the basis that, phosphate-solublizing bacteria produces organic and inorganic acids and/or CO_2 which dissolve the precipitated form of phosphate to available one. Thereby, offered adequate quantity of phosphorus in root media, promoting root growth to go forward and keep roots healthy [21, 7, 8]. Phosphate-solubilizing bacteria also secret many growth-promoting substances such as auxins, gibberellins and cytokinins [22]. These growth-promoting substances improve plant growth and stimulate beneficial microbial development in the rhizosphere zone [23]. Many investigators reported similar findings on broad bean (9), onion [24, 11], garlic [25], common bean [7] and pea [8].

Table 2: Effect of soil inoculation with phosphorien (Bio-P) and foliar application with magnesium (Mg; mM) on vegetative growth traits [i.e., stem length (cm), canopy dry weight (g $plant^{-1}$), number of leaves $plant^{-1}$, leaf area (dm^2 $leaf^{-1}$) and

total leaf area (dm^2 $plant^{-1}$)] of squash plants grown in 2013and 2014 seasons

Treatment		Stem length	Canopy DW	No. of leaves	Leaf area $leaf^{-1}$	Leaf area $plant^{-1}$
Bio-P	Mg (mM)					
		2013 season				
without	0	3.8d*	16.1d	7.8d	2.35c	18.4c
	0.5	4.3c	20.4c	8.6cd	2.61b	22.2b
	1	4.8b	24.9b	9.6ab	2.83a	27.1a
with	0	4.4c	21.4c	9.0bc	2.66b	24.1b
	0.5	5.0b	25.4b	10.0a	2.88a	28.7a
	1	5.5a	28.2a	10.4a	2.87a	29.5a
		2014 season				
without	0	4.0d	18.7d	8.0d	2.34c	18.8d
	0.5	4.6c	23.5c	8.8cd	2.56b	22.4c
	1	5.4b	29.2b	9.8ab	2.75a	27.0b
with	0	4.8c	24.8c	9.4bc	2.62b	24.6bc
	0.5	5.4b	29.5b	10.2ab	2.82a	28.7ab
	1	5.9a	32.4a	10.5a	2.83a	29.8a

*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 $P \leq 0.05$.

Application of Mg to plant foliage 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 [26]. The author 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. [27] on tomato, [7] on common bean and [8] on pea, stated that plant dry matter production increased as Mg concentration increased to a certain level. These results are in accordance with those obtained by [5] and [6].

The valuable effect of soil inoculation with phosphorien in combination with plant spray by Mg, particularly at 1 mM, on all various studied vegetative parameters compared to the untreated control, positively reflected in the yield characters (Table 5). The superiority of the combined treatment, which generated the best results might have come from improving the nutritional status of plants (Table 4), the abundant values of leaf pigments and free proline (Table 3) and the obvious shortage in Na^+ (Table 4) of this treatment, saving more osmolytes, which enable plant cells to maintain more water against the adverse conditions of the soil under study.

Leaf photosynthetic pigment, free proline and nutrient concentrations

Data in Tables 3 and 4 reveal that the concentrations of total chlorophyll, total carotenoids, free proline, N, P, K, Mg and Ca, and the ratio of Ca/Na significantly increased in plants, which received 0.5 or 1 mM Mg as foliar application compared to the control plants, which not received any rates of Mg. Plants applied with Mg at a rate of 1 mM had higher chlorophylls, carotenoids, free proline and nutrient concentrations and Ca/Na ratio than plants applied with Mg at a rate of 0.5 mM Mg. Further increased concentrations of these attributes were observed with soil inoculation with phosphorien. The concentration 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 2013 and 2014 seasons.

Table 3: Effect of soil inoculation with phosphorien (Bio-P) and foliar application with magnesium (Mg; mM) on Effect of soil inoculation with phosphorien (Bio-P) and foliar application with magnesium (Mg; mM) on the leaf contents of total chlorophyll (mg g–1 FW), total carotenoids (mg g–1 FW) and free proline (μg g–1 DW) of squash plants grown in 2013 and 2014 seasons

Treatment		Total chlorophyll	Total carotenoids	Free proline
Bio-P	Mg (mM)			
		2013		
without	0	1.08d*	0.34d	22.4c
	0.5	1.27c	0.44c	24.6b
	1	1.40b	0.56b	25.9b
with	0	1.15d	0.48c	25.5b
	0.5	1.43b	0.58b	28.7a
	1	1.53a	0.65a	29.7a

		2014		
without	0	1.13d	0.39d	24.6c
	0.5	1.33c	0.50c	26.2bc
	1	1.52b	0.58b	27.8b
with	0	1.19d	0.51c	26.9b
	0.5	1.47b	0.62b	30.3a
	1	1.60a	0.68a	31.1a

*Values are means (n = 4), 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 ≤ 0.05.

Table 4: Effect of soil inoculation with phosphorien (Bio-P) and foliar application with magnesium (Mg; mM) on leaf mineral content (N, P, K, Mg, Na and Ca; (mg g–1 DW) and the ratio of Ca/Na of squash plants grown in 2013 and 2014 seasons

Treatment		N	P	K	Mg	Na	Ca	Ca/Na ratio
Bio-P	Mg (mM)							
		2013						
without	0	17.7d*	2.61e	23.4d	1.45e	5.93a	2.23c	0.38c
	0.5	20.9c	3.08d	25.9cd	1.76d	5.61ab	2.56b	0.46bc
	1	24.0b	3.33c	27.5bc	2.04c	5.24bc	2.76b	0.53b
with	0	21.4c	3.30c	27.7bc	1.97c	5.01cd	2.64b	0.53b
	0.5	24.4b	3.94b	29.2ab	2.46b	4.59de	3.11a	0.68a
	1	26.7a	4.54a	30.3a	2.91a	4.36e	3.28a	0.75a
		2014						
without	0	18.2d	2.76e	24.0d	1.50e	5.78a	2.38c	0.41c
	0.5	20.9c	3.19d	26.6c	1.82d	5.46ab	2.77b	0.51bc
	1	24.3b	3.64c	28.2b	2.10c	5.09bc	2.92b	0.57b
with	0	21.0c	3.49c	28.4b	2.03c	4.88c	2.82b	0.58b
	0.5	25.4b	4.12b	31.5a	2.53b	4.40d	3.20a	0.73a
	1	28.5a	4.67a	32.5a	2.98a	4.27d	3.31a	0.77a

Table 5: Effect of soil inoculation with phosphorien (Bio-P) and foliar application with magnesium (Mg; mM) on yield and its components [i.e., number of fruits plant–1, average fruit weight (g), fruit weight plant–1 (g) and fruit yield (ton fed–1)] of squash plants grown in 2013 and 2014 seasons

Treatment		No. of fruits $plant^{-1}$	Average fruit weight	Fruit weight $plant^{-1}$	Fruit yield fed^{-1}
Bio-P	Mg (mM)				
		2013			
without	0	5.5c*	48.9a	271d	3.8d
	0.5	6.0bc	50.1a	301c	4.2c
	1	6.6ab	50.9a	340b	4.8b
with	0	5.9bc	50.0a	298c	4.2c
	0.5	6.8a	50.4a	349b	4.8b
	1	7.3a	51.0a	373a	5.2a
		2014			
without	0	5.7d	50.3a	248d	4.0d
	0.5	6.3cd	52.1a	330c	4.6c
	1	6.8bc	53.3a	363b	5.1b
with	0	6.2d	50.5a	314c	4.4c
	0.5	7.1ab	53.1a	379b	5.2b
	1	7.5a	53.2a	400a	5.6a

*Values are means (n = 4), 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 ≤ 0.05.

The favorable effects of phosphorien on the concentrations 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 are increased surface area per unit area of root, leading to an eventual increase in the uptake of nutrients from the soil. Therefore, more storage of energy in the form of ADP and ATP to transport the nutrient across the cell wall and the synthesis of nucleic acid and proteins as well as other photosynthates. These results are in accordance with those of [7], [8], [25], [28] and [29].

The positive linear relationship between foliar application of Mg and leaf Mg concentration and interrelationship between leaf Mg and P concentrations were due to that Mg acts as a carrier of P in plants, which were documented by [6], [7] and [8]. [26]. They stated that Mg is essential element that involved in the biosynthesis of chlorophyll and consequently the higher leaf Mg concentration and the higher leaf chlorophyll concentration. The combined treatment of soil inoculation with phosphorien plus foliar application of Mg at 1 mM significantly attained the highest leaf chlorophylls, carotenoids, N, P, K, Mg and Ca concentrations.

Fruit yield and its components

Data in Table 5 show that fruit yield and its components (number of fruits plant^{-1}, fruit weight plant^{-1} and fruit yield, except the average fruit weight) significantly increased in plants sprayed with Mg at the two rates (0.5 and 1 mM) compared to the control plants that not applied with Mg. Plants received Mg at a rate of 1 mM gave higher fruit yield and its components than plants received Mg at a rate of 0.5 mM Mg. Soil inoculation with phosphorien further increased fruit yield and its components. 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 2013 and 2014 seasons.

The enhancing effects of soil inoculation with phosphorien on number of fruits plant^{-1}, fruit weight plant^{-1} and fruit yield fed^{-1} can be attributed to the positive effects of phosphorien on plant growth traits (Table 2) and leaf photosynthetic pigment concentrations (Table 3), which may coupled togther to enhance photosynthesis to go forward. Also, phosphorien-treated soil improved plant nutritional status (Table 4) and probably reflected better partitioning of photosynthates to reproductive organs with an eventual result, increasing fruit yield and its quality. Many investigators reported similar trends on onion [10, 11], garlic [25], common bean [7] and pea [8].

The improving effects of Mg on studied fruit yield and its components was mainly attributed to its positive action on enhancing vegetative growth traits (Table 2), leaf photosynthetic pigments (Table 3) and plant nutritional status (Table 4) for sustenance of cells turgor, leading to maintenance of metabolic activities in plants at their highest levels. In this respect, on different crops, [5], [6], [7], [8] and [27] 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, positively 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 fruit yield than the un-inoculated ones. Likely, soil inoculation with phosphorien reflected the desirable effect of Mg on all studied parameters of fruit yield, particularly at 1 mM. The superiority of the best combined treatment on fruit yield 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 2), leaf photosynthetic pigments (Table 3) and nutritional status of plants (Table 4).

We concluded that, under the adverse conditions of sandy calcareous soils, phosphorien (phosphate-solubilizing bacteria) was capable to hydrolyze the insoluble phosphate into soluble one, which reflected in an increase in the efficiency of Mg foliar application to overcome the great deficiency of Mg in such soils. Therefore, the positive reflection of these applications on growth, fruit yield and chemical composition of squash (cv. Amjed hybrid) was expected.

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