



Biochemical and Productive Responses of Okra to Selected Biostimulants

Jelena Stojiljković¹, Biljana Šević¹, Dragana Stevanović², Aleksandra Rakonjac¹, Darko Jovanović¹, Milan Ugrinović¹, Emhemed Abourawi Daw³, Ivan Tupajić¹

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ABSTRACT

Background: Although okra [*Abelmoschus esculentus* (L.) Moench] is a thermophilic species that thrives in warm climates, it remains highly vulnerable to abiotic stress. This study aimed to evaluate the effectiveness of three biostimulants of natural origin as sustainable alternatives to chemical fertilizers for greenhouse production under high summer temperatures.

Methods: A two-year trial (2024 and 2025) was set up in the Institute for Vegetable Crops Smederevska Palanka on the okra. Fifteen days after planting treatments were applied weekly using both foliar application and fertigation methods. Yield per plant, protein content, nitrogen content, antioxidant activity and total phenols were measured.

Result: Biostimulants application significantly increased the yield ($p < 0.05$), with the MAB treatment showing the most significant effect (129.20 g/plant in 2025), followed by PBP (121.16 g/plant) and MBB (80.82 g/plant) compared to the control (<40 g/plant). The highest protein and nitrogen content were observed in the MAB and MBB groups. Antioxidant activity was highest in PBP treatment (3.74 mg TE g⁻¹ d.m. in 2025), followed by MAB (3.71 mg TE g⁻¹ d.m. in 2024). The interaction of year × treatment was significant for all measured parameters, except for total phenols.

Key words: Biostimulants, Climate change, Okra, Sustainable agriculture, Yield per plant.

INTRODUCTION

In the context of intensifying climate change and growing global food demands, sustainable agriculture has become increasingly important (Rajičić *et al.*, 2025). Unsustainable agricultural management, the excessive application of mineral fertilizers and the impacts of climate change have led to soil and water contamination (Nagegowda and Senthivel, 2021; Šević *et al.*, 2025). In this regard, microalgal biotechnology, alongside fertilizers derived from plant extracts and natural minerals, represent a promising approach for achieving sustainable development goals (Sutherland *et al.*, 2021). Biostimulants based on microalgae, plant extracts and natural minerals improve the biological, chemical and physical characteristics of the soil, facilitating plant development and nutrient cycling (Fiorentino *et al.*, 2025).

Although the precise geographical origin of okra remains a subject of ongoing debate (Ranga *et al.*, 2019), okra is currently cultivated worldwide in tropical, subtropical and warm-temperate regions. Okra represent a nutrient-rich and economically profitable plant species (Krishnakumar *et al.*, 2018; Gaur *et al.*, 2025; Belkhodja *et al.*, 2026). Recent studies by Platzer *et al.* (2022) have confirmed that phenolic compounds, primarily quaternary derivatives, catechin oligomers and hydroxycinnamic acid derivatives, have a preventive effects and a positive impact on the antioxidant status of plants.

The goal of the work

The goal of this research was to evaluate the effects of biostimulants derived from microalgae, plant extracts and natural minerals as sustainable alternatives to conventional mineral fertilizers on okra yield and chemical properties.

¹Institute for Vegetable Crops Smederevska Palanka, Karađorđeva 71, 11420 Smederevska Palanka, Serbia.

²“Tamiš” Research and Development Institute, 26000 Pančevo, Serbia.

³Faculty of Agriculture-University of Bani Waleed, Bani Waleed, Libya.

Corresponding Author: Jelena Stojiljković, Institute for Vegetable Crops Smederevska Palanka, Karađorđeva 71, 11420 Smederevska Palanka, Serbia. Email: jstojiljkovic@institut-palanka.rs

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Novelty of the work

Most studies focus on the potential of microalgae at the laboratory level, without addressing the practical challenges of integrating them into existing agricultural systems. The application of micro and macroalgae based preparations in agriculture has been investigated on vegetable species, while the effects of these preparations are not as well known and investigated on the growth and development of okra.

MATERIALS AND METHODS

The location of the survey and the plant material

A two-year trial was conducted 2024 and 2025 as a follow-up crop under production conditions in a greenhouse. The okra

genotype Nana was used as plant material. Seedlings were transplanted onto mulch foil. There was a 100 cm distance between the two mulch foils, intended for a path. Two rows of plants were formed on the mulch foil, which were 20 cm apart, while the distance between the plants in the row was 25 cm.

The experiment was set up in the greenhouse of the Institute for Vegetable Crops Smederevska Palanka, Serbia. Before starting the experiment, the soil's nutrient availability and mechanical composition (Table 1) were determined in the laboratory.

The soil has the highest clay content (49.3%), a slightly acidic pH (6.1) and high levels of accessible P_2O_5 and K_2O (> 40).

Biostimulant treatments

In the experiment, three treatments with biostimulants of natural origin and a control without application were applied (7-day intervals, starting on the 15th day post-transplant):

1. Control- without biostimulant application.
2. MAB- New generation biostimulant obtained by enzymatic hydrolysis of microalgae. The commercial name of the biostimulant is Algafert, (Biorizon Biotech, Almeria, Spain). The application was carried out foliarly and by watering in a concentration of 30 ml per 10 l of water.
3. PBP- Plant extracts containing phenolic compounds and substances with phytohormone effect. The commercial name of the preparation is Traiko, (Kobilje, Braničevski district, Serbia). The application was carried out foliarly and by watering at a concentration of 375 ml per 10 l of water.
4. MBB- Natural mineral fertiliser based on calcium, magnesium and boron. The commercial name of the preparation is Mineral Forte Plus (Azertrade d.o.o. Novi Sad, Serbia). The application was carried out foliarly and by watering in a concentration of 20 g per 10 l of water.

Experiment design and agrotechnical measures

The experiment was set up in a completely randomized block design with 3 replications of 120 plants each.

Standard agrotechnical practices were applied throughout the growing season, including drip irrigation and integrated pest and diseases management. Yield per plant, along with protein and nitrogen content, antioxidant activity and total phenol content, were also measured.

Environmental conditions

Air temperature (°C) and relative air humidity (RH %) were measured daily using a testo 608-H1 digital thermohygrometer (LGG, Romania).

Daily measurements revealed that average greenhouse temperatures in 2025 ranged from 17°C in October to 32°C in July, while in 2024 they were slightly higher and ranged from 21.1°C (October) to 35.1°C (July), (Fig 1). Maximum temperatures occasionally exceeded 40°C in July and August in both years.

To assess the outdoor meteorological conditions during the study period, average air temperature and relative humidity data were obtained from the Smederevska Palanka weather station.

Air temperatures in both study years were significantly higher than the long-term average (1991-2020), characterized by pronounced fluctuations both between months and across years (Fig 2). Relative air humidity also exhibited significant fluctuations between months and years.

Data from 2024 and 2025 demonstrate the intensifying meteorological variability in the region (RHMZ, 2026) and these conditions, which align with global trends of accelerated warming, necessitate the adaptation of agrotechnical measures to mitigate adverse effects on the growth and development of cultivated crops.

Sample preparation and laboratory analysis

Okra pod samples were collected from each plot (replication) during both growing seasons at full market maturity. The plant material was dried, ground and prepared for analysis (Fig 3).

The protein content was determined on fresh okra pod samples using the Kjeldahl method (AOAC, 2006).

Antioxidant activity was evaluated using a modified DPPH method and the measured values are shown in mg TE g⁻¹ d.m (Molyneux, 2004).

Total phenols were determined using the Folin-Ciocalteu method and values are expressed in mg GAE g⁻¹ d.m (Singleton and Rossi, 1965).

Statistical data analysis

Statistical analysis was performed using IBM SPSS Statistics, version 26.0. Data normality was verified using the *Anderson-Darling* test. Homogeneity of variances was assessed using *Levene's* test. Analysis of variance was

Table 1: Characteristics of the soil in the sample plot.

Provision of soil with nutrients					
Depth	Humus	N	Available (mg 100 g ⁻¹ soil)		pH
(cm)	(%)	(%)	P_2O_5	K_2O	KCl
0-30	3.41	0.17	> 40	> 40	6.1
Mechanical composition of soil					
Smederevska palanka	Sand	Fine sand	Powder		Clay
	5.1	21.1	24.5		49.3

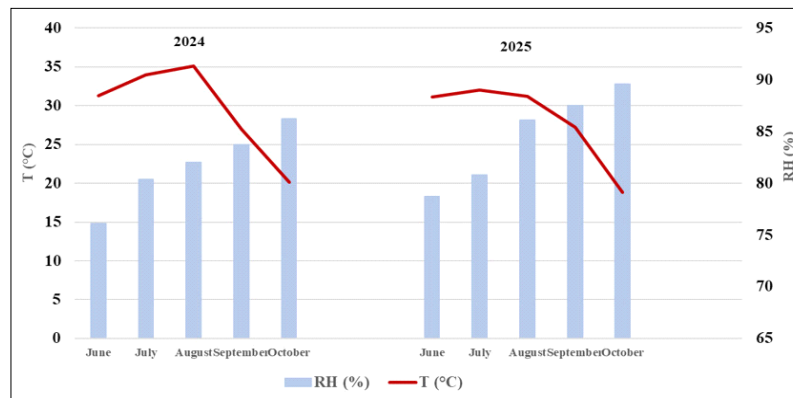


Fig 1: Average air temperature and relative humidity in the greenhouse (2024 and 2025).

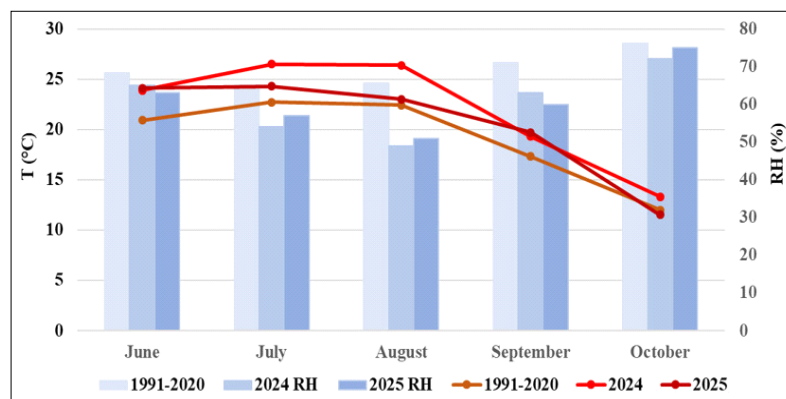


Fig 2: Average outdoor air temperature and relative humidity (2024 and 2025) compared to the multi-year average (1991-2020).



Fig 3: Plant material and laboratory analysis (Source: Author's).

applied to evaluate the effects of year and treatment at the significance level $p < 0.05$ and $p < 0.01$, followed by *Tukey's* (Honestly Significant Difference) post hoc test for mean comparison. The values obtained for the productive and qualitative properties of okra are presented graphically as averages for the research period. In addition, *Pearson's* correlation was conducted, which was represented via a heat map using the GraphPad Prism 11.0.0 program (trial version) to determine the relationship between the studied properties of okra.

RESULTS AND DISCUSSION

The vertical trend in total phenols indicates minimal variance and high homogeneity of experimental data for this parameter ($p < 0.005$). Such uniformity indicates a high stability of the parameter in the tested conditions, regardless of the applied treatments (Table 2).

It was determined that the data for all examined parameters follow a normal distribution, given that the values are within the 95% confidence interval and follow the trend line.

The analysis of variance revealed that year, treatment and their interaction had a statistically significant effect on yield per plant (Table 3, Fig 4). The highest average yield was observed in the second year of the study under the MAB and PBP treatments, which yielded 129.20 g/plant and 121.16 g/plant, respectively. The MAB treatment exhibited the most pronounced growth intensity, increasing by 119.17% in 2025. Similarly, the MBB treatment (80.82 g/plant) nearly doubled its output (95.17%), while the PBP treatment showed a substantial increase of 68.51%. In contrast, the control treatment had the lowest yields in both examined

years (below 40 g/plant) and showed a negative trend (15.67%) in 2025.

The MAB treatment showed the most significant increase, from 58.90 g/plant in 2024 to 129.20 g/plant in 2025 (Fig 4). Some studies have demonstrated that foliar application of microalgae-based biostimulants significantly increases productivity and plant growth of tomato, cucumber and lettuce (Barone *et al.*, 2019). The results clearly show that the application of biostimulants of natural origin yields significantly better results than the control under greenhouse conditions with extremely high temperatures ($> 40^{\circ}\text{C}$). This is further supported by the fact that elevated temperatures and their fluctuations align with broader climate change trends in Serbia and Southeast Europe. The greatest increase in yield in MAB may be explained by improved root system development and increased resistance to heat stress, which is consistent with the mechanisms of action of alginic extracts (increased synthesis of cytokinins and auxin-like substances) (Kabato *et al.*, 2025).

The analysis of variance revealed that year, treatment and their interaction had a statistically significant effect on the protein content of okra pods (Table 4, Fig 5). In the first year, the highest average protein content was observed in the MAB treatment (4.54%), whereas in the second year it was significantly lower (2.73%). In both research years, the control treatment had the lowest protein content (2.76% and 3.24%, respectively) compared to all other treatments. In 2024, the MBB treatment achieved the best result (4.08%). Analysis of okra pods shows that the year as an influencing factor significantly affects protein accumulation. The PBP treatment stood out in 2025, with a protein content increase of 57.51% compared to the 2024. In contrast, a decrease in protein content was observed in the MAB and MBB treatments (15.86% and 10.54%, respectively), which can be attributed to dilution effects resulting from the substantial increase in total pod weight. The elevated protein and nitrogen levels observed in the MAB, PBP and MBB treatments align with previous reports indicating that biostimulants enhance nitrogen concentration in okra pods, thereby improving overall fruit quality (Raza *et al.*, 2024).

The analysis of variance revealed significant effects of the treatment factor and the year $\times$ treatment interaction on nitrogen content (Table 5). The MAB treatment showed the most significant impact in the first year, achieving a nitrogen

Table 2: *Anderson-Darling* test for determining the normal distribution of data (*Gaussian distribution*).

Parameter	AD	p-value
Yield per plant	0.559	0.133
Protein	0.507	0.182
Nitrogen	0.138	0.971
Antioxidant activity	0.800	0.033
Total phenols	5.681	< 0.005

AD- Value test; p- Value < 0.05 the data do not follow a normal distribution; p- Value > 0.05 the data follow a normal (*Gaussian*) distribution.

Table 3: Analysis of variance and the significance of factors influencing yield per plant for the two-year research period (2024 and 2025).

Effect	SS	df	MS	F	Sign.
Year	8809.001667	1	8809.001667	53.62739188	**
Treatment	16216.25688	3	5405.418961	32.90707981	**
Year $\times$ Treatment	4611.834967	3	1537.278322	9.358634512	**
Total	32329.26758	23	1405.62033		
C.V. (%) = 17.98					

Note: SS- Sum of squares; df- Degree of freedom; MS- Mean squares; F test- F value calculated; **Highly significant at $p < 0.01$ probability level.

Table 4: Analysis of variance and the significance of factors influencing the protein content for the two-year research period (2024 and 2025).

Effect	SS	df	MS	F	Sign.
Year	0.302536215	1	0.302536215	71.81032898	**
Treatment	4.618100648	3	1.539366883	365.3858176	**
Year × Treatment	4.796891048	3	1.598963683	379.5317797	**
Total	9.865649098	23	0.428941265		

C.V. (%) = 1.78

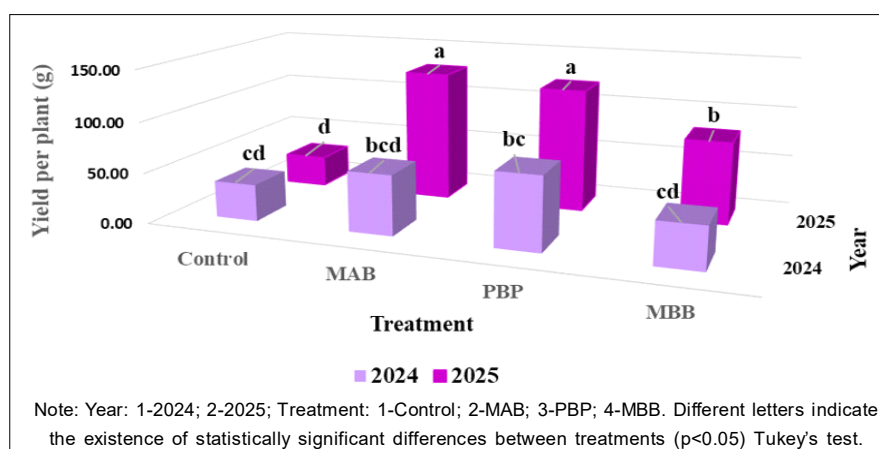
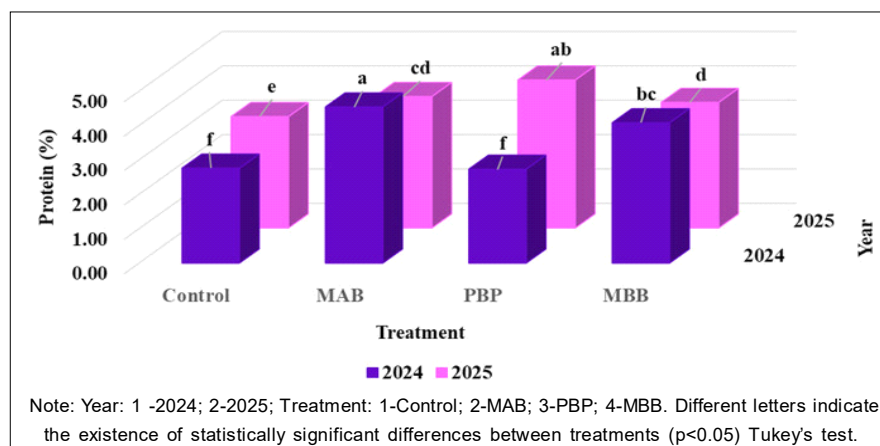
Note: SS- Sum of squares; df- Degree of freedom; MS- Mean Squares; F test- F value calculated; **Highly significant at $p < 0.01$ probability level.

Table 5: Analysis of variance and the significance of factors influencing nitrogen content for the two-year research period (2024 and 2025).

Effect	SS	df	MS	F	Sign.
Year	0.015105184	1	0.015105184	1.6578923	ns
Treatment	0.132159551	3	0.044053184	4.835123844	**
Year × Treatment	0.100800551	3	0.033600184	3.687839012	**
Total	0.408068226	23	0.017742097		

C.V. (%) = 16.17

Note: SS- Sum of Squares; df- Degree of freedom; MS- Mean Squares; F test- F value calculated; **Significant at $p < 0.01$ probability level; nsNon significant $p > 0.05$.


Fig 4: Average value for yield per plant (g) across different treatments during the years of research.

Fig 5: Average value for protein (%) across different treatments during the years of research.

content of 0.72%, which significantly decreased to 0.61% in the second year. In contrast, the MBB treatment proved to be the most stable, its results remain consistent regardless of the study year (0.65% and 0.66%), as illustrated by the blue line (Fig 6). In 2025, the PBP treatment stood out with a 58.14% increase in nitrogen content, indicating more intensive mobilisation of this element in okra pods. Conversely, during the year of maximum yield (2025), the MAB treatment showed a 15.28% decrease in nitrogen content, which can be explained by physiological dilution resulting from the sudden increase in okra pods weight.

The analysis of variance showed that both year and treatment factors, as well as their interaction, had statistically significant impact on antioxidant activity (Table 6). As a key indicator of plant quality and stress resistance, antioxidant activity reached its higher value in 2025 under the PBP treatment (3.74 mg TE g⁻¹ d.m.), while the MAB

treatment showed the highest value in 2024 (3.71 mg TE g⁻¹ d.m.). The MBB treatment proved to be the most stable. Although the MBB treatment did not yield “extreme” results, it offered the highest predictability across research years (Fig 7).

In the control, a 25% decrease in antioxidant activity was observed in the second year, whereas the PBP treatment showed an increase of 26.78%. High stability for this parameter was observed in the MBB treatment, while the slight decrease recorded in the MAB treatment can likely be attributed to the plant’s physiological trade-off on maximising biomass production rather than secondary metabolite accumulation.

The analysis of variance showed that year, treatment and their interaction did not have a statistically significant effect on the total phenols of okra pods (Table 7). In 2024, the PBP treatment achieved the highest average total phenol content (8.22 mg GAE g⁻¹ d.m.), while in 2025

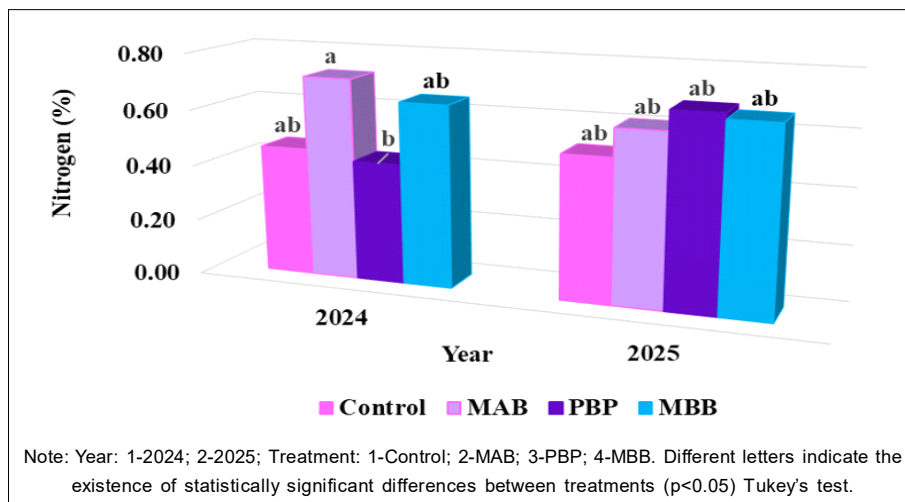


Fig 6: Average value for nitrogen (%) across different treatments during the years of research.

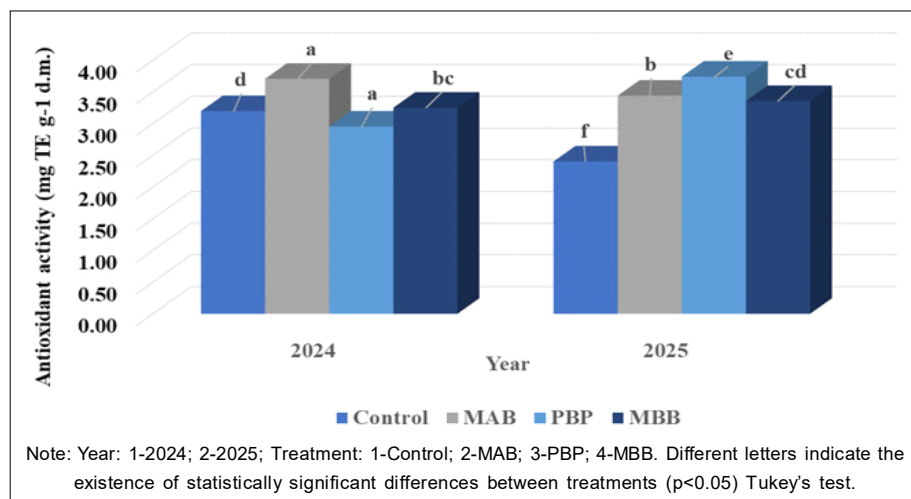


Fig 7: Average value for antioxidant activity (mg TE g⁻¹ d.m.) across different treatments during the years of research.

comparable values were observed in both the MAB and PBP treatments. The most noticeable increase in this parameter over the two-year research period was observed in the MBB treatment (from 5.38 to 7.87 mg GAE g⁻¹ d.m.). The environmental conditions in the second year particularly favoured phenol synthesis in the MBB treatment, potentially explaining the increased and stable antioxidant activity in that year (Fig 8). While the differences in phenolic content did not reach statistical significance in this study, the literature generally suggests that foliar biostimulant application enhances phenol levels in various vegetable species (Caruso *et al.*, 2019).

Phenols are secondary metabolites that play a crucial role in plant defence against abiotic stress, UV radiation and pathogens and are the primary drivers of antioxidant capacity. Although visual differences between treatments were observed (Fig 8), the high degree of variability within the samples precluded the confirmation of a statistically significant influence of either treatment or year on this parameter (Table 7).

The correlation heatmap (Fig 9) shows the strength and direction of linear association between yield per plant and biochemical parameters of okra pods. A strong positive correlation was found between protein content and nitrogen. Since nitrogen is a key component of amino acids, its accumulation directly parallels protein synthesis. Furthermore, a significant relationship between protein content and antioxidant activity suggests that treatments enhancing protein levels also tend to exhibit increased antioxidant capacity. A moderately strong correlation was found between antioxidant activity and yield per plant, whereas total phenols exhibited the weakest correlation with all other parameters. While there is a positive correlation between yield per plant and nitrogen, the weak correlation

suggests that nitrogen, though important, is not the only factor influencing overall plant productivity in this study.

The results of this research demonstrate that PBP and MAB treatments were the most effective, as they facilitated a multiple increase in yield in 2025 but also preserving or significantly enhancing nutritional parameters, specifically protein and nitrogen contents. These improvements in okra yield and quality align with previous research indicating that biostimulants regulate physiological processes via hormonal signaling and enhanced nutrient absorption (Johnson *et al.*, 2023). These results underscore that biostimulants offer a sustainable and eco-friendly alternative to conventional mineral fertilizers, significantly boosting okra's resilience to abiotic stresses such as extreme temperatures and declining soil fertility (Fernandes *et al.*, 2023). This enhanced adaptability mirrors observations in tomato plants, where biostimulants have been shown to improve tolerance to heat stress by strengthening antioxidant defense systems and maintaining nutritional quality (Francesca *et al.*, 2020).

The 2025 year underscored the dual benefits of the applied biostimulants, most notably the MAB and PBS treatments; they not only increased the yield, but also substantially enhanced fruit quality. Among the treatments, the PBP treatment emerged as the most balanced and effective, concurrently increasing yield, protein levels, nitrogen content and antioxidant activity. Conversely, the MBB treatment had a notable effect on increasing phenolic compounds by 46.28%, thereby significantly increasing the fruit's functional value during the second year. These findings confirm that through the strategic selection of biostimulants, it is possible to simultaneously optimize both crop productivity and nutritional profiles, even within highly variable agroecological environments.

Table 6: Analysis of variance and the significance of factors influencing antioxidant activity for the two-year research period (2024 and 2025).

Effect	SS	df	MS	F	Sign.
Year	0.010416667	1	0.010416667	4.939316963	*
Treatment	1.914583333	3	0.638194444	302.6154859	**
Year × Treatment	1.98565	3	0.661883333	313.8481513	**
Total	3.945783333	23	0.171555797		
C.V. (%) = 1.41					

Note: SS- Sum of Squares; df- Degree of freedom; MS- Mean squares; F test- F value calculated; **Highly significant at p<0.01 probability level; *Significant at p<0.05 probability level.

Table 7: Analysis of variance and the significance of factors influencing total phenols for the two-year research period (2024 and 2025).

Effect	SS	df	MS	F	Sign.
Year	0.357704167	1	0.357704167	0.135678682	ns
Treatment	7.0231125	3	2.3410375	0.887965288	ns
Year × Treatment	10.62897917	3	3.542993056	1.343872044	ns
Total	60.15359583	23	2.615373732		
C.V. (%) = 21.71					

Note: SS- Sum of squares; df- Degree of freedom; MS- Mean squares; F test- F value calculated; nsNon significant p>0.05.

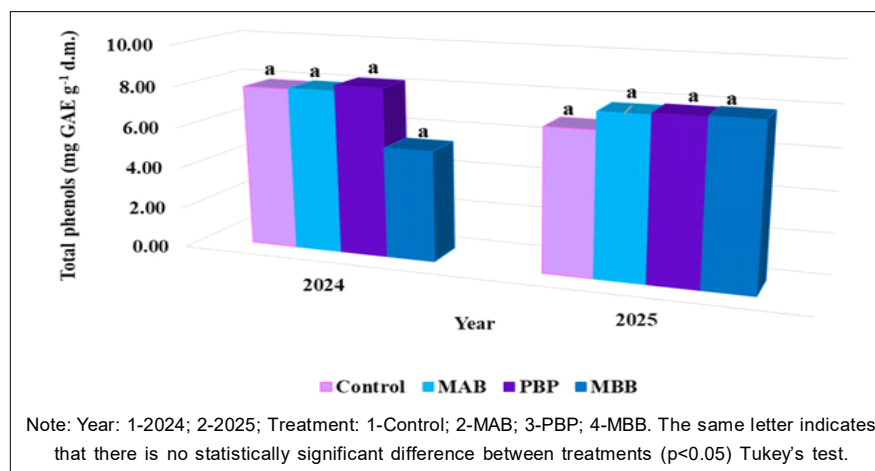


Fig 8: Average value for total phenols (mg GAE g⁻¹ d.m.) across different treatments during the years of research.

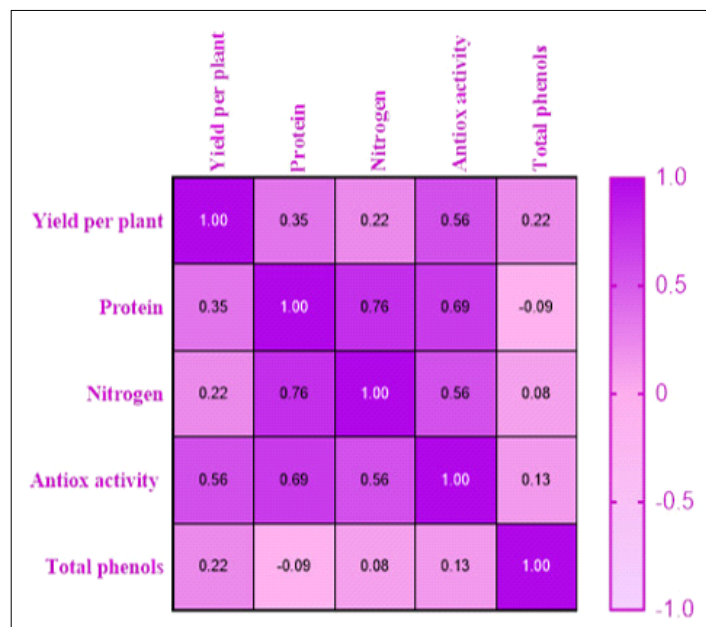


Fig 9: Heatmap for yield per plant and biochemical parameters of okra pods.

CONCULISON

Two-year study demonstrates that biostimulant application, particularly MAB and PBP, holds significant potential for enhancing okra productivity under thermal stress within protected environments. Notably, the MBB treatment emerged as a key factor in maintaining stability of quality parameters. Findings highlight the potential of these biostimulants to partially substitute mineral fertilizers, thereby promoting environmental sustainability and enhancing plants' physiological resistance to abiotic stress. These results support the integration of biostimulants into standard agrotechnical practices; however, further research is warranted to optimize dosages and application timing across diverse soil types and climatic conditions.

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Disclaimers

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Informed consent

Not applicable.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish, or preparation of the manuscript.

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