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Research Article

Muskmelon morpho-physiology and yield: The combined effect of biostimulants and phosphorus

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Abstract

Muskmelon (*Cucumis melo* L.), a hydrating fruit rich in antioxidants, vitamins, and minerals, is widely grown in tropical and subtropical regions where phosphorus (P) deficiency is common. P availability influences sugar and acid contents in melons because of its role in sugar acid phosphatase enzymes. Increasing phosphorus use efficiency through the use of biostimulants, particularly phosphorus-solubilizing bacteria, represents a promising approach for sustainable muskmelon production. These biostimulants solubilize inorganic P by releasing phosphatase enzymes and organic acids. This study examined the effects of three P levels (100%, 50%, and 0% P_2O_5) and biostimulants (control, GEA 1499- a formulation containing plant base biostimulant and the microbial species *Bacillus pumilus* and *Bacillus megaterium* at 2.5 kg ha^{-1}, and GEA 1499 at 5 kg ha^{-1}) on muskmelon morphology, physiology, biochemistry, and yield. The combination of 100% P_2O_5 with GEA 1499 at 2.5 kg ha^{-1} significantly improved the leaf count, vine length, photosynthesis, stomatal conductance, transpiration, chlorophyll index, marketable yield, and total soluble solids while reducing the undesirable traits rind thickness and seed cavity dimensions, indicating improvement in fruit quality. Phosphorus enhances gas exchange via ATP and the Calvin cycle, whereas biostimulants containing microbes and plant extracts improve nutrient availability, promoting better muskmelon growth, yield, and fruit quality. A combination of plant extracts provides phytohormones that complement the microbial action and improve the overall efficiency of the biostimulant.

Keywords: biostimulant; fruit quality; morphology; muskmelon; phosphorus; physiology; yield

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Introduction

Melons rank as the eleventh most important fruit crop globally, with a total production of approximately 28.56 million tons in 2022. India contributed about 1.50 million tons, following China and Turkey (FAO, 2022). Melons have a high-water content, ranging from 90% to 97% by weight. They are abundant in antioxidants, essential primary metabolites (such as proteins, lipids, and carbohydrates), a variety of vitamins (including vitamins A, B1, B2, B6, C, and E), and minerals such as potassium, calcium, iron, magnesium, and phosphorus. On average, melon fruits provide approximately 45 kcal per 100 g of fresh weight (Manchali and Murthy, 2020). Musk melon is cultivated widely in tropical and subtropical areas, where Pi deficiency is common (Li *et al*., 2022). The availability of macro- and micronutrients determines muskmelon growth, yield, and quality (Rajasekar *et al*., 2017). Fertilizers play a vital role in increasing the productivity of muskmelon by providing essential nutrients such as nitrogen, phosphorus, and potassium, which support plant growth, improve yields, and enhance overall crop quality (Song *et al*., 2025). Among the macronutrients, phosphorous is particularly important for muskmelon because it enhances sugar accumulation and sink strength by promoting sucrose metabolism (Lingle and Dunlap, 1987). The balanced application of nutrients ensures optimal soil fertility and sustainable agricultural practices (Beaton *et al*., 1999). Using chemical fertilizers can compromise agricultural sustainability by negatively impacting the environment and ecosystems over time (Zafar *et al*., 2024).

Biostimulants can replace chemical fertilizers, which have harmful effects on the environment (Boutahiri *et al*., 2024): "Biostimulants are any substance or microorganism applied to plants to increase nutrition use efficiency, abiotic stress tolerance, and/or crop quality traits, regardless of their nutrient content" (Kauffman *et al*., 2007). In horticultural systems, biostimulants promote plant growth, improve nutrient uptake, and enhance stress resilience (Boutahiri *et al*., 2024). Particularly, phosphorus-solubilizing biostimulants containing *Bacillus subtilis, B. megaterium*, or *Pseudomonas striata* enhance P availability by solubilizing inorganic P minerals through the secretion of phosphatase enzymes and organic acids, lowering soil pH and improving chelation capacity (Elhaissoufi *et al*., 2022).

Recent research primarily focuses on the independent evaluation of biostimulants or phosphorus under controlled conditions and the field studies examining the combined effect on morphological, physiological and yield traits of muskmelon are scarce and the existing findings are reported in crops such as tomato and lettuce (du Jardin 2015), while crop-specific responses in *Cucumis melo* L. under variable soil phosphorus regimes are less studied. Field-level evaluations are essential to address the efficacy of biostimulants in a controlled environment (Povero *et al*., 2016). Therefore, the present study aimed to evaluate the morpho-physiological responses and yield performance of muskmelon under varying phosphorus levels and biostimulants applications, with an emphasis on improving phosphorus-use efficiency through sustainable soil management.

Materials and Methods

Plant material and experimental design

Muskmelon (*Cucumis melo* L. cv. 'Golden Glory') seeds, obtained from Greenfields Company, were sown in 96-cell trays containing a coir peat and vermicompost mixture (3:1 v/v). Seedlings were irrigated by frequent sprinkling and maintained for 14 days in a nursery before field transplantation. The field experiment was conducted at the Eastern Block Farm, Tamil Nadu Agricultural University, Coimbatore, from March to May 2024, to evaluate the combined effects of phosphorus (P) and biostimulants on the morpho-physiological traits and yield of muskmelon. The experimental soil was classified as sandy clay loam, consisting of 39.6% sand, 42.3% clay, and 12.1% silt, with a pH of 7.13 and a cation exchange capacity (CEC) of 16.4 mEq/100 g. The soil contained 0.77% total organic carbon, 19.70 mg kg^{-1} total nitrogen, a C/N ratio of 4.05, and the following nutrient contents (mg kg^{-1}): total and available P (53.1 and 8.81), K (313 and 94.74), SO_4^{2-} (1.18), Fe (13.8 and 2.90), and Zn (23.66 and 0.48). The experiment was based on a split-plot design with four replications and 18 plants per plot; the main plot treatment included 100% (375 kg single superphosphate (SSP)/ha), 50% (188 kg SSP/ha), and 0% (0 kg SSP/ha) P_2O_5, whereas the 100% (M1), 50% (M2) and 0% P_2O_5 (M3) treatments included standard amounts of nutrients based on soil tests, simulations of nutrient-limited conditions and baselines to assess plant growth in the absence of P_2O_5 respectively, and the subplot biostimulant (plant base biostimulant and the microbial species *Bacillus pumilus* and *Bacillus megaterium*) treatments included a control (S1), GEA 1499 at 2.5 kg ha^{-1} (S2), and GEA 1499 at 5 kg ha^{-1} (S3). The phosphorus fertilizer at different levels was given as basal fertilizer, and the biostimulant was applied as a root application at 10 days after transplanting (DAT), 25 DAT, and 40 DAT. Fruits were harvested at 60 DAT and five fruits per subplot were used for sampling.

Morphological parameters

The vine length was measured from the soil surface to the growing tip of the longest branch. The length of the vine was recorded 50 days after transplanting (DAT) with the help of a measuring tape from the base of the plant to the apex of the main vine for each replication and treatment. The data were recorded in centimeters (cm).

The number of leaves per $plant^{-1}$ was counted for each replication and each treatment at 50 DAT.

Physiological parameters

The chlorophyll index was measured via a portable chlorophyll meter (Soil-Plant Analytical Development) SPAD Model 5020 Minolta (Konica Minolta, INC, Tokyo, Japan) under field conditions. A physiologically active third leaf was chosen, and five SPAD readings were taken from each replication, with the average being calculated.

The rates of photosynthesis, stomatal conductance, and transpiration were measured via a portable photosynthesis system (CID Bio-Science, CI-340). For each trial, three measurements were taken. A healthy leaf was selected, cleaned, and positioned inside a cuvette (6.0 cm^2) for measurement, with values recorded via the PPS between 10:00 a.m. and 12:00 noon on a clear sunny day. The photosynthetic rate was reported as μmol CO_2 m^{-2} s^{-1}, stomatal conductance was recorded as μmol H_2O m^{-2} s^{-1}, and the transpiration rate was quantified as mmol H_2O m^{-2} s^{-1}.

Yield and fruit traits

The muskmelon fruit weight was taken at the full-slip stage, and the individual fruit weight was taken from each treatment and replication and expressed in tonnes per hectare (t ha^{-1}).

The length of five fruits harvested at edible maturity was recorded from the base to the apex of the fruit and averaged and represented in cm.

The diameter of the edible fruits was recorded for the same five randomly selected fruits in each replication, and the length was measured. The fruit girth at the top, middle, and bottom portions of the fruit was measured with the help of a digital Vernier calliper in centimeters, and the mean value was determined.

The thickness of the fruit rind was determined via Vernier callipers on the same fruits used for measuring fruit length, and the average value was computed and presented in millimeters (mm).

The length of the seed cavity was measured in the vertical section of the fruit with the help of a scale at the edible maturity stage and is presented in cm.

The width of the seed cavity was measured in the horizontal section of the fruit with the help of a scale at the edible maturity stage and is presented in cm.

TSS of fruits was observed by using an ERMA hand refractometer (0-32%). The juice was squeezed from uniformly ripened muskmelon, and a few drops of juice were placed on the prism of the digital hand refractometer; it was expressed in °Brix. The instrument's prism was cleaned using distilled water after each sample.

A TA-XT2 texture analyser (Stable Micro Systems, UK) was utilized to assess the texture of the muskmelon. Penetrometer assessments were carried out using a 5 mm stainless steel probe, which penetrated 10 mm into the sample. The test was executed at a speed of 1 mm/s with a trigger force set at 10.01 N. The peak force recorded served as an indicator of firmness. Firmness was measured in Newtons (N).

Foliar P content

Leaf samples were collected at the fruit setting stage, and P content was analysed using the colorimetric method and expressed in g kg^{-1} (Piper, 1966) .

Statistical analysis

Data were analyzed under a split-plot design using analysis of variance (ANOVA). the ANOVA indicated significant treatment effects, mean separation was carried out using the Least Significant Difference (LSD) post-hoc test at the $p < 0.05$ significance level. Statistical analyses were conducted using SPSS version 16.0, and graphical visualizations were prepared with Microsoft Excel 2010.

Results

Morphological parameters

Vine length

The effects of different phosphorus levels and the application of the biostimulant GEA 1499 on muskmelon vine length (cm) are presented in Table 1 and ANOVA revealed significant differences for main plot (p levels), subplot (biostimulant levels), and their interaction ($p < 0.01$). Compared with that of the control, vine length was significantly influenced by phosphorus and root application of biostimulants, irrespective of dose. Among the three levels of P_2O_5, M1 had the highest vine length (101.67 cm), which was 29.2% greater than that of M3. Among the biostimulants, S2 resulted in the longest vine length of 100.33 cm. Among the interactions, M1S2 presented a maximum vine length of 110 cm compared with the other treatment combinations, and the reduction in P_2O_5 to 50% with the application of the biostimulant GEA 1499 at 2.5 kg ha^{-1} resulted in a 13.5% increase compared with that of M1S1.

Number of leaf plants $^{-1}$

The application of different levels of P_2O_5 and biostimulants and interaction significantly ($P < 0.01$) influenced the number of leaves per plant (Table 1). Among the phosphorus treatments, M1 recorded the mean maximum number of leaves at 43 plant^{-1} and the mean minimum number of leaves at 26 per plant at M3. A reduction in P_2O_5 application to 0% reduced the number of leaves by 39.5% compared with 100% P_2O_5. Among the biostimulants, the mean maximum number of leaves per plant was observed in S2, and the mean minimum number of leaves per plant was 32 per plant in S1. Compared with S1, the application of the biostimulant S2 increased the number of leaves by 25%. Among the interactions between different levels of P_2O_5 and the biostimulant, the M1S2 treatment resulted in a greater number of 46 leaves per plant.

Table 1. Effects of phosphorus and biostimulants on vine length and number of leaves plant^{-1} of muskmelon

Treatments	Vine length (cm)	Number of leaves plant^{-1}
M1S1	89.00 ± 6.23^{c}	40.00 ± 2.80^{d}
M1S2	110.00 ± 4.79^{a}	46.00 ± 3.22^{a}
M1S3	106.00 ± 7.42^{b}	43.00 ± 3.01^{b}
M2S1	78.00 ± 3.40^{g}	35.00 ± 1.53^{f}
M2S2	101.00 ± 7.07^{c}	42.00 ± 2.94^{c}
M2S3	93.00 ± 4.05^{d}	39.00 ± 1.70^{e}
M3S1	62.00 ± 4.34^{h}	21.00 ± 1.47^{i}
M3S2	90.00 ± 3.92^{e}	32.00 ± 1.39^{g}
M3S3	84.00 ± 5.88^{f}	25.00 ± 1.75^{h}
F stat	6.77 **	28.12**
p value	<0.01	<0.01
CD [A(B)]	2.65	0.84
CD[B(A)]	2.79	1.82
SE(d)	1.22	0.39

M1: 100% P_2O_5; M2: 50% P_2O_5; M3: 0% P_2O_5; S1: Control; S2: GEA 1499 at 2.5 kg ha^{-1}; S3: GEA 1499 at 5 kg ha^{-1}. The values represent mean ± SD. Treatments with the same letter grouping are not significantly different. * indicates significance at the 5% level; ** indicates significance at the 1% level; NS indicates non-significance

Physiological parameters

SPAD value (chlorophyll index)

The SPAD (Figure. 1) value decreased to 29.7% as the P_2O_5 level decreased from 0% to 100%, and biostimulant application increased the SPAD value by 6.2% in S2 compared with the control. The interaction effect between phosphorus and biostimulants was significant; a mean maximum SPAD value of 48 was recorded in M1S2, and a minimum of 27 was observed in M3S1.

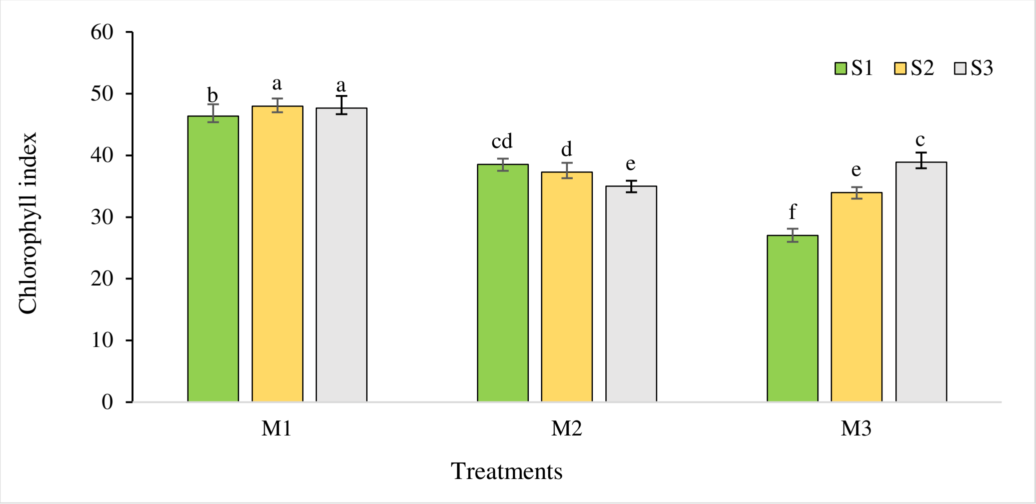

Figure 1. Effects of phosphorus and biostimulants on the chlorophyll index of muskmelon
M1: 100% P_2O_5; M2: 50% P_2O_5; M3: 0% P_2O_5; S1: Control; S2: GEA 1499 at 2.5 kg ha^{-1}; S3: GEA 1499 at 5 kg ha^{-1}. The values represent mean ± SD. Treatments with the same letter grouping are not significantly different. LSD ($p < 0.01$)

Gas exchange parameters

The effects of phosphorus and biostimulant treatments on gas exchange parameters such as the photosynthetic rate, stomatal conductance, and transpiration rate of muskmelon are shown in the Figure. 2, 3, and 4 respectively. Among the phosphorus treatments, M1 presented the highest photosynthetic rate (26.19 mmol CO_2 m^{-2} s^{-1}), stomatal conductance (317.11 mmol H_2O m^{-2} s^{-1}), and transpiration rate (5.83 mmol H_2O m^{-2} s^{-1}), and among the biostimulants, S2 presented the maximum photosynthetic rate (22.18 mmol CO_2 m^{-2} s^{-1}), stomatal conductance (219.56 mmol H_2O m^{-2} s^{-1}) and transpiration rate (4.37 mmol H_2O m^{-2} s^{-1}). Compared with M1S1, the combination of phosphorus and biostimulants (M1S2) significantly increased the photosynthetic rate, stomatal conductance, and transpiration rate by up to 19%, 17%, and 25%, respectively.

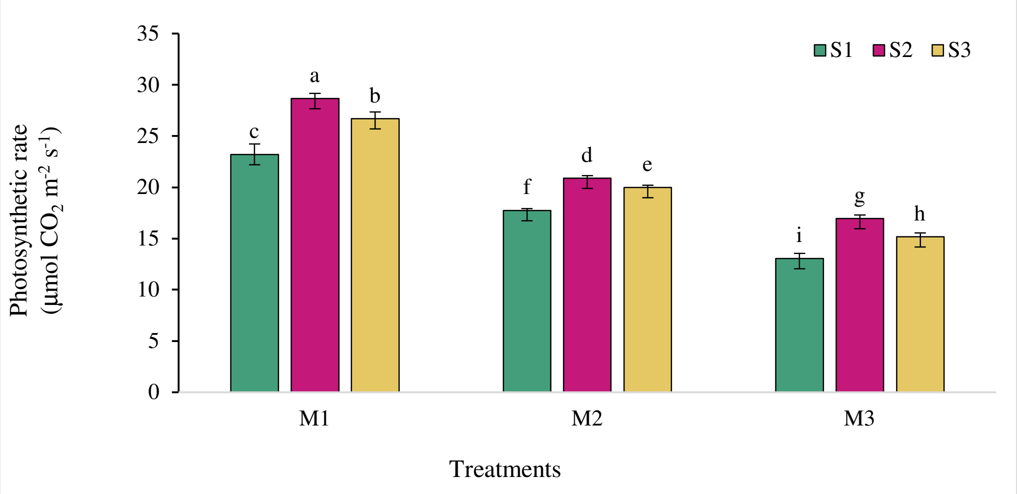

Figure 2. Effects of phosphorus and biostimulants on the photosynthetic rate of muskmelon
M1: 100% P_2O_5; M2: 50% P_2O_5; M3: 0% P_2O_5; S1: Control; S2: GEA 1499 at 2.5 kg ha^{-1}; S3: GEA 1499 at 5 kg ha^{-1}. The values represent mean ± SD. Treatments with the same letter grouping are not significantly different. LSD ($p < 0.01$)

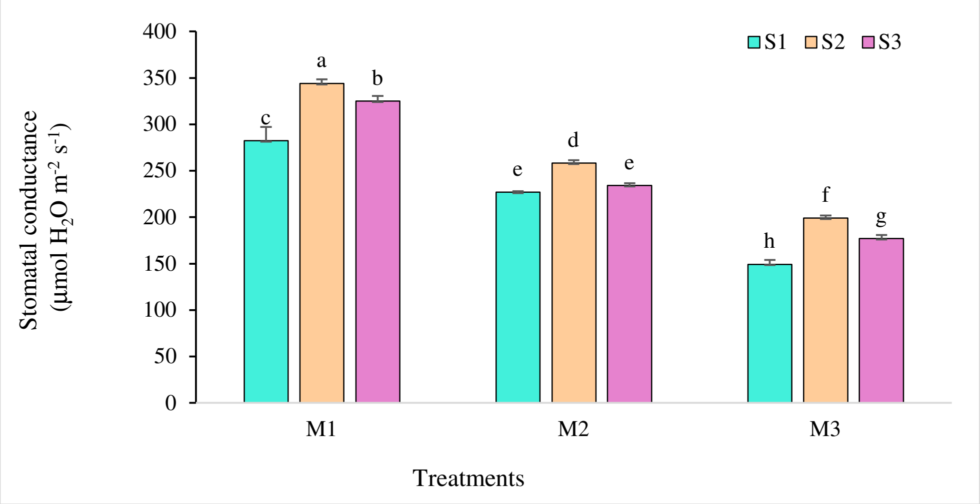

Figure 3. Effects of phosphorus and biostimulants on the stomatal conductance of muskmelon
M1: 100% P_2O_5; M2: 50% P_2O_5; M3: 0% P_2O_5; S1: Control; S2: GEA 1499 at 2.5 kg ha^{-1}; S3: GEA 1499 at 5 kg ha^{-1}. The values represent mean ± SD. Treatments with the same letter grouping are not significantly different. LSD ($p < 0.05$)

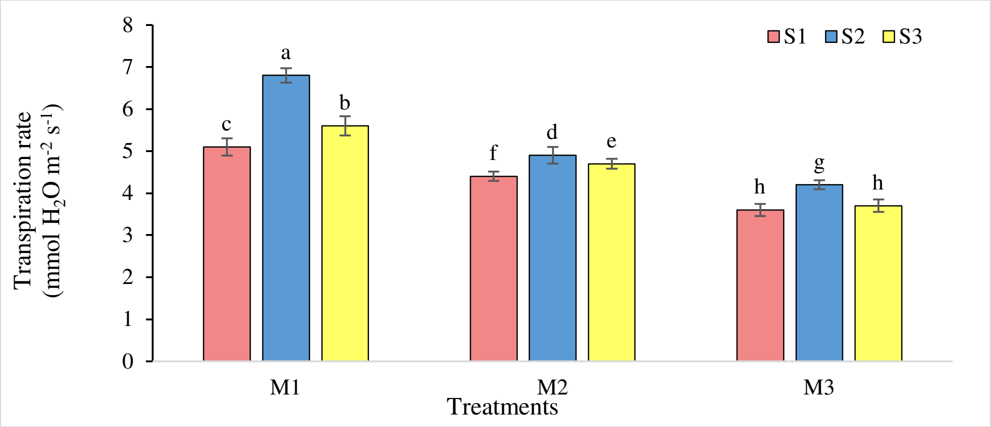

Figure 4. Effects of phosphorus and biostimulants on the transpiration rate of muskmelon
M1: 100% P_2O_5; M2: 50% P_2O_5; M3: 0% P_2O_5; S1: Control; S2: GEA 1499 at 2.5 kg ha^{-1}; S3: GEA 1499 at 5 kg ha^{-1}. The values represent mean ± SD. Treatments with the same letter grouping are not significantly different. LSD ($p < 0.01$)

Yield and fruit traits

Marketable yield

The impact of biostimulant and phosphorus on marketable yield of muskmelon per hectare was recorded, and the results are given in Table 2. There was a significant ($p < 0.001$) difference between phosphorus and biostimulant levels. Among the different doses of phosphorus applied treatments, the significantly greater marketable yield of 17.72 t ha^{-1} was observed in 100 % P_2O_5 (M1) applied treatment, followed by 50 % P_2O_5 (M2) with 14.18 tonnes per hectare and the lowest marketable yield per hectare was recorded in 0 % P_2O_5 (M3) treatment (10.18 t ha^{-1}). Plants treated with GEA at 2.5 kg ha^{-1} (S2) recorded a higher marketable yield of 15.65 t ha^{-1} compared to other treatments and the lowest marketable yield of 12.18 t ha^{-1} observed in S1. Among the phosphorus and biostimulant combination treatments, the combined application M1S2 registered the significantly highest marketable yield of 19.91 t ha^{-1} and the lesser marketable yield of 7.81 t ha^{-1} was noticed in M3S1.

Table 2. Effects of phosphorus and biostimulants on the yield and fruit quality of muskmelon

Treatments	**Marketableyield (t ha^{-1})**	**Fruit length (cm)**	**Fruit. diameter (cm)**	**Seed. cavity length (cm)**	**Seed cavity diameter (cm)**	**Firmness (N)**	**Rind thickness (mm)**	**TSS (°Brix)**
M1S1	15.74 ± 1.10^{c}	11.20 ± 0.78^{d}	12.90 ± 0.90^{e}	7.20 ± 0.50^{c}	5.90 ± 0.41^{cd}	4.03 ± 0.28^{c}	10.14 ± 0.71^{d}	9.60 ± 0.67^{de}
M1S2	19.91 ± 0.87^{a}	13.10 ± 0.57^{a}	14.50 ± 0.63^{a}	6.00 ± 0.26^{e}	5.50 ± 0.24^{e}	4.62 ± 0.20^{a}	9.03 ± 0.39^{e}	11.80 ± 0.51^{a}
M1S3	17.50 ± 1.22^{b}	12.30 ± 0.86^{bc}	13.40 ± 0.94^{cd}	7.10 ± 0.50^{c}	5.80 ± 0.41^{d}	4.30 ± 0.30^{b}	9.32 ± 0.65^{e}	10.60 ± 0.74^{b}
M2S1	12.98 ± 0.57^{f}	10.70 ± 0.47^{e}	11.90 ± 0.52^{g}	7.50 ± 0.33^{b}	6.10 ± 0.27^{b}	3.07 ± 0.13^{e}	10.16 ± 0.44^{d}	9.40 ± 0.41^{ef}
M2S2	15.09 ± 1.06^{d}	12.40 ± 0.87^{bc}	13.80 ± 0.97^{b}	5.00 ± 0.35^{f}	4.50 ± 0.32^{g}	4.40 ± 0.31^{b}	9.12 ± 0.64^{e}	10.80 ± 0.76^{b}
M2S3	14.48 ± 0.63^{e}	12.10 ± 0.53^{c}	13.50 ± 0.59^{bc}	6.20 ± 0.27^{e}	6.50 ± 0.28^{a}	3.45 ± 0.15^{d}	9.85 ± 0.43^{d}	10.10 ± 0.44^{c}
M3S1	7.81 ± 0.55^{i}	9.00 ± 0.63^{f}	10.00 ± 0.70^{h}	8.00 ± 0.56^{a}	6.50 ± 0.45^{a}	2.56 ± 0.18^{f}	12.99 ± 0.91^{a}	8.76 ± 0.61^{g}
M3xS2	11.94 ± 0.52^{g}	12.60 ± 0.55^{b}	13.10 ± 0.57^{de}	6.50 ± 0.28^{d}	5.30 ± 0.23^{f}	4.10 ± 0.18^{c}	11.29 ± 0.49^{c}	9.80 ± 0.43^{d}
M3xS3	10.79 ± 0.76^{h}	11.50 ± 0.80^{d}	12.50 ± 0.88^{f}	7.80 ± 0.55^{a}	6.00 ± 0.42^{bc}	3.16 ± 0.22^{e}	11.64 ± 0.81^{b}	9.20 ± 0.64^{f}
F stat	35.54**	23.41**	18.73**	25.61**	65.97**	38.95**	6.20 **	9.41 **
CD [A(B)]	0.36	0.35	0.39	0.24	0.18	0.13	0.34	0.3
CD[B(A)]	0.6	0.32	0.36	0.26	0.18	0.14	0.39	0.28
SE(d)	0.16	0.16	0.18	0.11	0.08	0.06	0.16	0.14

M1: 100% P_2O_5; M2: 50% P_2O_5; M3: 0% P_2O_5; S1: Control; S2: GEA 1499 at 2.5 kg ha^{-1}; S3: GEA 1499 at 5 kg ha^{-1}. The values represent mean±SD. Treatments with the same letter grouping are not significantly different. * Indicates significance at the 5% level; ** indicates significance at the 1% level; NS indicates non-significance

TSS

The effects of biostimulants and different doses of phosphorus on the TSS content of muskmelon fruit were tested, and the results are presented in Table 2. Among the three different phosphorus treatments, a maximum TSS of 10.67 °Brix was recorded in M1, and a lower TSS of 9.25 °Brix was recorded in the M3. Compared with biostimulant application, biostimulant (GEA 1499) application increased the TSS of muskmelon fruit and resulted in a maximum TSS of 10.80 °Brix in S2 and S1, which was a lower TSS of 9.25 °Brix than the other biostimulant treatments. Among the combination treatments, the combined application of 100% P_2O_5 + GEA 1499 at 2.5 kg ha^{-1} (M1S2) increased the TSS by 11.80 °Brix over the other combination treatments, and the lowest TSS was noted in the 0% P_2O_5 + biostimulant control (M3S1; 8.76 °Brix).

Physical fruit characteristics

Phosphorus and biostimulants influence fruit length and diameter, seed cavity length and diameter, and fruit firmness significantly. The results (**Table 2**) indicated that a reduction in phosphorus application from 100% to 0% reduced fruit length, fruit diameter, and firmness by 9.59%, 14.57%, and 24.31%, respectively, and increased the seed cavity length, seed cavity diameter, and rind thickness by 9.75%, 3.37%, and 26%, respectively. Compared with S1, Biostimulant S2 had greater fruit length (12.7 cm), fruit diameter (13.8 cm), and firmness (4.37 N) and reduced seed cavity length, seed cavity diameter, and rind thickness than S1. The. Compared with those of M3S1, the combined impacts of phosphorus and the biostimulant were greater in M1S2, with increased fruit length, fruit diameter, and firmness values of 45.56%, 45%, and 80.46%, respectively, and reduced seed cavity length, seed cavity diameter, and rind thickness values of 25%, 15%, and 30%, respectively.

Foliar P content

Effect of different levels of phosphorus, biostimulants and interactions on foliar P content given in Table 3. Different levels of P_2O_5 significantly ($p < 0.01$) influenced the P content in leaves. The M1 has the highest mean (2.54) and M3 has the lowest mean (1.26) with the 50% reduction compared to M1. Biostimulants also significantly ($p < 0.01$) influenced the P content in leaves with S2 has the highest mean (2.01) and S1 has the lowest mean (1.66). Interaction between phosphorus and biostimulants significantly ($p < 0.01$) influenced the P content. Among the interactions, M1S2 has the highest mean (2.89) and M3S1 has the lowest mean (1.19).

Table 3. Effects of phosphorus and biostimulants on the foliar P content of muskmelon

Treatments	**P (g kg^{-1})**
M1S1	2.24 ± 0.16^c
M1S2	2.89 ± 0.13^a
M1S3	2.50 ± 0.17^b
M2S1	1.55 ± 0.07^f
M2S2	1.79 ± 0.12^d
M2S3	1.63 ± 0.07^e
M3S1	1.19 ± 0.08^i
M3S2	1.34 ± 0.06^g
M3S3	1.26 ± 0.09^h
F stat	60.31**
CD [A(B)]	0.05
CD[B(A)]	0.1
SE(d)	0.02

M1: 100% P_2O_5; M2: 50% P_2O_5; M3: 0% P_2O_5; S1: Control; S2: GEA 1499 at 2.5 kg ha^{-1}; S3: GEA 1499 at 5 kg ha^{-1}. The values represent mean ± SD. Treatments with the same letter grouping are not significantly different. * Indicates significance at the 5% level; ** indicates significance at the 1% level; NS indicates non-significance

Discussion

Morphological parameters

Vine length and the number of leaves are critical determinants of muskmelon growth, development, and yield because they influence the plant's photosynthetic capacity and structural support (Meiri *et al.*, 1995). The observed reduction in vine length and the number of leaves as the phosphorus level decreased was due to its effect on cell division and proliferation in the meristematic region, thus producing small leaves (Kavanová *et al.*, 2006). Phosphorus deficiency reduces the production of ATP synthesis due to reduced activity of ATP synthase in chloroplasts and ultimately limits the Calvin cycle (Rao and Terry, 1989). Biostimulant application significantly improved vine length and the number of leaves, as it is able to produce phytohormones such as auxins and cytokinins and to increase nutrient availability, that collectively stimulate cell division and vegetative growth (Gedeon *et al.*, 2022). Similar positive responses to natural biostimulants application on vine elongation have also been documented in muskmelon and other cucurbits (Dhkal *et al.*, 2022). The microbial components of the biostimulants, *Bacillus megaterium* solubilize the inorganic phosphorus, produce phytohormones, enhance root development (Yavarian *et al.*, 2023), and *Bacillus pumilus* facilitates nitrogen assimilation and improves vegetative growth (Masood *et al.*, 2020).

Physiological parameters

The SPAD value indicates the presence of a chlorophyll pigment, an essential compound involved in photosynthesis. In our current study, a reduced level of phosphorus application reduced the chlorophyll index (Pallavolu *et al.*, 2023). Phosphorus is vital for regulating chlorophyll content, as it is an essential component of important molecules within the photosynthetic system. A lack of phosphorus directly restricts chlorophyll production, lowering chlorophyll levels (Kayoumu *et al.*, 2023). Biostimulant application increased the chlorophyll index of muskmelon at all phosphorus application levels, as it increased nutrient uptake and reduced chlorophyll degradation and leaf senescence (Dhkal *et al.*, 2022). *Bacillus megaterium* and *Bacillus pumilus*, which enhance nitrogen metabolism and uptake, are crucial for chlorophyll synthesis, and improve chloroplast integrity by modulating nitrite transport and producing growth-promoting metabolites such as phytohormones and antioxidants, thereby increasing photosynthetic efficiency and stress resilience (Masood *et al.*, 2020; Yavarian *et al.*, 2023).

Mechanistic insights into the mechanisms underlying carbon and water fluxes in plant leaves are made possible by gas exchange measurements, which in turn help to clarify associated processes ranging from individual cells to large ecosystems (Busch *et al.*, 2024). The photosynthetic rate determines plant growth and biomass production and is reduced under low phosphorus availability, as it regulates the enzymes involved in photosynthesis and the electron transport chain (Li *et al.*, 2022.). Similar report on cucumber indicating the reduction in photosynthetic capacity with low phosphorus condition (Wang *et al.*, 2022). Our results also revealed a reduction in photosynthesis as phosphorus application decreased. The biostimulant GEA 1499 invariably increased photosynthesis at all phosphorus levels. The increase in photosynthesis caused by biostimulants is due to increased production of chlorophyll, nutrient uptake and reduced stress factors that hinder carbon fixation (Raza *et al.*, 2022) and the results were consistent with the influence of *Bacillus megaterium* on the cucumber (Zhao *et al.*, 2021).

Stomatal conductance quantifies the rate at which water vapour transpires through a plant's stomata. This rate is directly influenced by the size of the stomatal aperture, with larger apertures generally facilitating greater water loss (Harrison *et al.*, 2020). The present study revealed a reduction in stomatal conductance in response to 0% P_2O_5 compared with 100% P_2O_5. Reduced P results in a smaller vascular bundle area and xylem conduit area and lower leaf hydraulic conductance, hence reducing stomatal conductance (Shu *et al.*, 2023). *Bacillus megaterium* and *Bacillus pumilus* have been associated with increased stomatal conductance and enhanced metabolic activity (Sharma *et al.*, 2025), thereby optimizing the water and carbon fluxes needed for robust growth and yield in muskmelon.

Yield and fruit traits

Muskmelon fruit yield relies on varietal selection, environmental factors and nutrient availability. A reduction in phosphorus application affects the fruit yield of muskmelon, and similar results have been reported (Martuscelli *et al.*, 2016). In short, sufficient phosphorus helps plants produce more high-quality fruits by supporting root growth, flower development, and seed maturation (Khan *et al.*, 2023). Phosphorus plays a critical role in plant cell division, energy transfer, and fruit development, especially in the later stages of growth, when high energy is required for seed and fruit formation (Zhu *et al.*, 2017). Biostimulants improve the fruit yield of muskmelon, as they help improve root development and nutrient uptake and increase flower and fruit setting (Fernandes *et al.*, 2023). Fruit length and diameter determine the size of the fruit and are affected by 0% P_2O_5. Seed cavity length and diameter are important because they reduce the pulp thickness of the fruit (Akrami and Arzani, 2019). Our results indicated that a reduction in P fertilization increased the seed cavity size, and biostimulant application invariably reduced the seed cavity size at all P_2O_5 levels (**Table 2**). The rind thickness and firmness of the fruit determine the shelf-life of the muskmelon. Low-phosphorus conditions can cause “low-temperature breakdown,” which results in fruits becoming less firm and squashy (Knowles *et al.*, 2001). The TSS is a fruit quality parameter that determines consumer preference. A reduced TSS in 0% P_2O_5 compared with 100% P_2O_5 was observed. P stimulates sucrose accumulation by increasing the synthesis of sucrose phosphate synthase (SPS), whose activity is crucial for controlling the amount of soluble sugars in fruit in many species (Lester *et al.*, 2001).

Foliar P content

Phosphorus, one of the three primary macronutrients alongside nitrogen (N) and potassium (K) and it is an important component of biomolecules (Jiaying *et al.*, 2022). Reduction in P content in leaves with the reduction of P_2O_5 levels from 100% to 0% due to reduction in the concentration of plant available phosphate in the root zone. P, immobile in soil and mainly enters the plant through diffusion. Under low P_2O_5, the diffusion gradient weakens, reducing the P uptake by the plant. Phosphorus deficiency also suppresses root growth, lateral root formation, and root hair density, reducing the absorbing surface area needed for efficient P acquisition (Gerke, 2024). Increased P content in the biostimulants applied to plants was due to under low P_2O_5, *Bacillus megaterium* produces organic acids such as citric and gluconic acids, which convert inorganic phosphorus into plant-available forms. Additionally, it regulates the expression of phosphate transporters (*PHT1;1* and *PHT1;4*) in plant roots, thereby improving phosphorus uptake efficiency (Singh *et al.*, 2024).

This study was conducted at a single location and within a single season, which may limit the broader applicability of the findings. In addition, microbial verification of the biostimulant strains was not performed, so their persistence and activity in the field could not be confirmed. Future multi-location and multi-season studies with microbial characterization would strengthen the reliability of these results.

Conclusion

In this study, we investigated the combined effects of phosphorus (P_2O_5) and biostimulants on the morphophysiological characteristics, yield, and fruit quality traits of muskmelon. The application of the biostimulant GEA 1499 at 2.5 kg ha^{-1} consistently resulted in superior performance across all P_2O_5 levels. Notably, the combination of 100% recommended P_2O_5 along with GEA 1499 at 2.5 kg ha^{-1} resulted in significant improvements in vine length, number of leaves, and key physiological parameters, such as the photosynthetic rate, stomatal conductance, and transpiration rate, as well as yield and fruit quality traits, compared with those of the other treatment combinations. Although 50% P_2O_5 reduced morphophysiological traits and yield, GEA 1499 at 2.5 kg ha^{-1} mitigated these effects and significantly improved yield.

Authors' Contributions

Conceptualization: BG, JP, RV, KV, DS and AL; Data curation: BG; Formal analysis: BG; Funding acquisition: JP; Investigation: RV, JP; Methodology: JP, RV, BG; Project administration: JP, RV; Resources: JP, RV; Software: BG; Supervision: JP, RV, KV, DS and AL; Validation: JP, RV; Visualization: BG; Roles/Writing - original draft: BG; and Writing - review & editing: JP, RV, KV, DS and AL. All authors read and approved the final manuscript.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

References

Akrami M, Arzani A (2019). Inheritance of fruit yield and quality in melon (*Cucumis melo* L.) grown under field salinity stress. Scientific Reports 9(1):7249. *https://doi.org/10.1038/S41598-019-43616-6*

Beaton JD, Tisdale SL, Nelson WL (1999). Soil fertility and fertilizers: an introduction to nutrient management. Prentice-Hall Inc., USA. *https://www.sidalc.net/search/Record/KOHA-OAI-TEST:32446/Description*

Boutahiri S, Benrkia R, Tembeni B, Idowu OE, Olatunji OJ (2024). Effect of biostimulants on the chemical profile of food crops under normal and abiotic stress conditions. Current Plant Biology 40:100410. *https://doi.org/10.1016/j.cpb.2024.100410*

Busch FA, Ainsworth EA, Amtmann A, Cavanagh AP, Driever SM, Ferguson JN, … Papanatsiou M (2024). A guide to photosynthetic gas exchange measurements: Fundamental principles, best practice and potential pitfalls. Plant, Cell & Environment 47(9):3344-3364. *https://doi.org/10.1111/PCE.14815*

Dhkal M, Sharma A, Sharma SP (2022). Biostimulants an important nonchemical alternative to pesticides for management of virus disease in Muskmelon. Egyptian Journal of Biological Pest Control 32(1):61. *https://doi.org/10.1186/S41938-022-00560-4*

Du Jardin P (2015). Plant biostimulants: Definition, concept, main categories and regulation. Scientia Horticulturae 196:3-14. *https://doi.org/10.1016/j.scienta.2015.09.021*

Elhaissoufi W, Ghoulam C, Barakat A, Zeroual Y, Bargaz A (2022). Phosphate bacterial solubilization: A key rhizosphere driving force enabling higher P use efficiency and crop productivity. Journal of Advanced Research 38:13-28. *https://doi.org/10.1016/J.JARE.2021.08.014*

FAO (2022). World Food and Agriculture – Statistical Yearbook 2022. Rome

Fernandes Â, Polyzos N, Mandim F, Pereira C, Petrović J, Soković M, Petropoulos SA (2023). Combined effect of biostimulants and mineral fertilizers on crop performance and fruit quality of watermelon plants. Horticulturae 9(7):838. *https://doi.org/10.3390/horticulturae9070838*

Gedeon S, Ioannou A, Balestrini R, Fotopoulos V, Antoniou C (2022). Application of biostimulants in tomato plants (*Solanum lycopersicum*) to enhance plant growth and salt stress tolerance. Plants 11(22):3082. *https://doi.org/10.3390/plants11223082*

Gerke J (2024). Improving phosphate acquisition from soil via higher plants while approaching peak phosphorus worldwide: A critical review of current concepts and misconceptions. Plants 13(24):3478. *https://doi.org/10.3390/plants13243478*

Harrison EL, Arce Cubas L, Gray JE, Hepworth C (2020). The influence of stomatal morphology and distribution on photosynthetic gas exchange. The Plant Journal 101(4):768-779. *https://doi.org/10.1111/TPJ.14560*

Jiaying MA, Tingting C, Jie L, Weimeng FU, Baohua F, Guangyan L, Hubo L, Juncai L, Zhihai W, Longxing T, Guanfu F (2022). Functions of nitrogen, phosphorus and potassium in energy status and their influences on rice growth and development. Rice Science 29(2):166. *https://doi.org/10.1016/j.rsci.2022.01.005*

Kauffman GL, Kneivel DP, Watschke TL (2007). Effects of a biostimulant on the heat tolerance associated with photosynthetic capacity, membrane thermostability, and polyphenol production of perennial ryegrass. Crop Science 47(1):261-267. *https://doi.org/10.2135/CROPSCI2006.03.0171*

Kavanová M, Lattanzi FA, Grimoldi AA, Schnyder H (2006). Phosphorus deficiency decreases cell division and elongation in grass leaves. Plant Physiology 141(2):766-775. *https://doi.org/10.1104/pp.106.079699*

Kayoumu M, Iqbal A, Muhammad N, Li X, Li L, Wang X, Dong Q (2023). Phosphorus availability affects the photosynthesis and antioxidant system of contrasting low-P-tolerant cotton genotypes. Antioxidants 12(2):466. *https://doi.org/10.3390/antiox12020466*

Khan F, Siddique AB, Shabala S, Zhou M, Zhao C (2023). Phosphorus plays key roles in regulating plants' physiological responses to abiotic stresses. Plants 12(15):2861. *https://doi.org/10.3390/PLANTS12152861*

Knowles L, Trimble MR, Knowles NR (2001). Phosphorus status affects postharvest respiration, membrane permeability and lipid chemistry of European seedless cucumber fruit (*Cucumis sativus* L.). Postharvest Biology and Technology 21(2):179-188. *https://doi.org/10.1016/S0925-5214(00)00144-7*

Lester GE, Arias LS, Gomez-Lim M (2001). Muskmelon fruit soluble acid invertase and sucrose phosphate synthase activity and polypeptide profiles during growth and maturation. Journal of the American Society for Horticultural Science 126(1):33-36. *https://doi.org/10.21273/JASHS.126.1.33*

Li P, Weng J, Rehman A, Niu Q (2022). Root morphological and physiological adaptations to low phosphate enhance phosphorus efficiency at melon (*Cucumis melo* L.) seedling stage. Horticulturae 8(7):636. *https://doi.org/10.3390/horticulturae8070636*

Lingle SE, Dunlap JR (1987). Sucrose metabolism in netted muskmelon fruit during development. Plant Physiology 84(2):386-389. *https://doi.org/10.1104/PP.84.2.386*

Manchali S, Murthy KNC, (2020). Muskmelon. In Jaiswal AK (Ed). Nutritional Composition and Antioxidant Properties of Fruits and Vegetables. Academic Press pp 533-546. *https://doi.org/10.1016/b978-0-12-812780-3.00033-7*

Martuscelli M, Di Mattia C, Stagnari F, Speca S, Pisante M, Mastrocola D (2016). Influence of phosphorus management on melon (*Cucumis melo* L.) fruit quality. Journal of the Science of Food and Agriculture 96(8):2715-2722. *https://doi.org/10.1002/JSFA.7390*

Masood S, Zhao XQ, Shen RF (2020). *Bacillus pumilus* promotes the growth and nitrogen uptake of tomato plants under nitrogen fertilization. Scientia Horticulturae 272:109581. *https://doi.org/10.1016/j.scienta.2020.109581*

Meiri A, Lauter DJ, Sharabani N (1995). Shoot growth and fruit development of muskmelon under saline and non-saline soil water deficit. Irrigation Science 16(1):15-21. *https://doi.org/10.1007/BF00208391*

Pallavolu LA, Pasala R, Kulasekaran R, Pandey BB, Virupaksham U, Perika S (2023). Analysing the SPAD dynamics of water-stressed vs. well-watered sesame (*Sesamum indicum* L.) accessions and establishing their relationship with seed yield. PeerJ 11:e14711. *https://doi.org/10.7717/PEERJ.14711/SUPP-2*

Piper CS (1966). Soil and plant analysis: A laboratory manual of methods for the examination of soils and the determination of the inorganic constituents of plants. Hans Publishers, Bombay, India.

Povero G, Mejia JF, Di Tommaso D, Piaggesi A, Warrior P (2016). A systematic approach to discover and characterize natural plant biostimulants. Frontiers in Plant Science 7:435. *https://doi.org/10.3389/fpls.2016.00435*

Rajasekar M, Nandhini D, Swaminathan V, Balakrishnan K (2017). A review on role of macro nutrients on production and quality of vegetables. academia. International Journal of Chemical Studies 5(3):304-309.

Rao IM, Terry N (1989). Leaf phosphate status, photosynthesis, and carbon partitioning in sugar beet: I. Changes in growth, gas exchange, and Calvin cycle enzymes. Plant Physiology 90(3):814-819. *https://doi.org/10.1104/PP.90.3.814*.

Raza QUA, Bashir MA, Rehim A, Ejaz R, Raza HMA, Shahzad U, Geng Y (2022). Biostimulants induce positive changes in the radish morpho-physiology and yield. Frontiers in Plant Science 13:950393. *https://doi.org/10.3389/fpls.2022.950393*

Sharma A, Das N, Pandey P, Shukla P (2025). Plant-microbiome responses under drought stress and their metabolite-mediated interactions towards enhanced crop resilience. Current Plant Biology 43:100513. *https://doi.org/10.1016/J.CPB.2025.100513*

Shu Y, Huang G, Zhang Q, Peng S, Li Y (2023). Reduction of photosynthesis under P deficiency is mainly caused by the decreased CO_2 diffusional capacities in wheat (*Triticum aestivum* L.). Plant Physiology and Biochemistry 198:107680. *https://doi.org/10.1016/j.plaphy.2023.107680*

Singh S, Chhabra R, Sharma A, Bisht A (2024). Harnessing the power of zinc-solubilizing bacteria: a catalyst for a sustainable agrosystem. Bacteria 3(1):15-29. *https://doi.org/10.3390/bacteria3010002*

Song J, Yang H, Yu X, Chen Y, Yang C, He Y, Wang H (2025). Effects of combined application of nitrogen, phosphorus, and potassium fertilizers on seed yield, seed quality and economic returns of *Elymus nutans* in alpine region. BMC Plant Biology 25(1):130. *https://doi.org/10.1186/s12870-025-06126-4*

Wang S, Zheng S, Bian T, Wu T, Li X, Fu H, Li T (2022). Photosynthetic characteristics combined with metabolomics analysis revealed potential mechanisms of cucumber (*Cucumis sativus*) yield reduction induced by different phosphorus stresses. Scientia Horticulturae 302:111156. *https://doi.org/10.1016/j.scienta.2022.111156*

Yavarian S, Jafari P, Akbari N (2023). *Bacillus megaterium* RTS1 enhances resistance of *Lycopersicon esculentum* to salinity stress through the improvement of antioxidant defenses. Iranian Journal of Microbiology 15(5):685. *https://doi.org/10.18502/ijm.v15i5.13874*

Zafar S, Bilal M, Ali MF, Mahmood A, Kijsomporn J, Wong LS, Alotaibi SS (2024). Nano-biofertilizer an eco-friendly and sustainable approach for the improvement of crops under abiotic stresses. Environmental and Sustainability Indicators 24:100470. *https://doi.org/10.1016/j.indic.2024.100470*

Zhao Y, Mao X, Zhang M, Yang W, Di HJ, Ma L, Li B (2021). The application of *Bacillus Megaterium* alters soil microbial community composition, bioavailability of soil phosphorus and potassium, and cucumber growth in the plastic shed system of North China. Agriculture, Ecosystems & Environment 307:107236. *https://doi.org/10.1016/j.agee.2020.107236*

Zhu Q, Ozores-Hampton M, Li Y, Morgan K, Liu G, Mylavarapu RS (2017). Effect of phosphorus rates on growth, yield, and postharvest quality of tomato in a calcareous soil. HortScience 52(10):1406-1412. *https://doi.org/10.21273/HORTSCI12192-17*