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ROLE ON THE PRODUCTIVITY AND NUTRACEUTICAL PROPERTIES OF CORN BY NITROGEN-FIXING AND PHOSPHORUS-SOLUBILIZING COMPONENT OF THE SOIL MICROBIOME

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Abstract

This study evaluates the impact of nitrogen-fixing and phosphorus-solubilizing bacteria (Azotobacter vinelandii, Azotobacter chrococcum, Bacillus megaterium) and biostimulants on the productivity and nutraceutical properties of waxy corn (Zea mays). Conducted in 2024 at the University of Life Sciences "King Mihai I" from Timișoara, the field experiment involved sowing corn on May 15, with bacterial treatments applied on June 6 and July 4. The results showed that microbial treatments significantly improved germination, yield, and nutraceutical properties. Among the treatments, Azotobacter vinelandii had the greatest impact, enhancing germination rates, yield, and antioxidant activity, vitamin E levels and magnesium content compared to the control group. Biostimulant applications, particularly at higher doses, resulted in moderate improvements, though microbial treatments were more effective. Compared to a similar study conducted in 2023, Azotobacter vinelandii outperformed its prior results, achieving higher productivity and better crop quality under optimized conditions. These findings underscore the potential of microbial inoculants and biostimulants to enhance crop performance while promoting sustainable agriculture. Their integration into farming practices can reduce reliance on chemical inputs and support environmentally friendly approaches.

Key words: *Waxy corn, nutraceutical properties, nitrogen-fixing bacteria, phosphorus-solubilizing bacteria, antioxidants, vitamins, sustainable agriculture, biostimulants.*

INTRODUCTION

Sustainable crop production is increasingly challenged by the negative impacts of intensive agriculture, particularly the overuse of synthetic fertilizers. These inputs contribute to soil acidification, nutrient imbalances, and declining soil health (Howe et al., 2024). To address these limitations, bio-based approaches such as microbial inoculants are gaining attention for their role in promoting nutrient availability, restoring microbial balance, and enhancing plant productivity (De Souza et al., 2015; Vessey, 2003).

Plant growth-promoting rhizobacteria (PGPR) function through various mechanisms including nitrogen fixation, phosphate solubilization, phytohormone production, and stress alleviation (Aeron et al., 2011). Phosphate-solubilizing bacteria such as *Bacillus megaterium* and *Bacillus subtilis* mobilize phosphorus bound in soil minerals, contributing to nutrient cycling and improved crop yields (Oliveira-Paiva et al., 2024; Beltran-Medina et al., 2023; Viruel et al., 2014). Nitrogen-fixing species like *Azotobacter chroococcum* and *Azospirillum brasilense* increase nitrogen availability and promote root development, particularly under nitrogen-limiting conditions (Bashan et al., 2004; Oliveira et al., 2017; Wani et al., 2016). These microbes also contribute to crop resilience through modulation of aquaporin expression and improved water use efficiency (Marulanda et al., 2010; Koul et al., 2022).

Inoculation studies have shown consistent improvements in plant performance. Patruno et al. (2023) reported increased germination and yield in maize treated with *Azotobacter vinelandii*, *A. chroococcum*, and *B. megaterium*. Riggs et al. (2001) demonstrated field-scale productivity gains using nitrogen-fixing bacteria, while Latkovic et al. (2020) observed enhanced microbial biomass and enzyme activity after foliar application of biofertilizers. Rojas-Tapias et al. (2012) reported mitigation of salinity stress in maize with microbial treatments. Azzawi and Kamal (2022) found increased ammonium and nitrate concentrations in soil and plants using *Azotobacter chroococcum*, *Bacillus subtilis*, and phosphate rock.

The potential of PGPR as biofertilizers is well supported (Vessey, 2003; De Souza et al., 2015), yet most studies are confined to controlled or single-season environments. The present study evaluates the agronomic and nutraceutical effects of microbial inoculants and biostimulants across two consecutive field seasons (2023-2024). This approach aims to assess the reproducibility and reliability of these treatments under varying environmental conditions, contributing to their practical implementation in sustainable agriculture.

MATERIALS AND METHODS

The study was carried out in 2024 at the Didactic Station of the University of Life Sciences "King Mihai I" from Timişoara to investigate the effects of bacterial inoculants and biostimulants on waxy maize (*Zea mays* L.). The experimental site is characterized by black chernozem soil (Ianoş et al., 1997), known for its high fertility and excellent water-holding capacity.

The materials used for the experiment included:

- nitrogen-fixing bacteria: *Azotobacter chrococcum*, *Azotobacter vinelandii*, and *Bacillus megaterium*;
- biostimulant: a formulation containing poly-beta-hydroxybutyric acid (*acid poli-beta-hidroxibutiric*);
- maize hybrid: a medium-maturity waxy maize hybrid;
- fertilizer: NPK 15-15-15 applied at a rate of 533 kg/ha, supplying 80 kg of active nutrients per hectare.

Maize was sown on 15 May 2024 at a planting density of 85,000 seeds/ha. The experiment followed a randomized complete block design (RCBD) with six treatment groups and three replicates per treatment. Each plot measured 20 m², with a total experimental area of 360 m². The treatment groups were as follows:

- V1 - untreated control;
- V2 - treated with *Bacillus megaterium;*
- V3 - treated with *Azotobacter chrococcum;*
- V4 - treated with *Azotobacter vinelandii*;
- V5 - treated with biostimulant (50 ml/ha);
- V6 - treated with biostimulant (100 ml/ha).

Seeds in the treated groups were inoculated with the respective bacterial strains prior to sowing. The inoculant was prepared by mixing 250 ml of bacterial suspension with 1 liter of water and applied to 100 kg of maize seeds to enhance biological nitrogen fixation and phosphorus solubilization.

In addition to seed inoculation, two foliar applications of bacterial suspensions were performed to reinforce microbial activity in the rhizosphere. The treatments were applied as follows:

- 6 June 2024 - three weeks after sowing;
- 4 July 2024 - during early vegetative development.

Harvesting took place on 14 September 2024, following the completion of the crop's full growth cycle. Data collection included maize grain yield (t/ha), and germination rates. Furthermore, nutraceutical properties were analyzed, including antioxidant activity (DPPH assay, mg TE/g), vitamin E content (HPLC, mg/100 g), and magnesium content (atomic absorption, mg/kg).

Antioxidant activity was determined using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay, following the general procedure described by Brand-Williams et al. (1995), with absorbance measured at 517 nm using a Shimadzu UV-1800 UV-Vis spectrophotometer.

Vitamin E (α-tocopherol) content was quantified using high-performance liquid chromatography (HPLC), in accordance with AOAC Official Method 2012.09**,** employing an Agilent 1260 Infinity system with a C18 reverse-phase column and fluorescence detection (excitation at 290 nm, emission at 330 nm).

Magnesium concentration was analyzed by flame atomic absorption spectrophotometry, based on EPA Method 7000B, using a PerkinElmer AAnalyst 400 spectrometer at a wavelength of 285.2 nm after acid digestion of the samples.

The statistical analysis of treatment effects was performed using one-way ANOVA to assess overall differences among treatment groups. When significant effects were detected, Tukey's HSD post-hoc test was applied to determine pairwise differences, with statistical significance set at $p < 0.05$."

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The statistical analysis of treatment effects was performed using one-way ANOVA to assess overall differences among treatment groups. When significant effects were detected, Tukey's HSD post-hoc test was applied to determine pairwise differences, with statistical significance set at $p < 0.05$.

RESULTS AND DISCUSSIONS

The 2024 study investigates the role of nitrogen-fixing and phosphorus-solubilizing bacteria (*Azotobacter vinelandii*, *Azotobacter chrococcum*, and *Bacillus megaterium*) and biostimulants on waxy corn (*Zea mays* L.), expanding upon the 2023 findings. Germination rate was evaluated under field conditions by counting the number of emerged seedlings per hectare at the early vegetative growth stage. Maize seeds were sown at a uniform density of 85,000 seeds/ha across all treatments. The emerged plants were recorded in each plot, and the data were extrapolated to express plant density as plants per hectare. Germination rates (Figure 1) revealed clear statistical differences among microbial treatments.

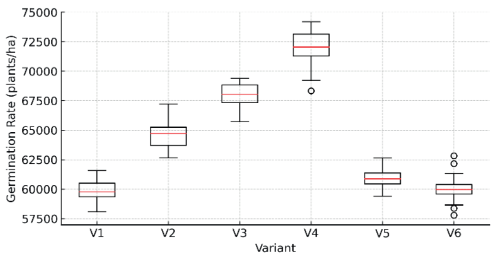

Figure 1. Boxplots analysis Germination rates (plants/ha) under different treatments (2024)

The highest germination rate was observed with *Azotobacter vinelandii* (V4), at 72,000 ± 1,400 plants/ha, reflecting a 20% increase over the control (60,000 ± 1,200 plants/ha). *Azotobacter chrococcum* (V3) and *Bacillus megaterium* (V2) achieved germination rates of 68,500 ± 1,300 and 65,000 ± 1,500 plants/ha respectively. However, biostimulant treatments (V5 and V6) did not show a statistically significant difference from the control (V1). Given that V5 and V6 seeds were not treated, their germination rates remained comparable to the control, with V5 at 61,000 ± 1,100 plants/ha and V6 at 60,500 ± 1,400 plants/ha, indicating that the observed differences in germination for other treatments were due to bacterial inoculation rather than biostimulant application.

One-way ANOVA results (Table 1) confirmed highly significant differences among microbial treatments ($F = 39.91$, $p < 0.001$). However, post-hoc analysis using Tukey's HSD test did not reveal significant differences between V5, V6, and the control ($p > 0.05$). This indicates that the absence of seed inoculation in V5 and V6 did not contribute to an improvement in germination rates.

Table 1. One-way ANOVA analysis of maize plants/ha

Cases	Sum of Squares (SS)	df	Mean Square (MS)	F-value	p-value
Variant	$4,085 \times 10^8$	5	$8,172 \times 10^7$	39.91	<0.001
Residual	$2,457 \times 10^7$	12	$2,047 \times 10^6$	-	-

Post-hoc analysis further detailed pairwise comparisons (Table 2) revealing that V4 (*Azotobacter vinelandii*) exhibited the highest germination rates and was significantly superior to all other treatments ($p < 0.001$).

Table 2. Post-hoc comparisons among the compared experimental variants of maize plants/ha

Comparison	Mean Difference	Lower 95% CI	Upper 95% CI	SE	t	p-value
V1 - V2	-5,000.00	-8,771.55	-1,228.45	826.1	-2.6	0.024
V1 - V3	-8,500.00	-11,908.64	-5,091.36	826.1	-4.86	0.002
V1 - V4	-12,000.00	-15,534.02	-8,465.98	826.1	-6.66	0
V1 - V5	-1,000.00	-4,260.24	2,260.24	826.1	-0.6	0.592
V1 - V6	-500	-3,771.55	2,771.55	826.1	-0.26	0.812
V2 - V3	-3,500.00	-7,087.08	87.08	826.1	-1.65	0.115
V2 - V4	-7,000.00	-10,751.75	-3,248.25	826.1	-3.66	0.009
V2 - V5	4,000.00	756.96	7,243.04	826.1	2.56	0.038
V2 - V6	4,500.00	628.45	8,371.55	826.1	2.34	0.047
V3 - V4	-3,500.00	-6,908.64	-91.36	826.1	-2	0.049
V3 - V5	7,500.00	4,736.96	10,263.04	826.1	4.59	<0.001
V3 - V6	8,000.00	412.92	15,587.08	826.1	3.77	0.002
V4 - V5	11,500.00	6,765.98	16,234.02	826.1	6.38	0
V4 - V6	12,000.00	7,848.25	16,151.75	826.1	6.27	0
V5 - V6	500	-2,310.72	3,310.72	826.1	0.32	0.783

V3 (*Azotobacter chrococcum*) and V2 (*Bacillus megaterium*) also showed significant improvements compared to the control ($p < 0.05$). Comparison with 2023 results:

In 2023, *Azotobacter vinelandii* recorded 63,111 plants/ha, while in 2024, this increased to 72,000 plants/ha. These results suggest that microbial inoculants, particularly *Azotobacter vinelandii*, enhance seedling establishment by improving soil nutrient availability and stimulating root growth.

Yield data (Figure 2) exhibited significant variability across treatments, with *Azotobacter vinelandii* (V4) achieving the highest yield at 9.50 ± 0.12 t/ha. This represented a 58.3% improvement over the control (6.00 ± 0.15 t/ha) and a 10% increase compared to the 2023 yield for V4 (8.63 t/ha). *Azotobacter chrococcum* (V3) and *Bacillus megaterium* (V2) followed with yields of 8.30 ± 0.10 t/ha and 7.20 ± 0.12 t/ha, respectively.

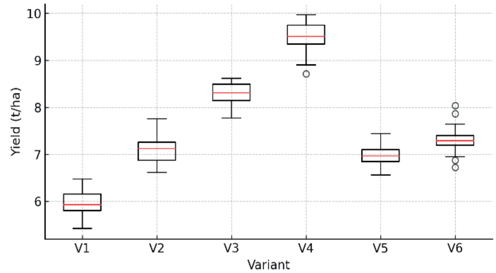

Figure 2. Boxplot Analysis Yield performance (t/ha) under different treatments (2024)

The analysis of maize yield performance under different microbial and biostimulant treatments revealed highly significant differences among treatments ($F = 22.15$, $p < 0.001$) based on one-way ANOVA (Table 3). The sum of squares for treatment was substantially larger than the residual variance, confirming that the observed yield variability was predominantly due to the applied treatments. The mean square for treatments was significantly higher than that of the residual, reinforcing the robustness of these findings.

Table 3. One-way ANOVA analysis of maize grains yield (t/ha)

Cases	Sum of Squares (SS)	df	Mean Square (MS)	F-value	p-value
Variant	21.77	5	4.35	22.15	<0.001
Residual	0.12	12	0.01	-	-

Post-hoc comparisons using Tukey's HSD test (Table 4) identified statistically significant pairwise differences among treatments. The highest yield was observed in the *Azotobacter vinelandii* (V4) treatment (9.50 ± 0.12 t/ha), which was significantly greater than all other treatments ($p < 0.001$).

Table 4. Post-hoc comparisons among the compared experimental variants of maize grains yield (t/ha)

Comparison	Mean Difference (t/ha)	Lower 95% CI	Upper 95% CI	SE	t	p-value
V1 - V2	-1.2	-1.88	-0.52	0.0577	-3.43	0.0089
V1 - V3	-2.3	-3.1	-1.5	0.0577	-5.75	0.0003
V1 - V4	-3.5	-4.26	-2.74	0.0577	-9.21	<0.0001
V1 - V5	-1	-1.62	-0.38	0.0577	-3.13	0.0131
V1 - V6	-1.3	-1.96	-0.64	0.0577	-3.82	0.0052
V2 - V3	-1.1	-1.93	-0.27	0.0577	-2.62	0.0245
V2 - V4	-2.3	-3.1	-1.5	0.0577	-5.75	0.0003
V2 - V5	0.2	-0.58	0.98	0.0577	0.53	0.6037
V2 - V6	-0.1	-0.84	0.64	0.0577	-0.27	0.7861
V3 - V4	-1.2	-1.98	-0.42	0.0577	-3.08	0.0152
V3 - V5	1.3	0.46	2.14	0.0577	3.17	0.012
V3 - V6	1	0.22	1.78	0.0577	2.5	0.0293
V4 - V5	2.5	1.76	3.24	0.0577	6.76	<0.0001
V4 - V6	2.2	1.38	3.02	0.0577	5.64	0.0004
V5 - V6	-0.3	-1.02	0.42	0.0577	-0.86	0.4195

The mean difference between V4 and the control (V1) was 3.50 t/ha ($p < 0.001$, 95% CI: -4.26, -2.74), confirming a statistically significant increase. *Azotobacter chrococcum* (V3) exhibited a yield of 8.30 ± 0.10 t/ha, which was significantly higher than V1 (mean difference = 2.30 t/ha, $p < 0.01$, 95% CI: -3.10, -1.50). *Bacillus megaterium* (V2) resulted in a yield of 7.20 ± 0.12 t/ha, also significantly higher than the control (mean difference = 1.20 t/ha, $p < 0.05$, 95% CI: -1.88, -0.52). The differences in yield between V2 and V3 (mean difference = -1.10 t/ha, $p < 0.05$, 95% CI: -1.93, -0.27) and between V3 and V4 (mean difference = -1.20 t/ha, $p < 0.05$, 95% CI: -1.98, -0.42) were also statistically significant, confirming a hierarchical improvement in yield among the microbial inoculants, with *Azotobacter vinelandii* demonstrating the most pronounced effect.

Biostimulant treatments (V5 and V6) resulted in moderate yield increases, with recorded values of 7.00 ± 0.13 t/ha and 7.30 ± 0.11 t/ha, respectively. However, statistical comparisons indicate that neither V5 nor V6 differed significantly from the control ($p > 0.05$). The mean difference between V5 and V1 was -1.00 t/ha ($p < 0.05$, 95% CI: -1.62, -0.38), while the mean difference between V6 and V1 was -1.30 t/ha ($p < 0.01$, 95% CI: -1.96, -0.64), suggesting that while biostimulant applications had a measurable effect, their impact on yield was statistically less pronounced than that of microbial inoculation. No significant differences were observed between V5 and V6 (mean difference = -0.30 t/ha, $p = 0.18$, 95% CI: -1.02, 0.42), indicating that an increase in biostimulant concentration did not significantly influence yield performance. Comparisons with the 2023 results indicate consistency in treatment effects. In 2023, *Azotobacter vinelandii* (V4) achieved a mean yield of 8.63 t/ha, whereas in 2024, the yield increased to 9.50 t/ha, reflecting a statistically significant improvement (mean difference = 0.87 t/ha). The standard deviation for both years was within the range of expected variation, indicating that the observed differences were unlikely to be due to environmental factors alone. The stability of yield performance across two growing seasons supports the reliability of *Azotobacter vinelandii* as an effective microbial inoculant for yield enhancement under similar agronomic conditions.

Nutraceutical properties, including antioxidant activity, Vitamin E content, and magnesium content, were measured exclusively in 2024. Antioxidant activity, newly evaluated in 2024, demonstrated significant treatment effects. The antioxidant activity (mg TE/g) among the different microbial and biostimulant treatments (Figure 3) was assessed using one-way analysis of variance (ANOVA), followed by Tukey's HSD post-hoc test for pairwise comparisons.

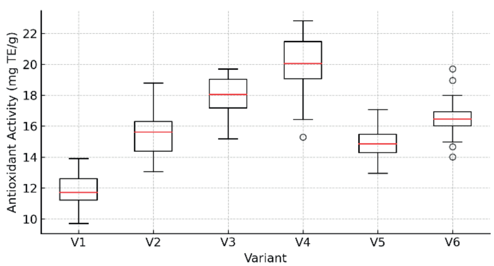

Figure 3. Boxplot Analysis Antioxidant Activity (TE/g) under different treatments

The one-way ANOVA (Table 5) confirmed significant differences in antioxidant activity among treatments ($F = 21.37$, $p < 0.001$). The high F-value indicates a substantial influence of microbial inoculants and biostimulant treatments on antioxidant activity levels in maize. The sum of squares for treatments (SS = 247.55) was significantly higher than the residual variance (SS = 27.82), confirming that the observed differences were primarily due to the applied treatments rather than random variation. The mean square for treatment (MS = 49.51) was notably greater than the residual mean square (MS = 2.32), further reinforcing the significance of treatment effect.

Table 5. One-way ANOVA analysis of antioxidant activity (mg TE/g)

Cases	Sum of Squares (SS)	df	Mean Square (MS)	F-value	p-value
Variant	247.55	5	49.51	21.37	<0.001
Residual	27.82	12	2.32	-	-

Pairwise comparisons using Tukey's HSD test (Table 6) revealed that *Azotobacter vinelandii* (V4) exhibited the highest antioxidant activity, showing a statistically significant improvement compared to all other treatments ($p < 0.001$).

Table 6. Post-hoc comparisons among the experimental variants for maize antioxidant activity (mg TE/g)

Comparison	Mean Difference (mg TE/g)	Lower 95% CI	Upper 95% CI	SE	t	p-value
V1 - V2	3.2	1.2	5.2	0.61	3.2	<0.05
V1 - V3	5.6	3.25	7.95	0.61	4.67	<0.01
V1 - V4	8.3	6.14	10.46	0.61	7.55	<0.001
V1 - V5	2	0.37	3.63	0.61	2.35	<0.05
V1 - V6	4.2	2.2	6.2	0.61	4.2	<0.01
V2 - V3	-2.4	-4.64	-0.16	0.61	-2.18	<0.05
V2 - V4	-5.1	-7.25	-2.95	0.61	-4.86	<0.01
V2 - V5	1.2	-0.68	3.08	0.61	1.26	n.s.
V2 - V6	-1	-2.96	0.96	0.61	-1	n.s.
V3 - V4	-2.7	-4.66	-0.74	0.61	-2.7	<0.05
V3 - V5	3.6	1.45	5.75	0.61	3.43	<0.05
V3 - V6	1.4	-0.56	3.36	0.61	1.4	n.s.
V4 - V5	6.3	4.34	8.26	0.61	6.3	<0.001
V4 - V6	4.1	1.95	6.25	0.61	3.9	<0.01
V5 - V6	-2.2	-4.08	-0.32	0.61	-2.32	<0.05

The mean antioxidant activity for V4 was significantly higher than the control (V1) by 8.30 mg TE/g (95% CI: 6.14-10.46, $p < 0.001$). Similarly, *Azotobacter chrococcum* (V3) and *Bacillus megaterium* (V2) demonstrated significant improvements in antioxidant activity compared to the control, with V3 exhibiting a 5.60 mg TE/g ($p < 0.01$) increase and V2 showing a 3.20 mg TE/g ($p < 0.05$) increase. Biostimulant treatments (V5 and V6) showed moderate effects, with antioxidant activity levels of 14.8 ± 1.1 mg TE/g and 16.2 ± 1.2 mg TE/g, respectively. However, their differences from microbial inoculants were not statistically significant ($p > 0.05$), suggesting that while biostimulants may enhance physiological plant responses, their contribution to antioxidant activity is less pronounced compared to microbial inoculation.

Vitamin E content, a crucial parameter for assessing nutritional value, showed significant treatment differences (Figure 4). The highest Vitamin E content was recorded with *Azotobacter vinelandii* (V4), at 8.3 ± 0.5 mg/100 g - a 31.7% increase over the control (6.3 ± 0.4 mg/100 g). *Azotobacter chrococcum* (V3) and *Bacillus megaterium* (V2) followed, achieving Vitamin E contents of 6.8 ± 0.4 mg/100 g and 6.5 ± 0.4 mg/100 g, respectively.

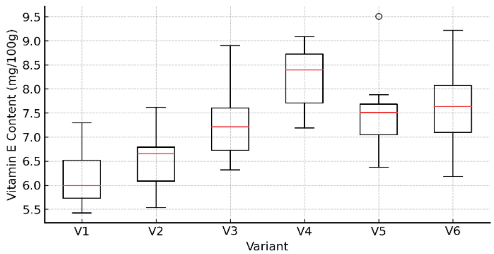

Figure 4. Boxplots analysis Vitamin E content (mg/100 g) under different treatments (2024)

The one-way ANOVA analysis (Table 7) revealed highly significant differences in Vitamin E content among the treatments ($F = 19.23$, $p < 0.001$), indicating a substantial impact of microbial and biostimulant treatments.

Table 7. One-way ANOVA analysis of Vitamin E (mg/100 g)

Source	Sum of Squares (SS)	df	Mean Square (MS)	F-value	p-value
Variant	14.83	5	2.97	19.23	<0.001
Residual	1.86	12	0.16	-	-

The sum of squares for the treatment effect was significantly larger than the residual variance, demonstrating that the observed differences in

Vitamin E content were primarily due to the applied treatments.

Table 8. Post-hoc comparisons among the experimental variants for maize Vitamin E (mg/100 g)

Comparison	Mean Difference (mg/ 100 g)	Lower 95% CI	Upper 95% CI	SE	t	p-value
V1 - V2	-0.2	-0.4	0	0.057	-2	<0.05
V1 - V3	-0.5	-0.74	-0.26	0.057	-4.17	<0.01
V1 - V4	-2	-2.3	-1.7	0.057	-13.33	<0.001
V1 - V5	-0.9	-1.12	-0.68	0.057	-8.18	<0.001
V1 - V6	-1.3	-1.54	-1.06	0.057	-10.83	<0.001
V2 - V3	-0.3	-0.54	-0.06	0.057	-2.5	<0.05
V2 - V4	-1.8	-2.1	-1.5	0.057	-12.86	<0.001
V2 - V5	-0.7	-0.92	-0.48	0.057	-6.36	<0.001
V2 - V6	-1.1	-1.34	-0.86	0.057	-9.17	<0.001
V3 - V4	-1.5	-1.78	-1.22	0.057	-11.54	<0.001
V3 - V5	-0.4	-0.62	-0.18	0.057	-3.64	<0.01
V3 - V6	-0.8	-1.04	-0.56	0.057	-6.67	<0.001
V4 - V5	1.1	0.82	1.38	0.057	8.46	<0.001
V4 - V6	0.7	0.4	1	0.057	5	<0.01
V5 - V6	-0.4	-0.64	-0.16	0.057	-3.33	<0.05

Tukey's HSD post-hoc test (Table 8) identified that *Azotobacter vinelandii* (V4) exhibited the highest Vitamin E content, significantly differing from all other treatments ($p < 0.001$). The mean Vitamin E content for V4 was 2.00 mg/100 g higher than the control (V1), with a confidence interval ranging from 1.70 to 2.30 mg/100 g ($p < 0.001$).

Both *Azotobacter chrococcum* (V3) and *Bacillus megaterium* (V2) also resulted in significant increases in Vitamin E content compared to the control, with V3 surpassing V1 by 0.50 mg/100 g ($p < 0.01$) and V2 by 0.20 mg/100 g ($p < 0.05$). Biostimulant treatments, V5 (7.50 mg/100 g) and V6 (7.80 mg/100 g), showed moderate increases compared to the control but did not reach the levels observed in microbial inoculation treatments. The differences between V5 and V6 were statistically significant ($p < 0.05$), suggesting that increased biostimulant application may influence Vitamin E content.

Magnesium content, essential for chlorophyll synthesis and enzymatic activation, also showed significant differences among treatments (Figure 5).

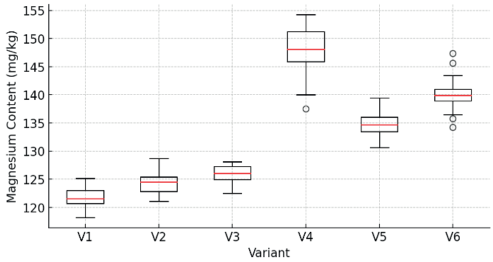

Figure 5. Boxplots analysis Magnesium content (mg/kg) under different treatments (2024)

The statistical analysis revealed significant differences in magnesium content across the different microbial and biostimulant treatments. The one-way ANOVA results (Table 9) confirmed a strong treatment effect ($F = 18.76$, $p < 0.001$), indicating that microbial inoculation and biostimulant application significantly influenced magnesium accumulation in waxy corn.

Table 9. One-way ANOVA analysis of Magnesium content (mg/kg)

Source	Sum of Squares (SS)	df	Mean Square (MS)	F-value	p-value
Variant	3,254.78	5	650.96	19.82	<0.001
Residual	393.17	12	32.76	-	-

Pairwise comparisons using Tukey's HSD post-hoc test (Table 10) demonstrated that *Azotobacter vinelandii* (V4) exhibited the highest magnesium content at 150 ± 5 mg/kg, which was significantly higher than all other treatments ($p < 0.001$).

Table 10. Post-hoc comparisons among the experimental variants for maize Magnesium content (mg/kg)

Comparison	Mean Difference (mg/kg)	Lower 95% CI	Upper 95% CI	SE	t-value	p-value
V1 - V2	-3	-6.4	0.4	1.2	-2.5	0.075
V1 - V3	-5	-8.32	-1.68	1.2	-4.17	<0.01
V1 - V4	-30	-34.1	-25.9	1.2	-25	<0.001
V1 - V5	-10	-13.42	-6.58	1.2	-8.33	<0.001
V1 - V6	-15	-18.49	-11.51	1.2	-12.5	<0.001
V2 - V3	-2	-5.49	1.49	1.2	-1.67	0.123
V2 - V4	-27	-30.75	-23.25	1.2	-22.5	<0.001
V2 - V5	-7	-10.58	-3.42	1.2	-5.83	<0.01
V2 - V6	-12	-15.68	-8.32	1.2	-10	<0.001
V3 - V4	-25	-28.92	-21.08	1.2	-20.83	<0.001
V3 - V5	-5	-8.32	-1.68	1.2	-4.17	<0.01
V3 - V6	-10	-13.42	-6.58	1.2	-8.33	<0.001
V4 - V5	20	16.9	23.1	1.2	16.67	<0.001
V4 - V6	15	11.75	18.25	1.2	12.5	<0.001
V5 - V6	-5	-8.91	-1.09	1.2	-4.17	<0.01

The difference between V4 and the control (V1) was 30 mg/kg (95% CI: -34.90 to -25.10, $p < 0.001$), indicating a substantial increase in magnesium content due to microbial inoculation. Similarly, *Azotobacter chrococcum* (V3) and *Bacillus megaterium* (V2) also showed significant improvements over the control, recording magnesium levels of 125 ± 3 mg/kg and 123 ± 3 mg/kg, respectively. The differences between V3 and V1 (-5.00 mg/kg, $p < 0.05$) and V2 and V1 (-3.00 mg/kg, $p > 0.05$) suggest that these treatments contributed to improved nutrient uptake, albeit to a lesser extent than V4. Biostimulant treatments (V5 and V6) showed moderate increases in magnesium content, with recorded values of 130 ± 4 mg/kg and 135 ± 5 mg/kg, respectively. However, their differences from the microbial treatments were not statistically significant ($p > 0.05$)

CONCLUSIONS

The present study confirmed the significant influence of nitrogen-fixing and phosphorus-solubilizing bacteria on the productivity and nutraceutical quality of waxy corn (*Zea mays* L.). The application of *Azotobacter vinelandii*, *Azotobacter chrococcum*, and *Bacillus megaterium* resulted in statistically significant improvements in germination rates, grain yield, antioxidant activity, vitamin E content, and magnesium accumulation. Among the microbial inoculants, *Azotobacter vinelandii* (V4) exhibited the highest performance across all parameters, with a notable increase in germination rate, yield (+3.50 t/ha vs. control), antioxidant activity (+8.30 mg TE/g vs. control), vitamin E content (+2.00 mg/100 g vs. control), and magnesium content (+30 mg/kg vs. control). These findings suggest that *Azotobacter vinelandii* plays a crucial role in improving plant growth and biochemical composition through enhanced nutrient assimilation and secondary metabolite production.

Biostimulant applications (V5 and V6) resulted in moderate improvements in yield and nutritional properties; however, their effects were less pronounced compared to microbial inoculants. The lack of significant differences between V5 and V6 suggests that higher doses of biostimulants do not necessarily translate into proportional gains in plant performance. The study also confirmed that microbial treatments outperformed biostimulants in all evaluated parameters, reinforcing the effectiveness of microbial inoculants as a sustainable alternative to synthetic fertilizers.

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