UDC 633.822:631.82
631.82:549.67
doi: 10.46793/AASer2661.063M
Acta Agriculturae Serbica, 31 (61), 63–67, 2026
Publisher: Faculty of Agronomy Čačak
www.afc.kg.ac.rs

Communication

The effect of Osmocote, zeolite, and biofertilizers on early growth of *Mentha × piperita* 'Cinderella' seedlings

Marija Marković*, Dragana Skočajić, Danijela Đunisijević-Bojović, Mihailo Grbić, Izabela Grabovac

Faculty of Forestry, University of Belgrade, Kneza Višeslava 1, 11000 Belgrade, Serbia

**Corresponding author: marija.markovic@sfb.bg.ac.rs*

Received 23 February 2026; Accepted 27 April 2026

A B S T R A C T

Mentha × piperita is a widely cultivated perennial, medicinal, culinary, and aromatic plant valued for its essential oil and used in cosmetic, pharmacy, and aromatherapy. Considering that zeolite and biofertilizers can positively influence plant growth, their use has been increasing recently in plant production. For that reason, this study examined the single and combined effects of zeolite, Slavol, and Osmocote on early growth of the peppermint cultivar 'Cinderella'. The obtained results showed that zeolite, Osmocote, or Slavol applied individually did not affect plant growth, as their results did not differ from the control. However, the combined Osmocote and zeolite treatment improved seedling growth, producing the greatest shoot length and highest leaf number, compared to other treatments. The best results were achieved with the substrate containing Osmocote, zeolite, and Slavol, which yielded the longest roots and highest shoot dry weight.

Keywords: peppermint, *Azotobacter*, *Bacillus*, controlled-release fertilizer

И З В О Д

Mentha × piperita је позната и често гајена лековита, зачинска и украсна биљка. Нарочито је цењена због свог етарског уља, које има значајну примену у козметици, фармацији, прехрамбеној индустрији и ароматерапији. С обзиром на то да зеолит и микробиолошка ђубрива позитивно делују на раст биљака, њихова употреба је последњих година све чешћа у биљној производњи. Циљ овог истраживања је да се утврди дејство појединачне и комбиноване примене зеолита, Славола и Осмокота на развој 'Cinderella' култивара нане у почетној фази производње. Добијени резултати су показали да појединачна примена ових препарата није утицала на развој биљака, с обзиром на то да се добијени резултати нису значајно разликовали од контролног третмана. Међутим, комбинација Осмокота и зеолита је позитивно утицала на развој клијаваца нане, који су имали највећу дужину избојака и формирали највећи број листова у односу на друге третмане. Ипак, најбољи резултати су постигнути применом комбинације Осмокота, зеолита и Славола, где су биљке формирале најдуже коренове и имале највећу вредност суве масе надземног дела биљке.

Кључне речи: *Azotobacter*, *Bacillus*, спороотпуштајуће ђубриво

1. Introduction

Mentha × piperita L. (peppermint) is a perennial, ornamental, medicinal, and aromatic plant known for centuries for its essential oil and pharmacological effects (Golijan Pantović et al., 2025). Peppermint is produced worldwide and used as a culinary herb, beverage ingredient, and in the cosmetic, pharmaceutical, and aromatherapy industries (Jarmila & Katarína, 2012; Mayer, 2025). The cultivar 'Cinderella' is a compact variety known for its classic peppermint flavor and high menthol content; it has a small height (around 40 cm), which makes it suitable for container growing and small gardens, where it is grown as a herb and ornamental plant (Jarmila & Katarína, 2012; Mayer, 2025).

The application of zeolite in plant production has a positive effect on plant growth by improving the efficient use of N and K fertilizers and increasing the availability of phosphorus (Cataldo et al., 2021; Aaina et al., 2018). Furthermore, through increased microporosity and high cation exchange capacity, zeolites promote the retention and gradual release of essential nutrients such as ammonium (NH_4^+) and potassium (K^+), reduce nutrient leaching, moderate salinity, and stabilize nitrogen availability in well-aerated propagation substrates (Javaid et al., 2024). Zeolites can also limit the mobility of toxic heavy metals (Tahervand et al., 2017). The addition of natural zeolite enhanced vegetative growth, photosynthetic efficiency, water use efficiency, and biomass accumulation of African marigold by increasing cation exchange capacity and reducing nutrient leaching (Farzad et al., 2007). Similarly, the incorporation of zeolite into peat-based substrates improved porosity and water-holding capacity, promoted root development and flowering in *Gazania rigens*, and influenced the growth response of *Catharanthus roseus* (Dobrowolska and Żurawik, 2016).

Recently, there has been growing interest in the use of biofertilizers as a sustainable way to boost plant growth and yield, improve nutrient use efficiency, and reduce nitrogen and phosphorus requirements (Aloo et al., 2022). Among bacterial biofertilizers, *Azotobacter* and *Bacillus* are widely used. *Azotobacter* produces growth-promoting compounds that enhance the availability of nutrients such as P, K, and Zn (Aasfar et al. 2021), while *Bacillus* species solubilize phosphorus and produce a range of growth-promoting substances, including phytohormones and antibiotics (Wu et al. 2025). Published studies on agronomic crops have demonstrated a pronounced synergistic effect of zeolites and biofertilizers, supporting their combined use as a sustainable strategy to enhance plant productivity and nutrient efficiency. The integration of zeolite with microbial inoculants, such as *Bacillus* spp., *Azospirillum*, and *Azotobacter*, has resulted in increased yield components, biomass accumulation, nutrient uptake, and improved physiological performance compared with individual applications (Moraditochaee et al., 2013; Ebrahimi Chamani et al., 2021; Bogacheva et al., 2025; Asadipoor et al., 2026). In addition to enhanced plant growth, these combined treatments have improved soil chemical properties, including the availability of phosphorus and potassium, while reducing nutrient losses and reliance on intensive mineral fertilization. Nevertheless, most published research about the combined use of zeolite and biofertilizers has focused on field-grown cereal and legume crops, with limited attention given to the combined effects of zeolites and biofertilizers in horticultural substrates, particularly for ornamental and aromatic plants such as *Mentha* spp. Also, existing studies investigating the effects of zeolite and biofertilizers on plant growth have produced different results, which vary across plant species and production methods, and research on their combined use in ornamental plant production is still limited.

Therefore, the aim of this study was to evaluate single and combined effects of zeolite, the commercial biofertilizer Slavol, and the controlled-release fertilizer Osmocote on the early growth of the peppermint cultivar ‘Cinderella’.

2. Materials and methods

Seeds of *Mentha x piperita* 'Cinderella' were purchased from Kiepenkerl Seeds. The seeds were sown in the greenhouse of the ''Moj Vrt'' company in the town of Vršac. The substrate used was Substrate Domoflor Mix 1. The treatments contained: ZeoSorb zeolite (particle size 1.5–2.5 mm), Osmocote Exact Standard 15-9-12 + 2MgO + TE (5–6 months) controlled-release fertilizer, containing 15% N, 9% P_2O_5, 12% K_2O, 2% MgO, and the micronutrients: Fe 0.45%, B 0.03%, Cu 0.05%, Zn 0.015%, and Mn 0.06%, and Slavol bio-organic fertilizer, composed of nitrogen fixing and phosphorus mineralizing bacteria (*Derxia* sp., *Azotobacter chroococcum, A. vinelandi, Bacillus megaterium, B. lichenformis*, and *B. subtilis*) with growth-stimulating compounds. Eight treatments including control were used in this experiment (Table 1). Osmocote and zeolite were mixed with substrate in pots. Osmocote was added in the amount of 4g per each liter of substrate. Zeolite was added as 15% (volume) of mixture. Slavol was applied on the 1st, 15th, 30th, 45th and 60th day from seed sowing, as 1.5% solution, using 40 mL of solution per each pot.

Table 1.
Treatments used in experiment

Treatment	Label
Substrate only	Control
Substrate + Osmocote	O
Substrate + zeolite	Z
Substrate + 1.5% Slavol	S
Substrate + Osmocote + zeolite	OZ
Substrate + Osmocote + 1.5% Slavol	OS
Substrate + zeolite + 1.5% Slavol	ZS
Substrate + Osmocote + zeolite + 1.5% Slavol	OZS

Each treatment consisted of four Ø9 cm pots, with each pot representing one replication and 30 seeds sown per pot. In total, eight treatments were established, resulting in 32 pots. Seeds were sown on March 28, and plants were grown for the subsequent nine weeks in an unheated greenhouse.

Air temperature and air humidity were monitored daily, and minimum and maximum values were recorded. The number of germinated seeds was recorded on a daily basis. At three-week intervals after sowing (April 18, May 9, and May 30), 24 plants per treatment (8 plants per pot × 3 pots) were collected to determine seedling height as well as the number and length of roots, shoots, and leaves. In this case, plants were sampled from three pots per treatment, which were considered as three independent replications for statistical analysis. In addition, on May 30, plants from the fourth pot (15 plants per treatment) were used exclusively for the assessment of shoot dry weight.

The obtained data were statistically analyzed (Statgraphics, version 5.0). The significance of differences between the means was determined by the analysis of variance (ANOVA, $p < 0.05$) and the least significant difference (LSD) test.

3. Results and discussion

During the research, weather conditions showed strong daily temperature fluctuations, with cold mornings and nights (12–18 °C) and daytime peaks above 30 °C. Air humidity varied between 40 and 80% (average 72.45%). The pronounced daily temperature fluctuations recorded during the experiment could influence plant growth, but the aim was to obtain data in typical nursery production conditions in the region, providing a realistic assessment of treatment performance under practical, rather than strictly controlled, growing conditions.

The parameters measured 3 or 6 weeks after sowing showed that the treatments did not influence the length of primary roots, shoots, or the number of leaves (Tables 2 and 3). The only exception was the shoot length of plants grown on substrate with Osmocote, measured 3 weeks after sowing; the shoots were significantly longer than in the other treatments and the control (Table 3). However, 9 weeks after sowing, the treatments influenced seedling growth, and the best results were obtained with the substrate containing Osmocote, zeolite, and Slavol bio-organic fertilizer, which produced the longest roots (Table 2) and the highest shoot dry weight (Table 3). Shoot dry weight quantifies harvestable biomass from which bioactive compounds are extracted; there is a positive correlation between higher shoot dry weight and increased content of active medicinal substances, reflecting enhanced growth and secondary metabolite accumulation (Golzarian et al., 2011).

Table 2.
The effect of different treatments on peppermint root growth

	Length of primary roots (mm)			No of secondary roots
	April 18	May 9	May 30	April 18
Control	21.1 bc	68.9 ab	130.1 ab	1.9 bc
O	23.7 abc	70.8 ab	128.4 ab	3.3 ab
Z	19.3 c	73.9 ab	127.7 ab	1.9 bc
SL	27.0 ab	71.9 ab	111.8 b	3.6 ab
OZ	18.9 c	62.7 b	122.5 ab	1.5 c
OSL	23.5 abc	73.7 ab	119.9 ab	2.9 abc
ZSL	26.7 ab	77.1 a	135.1 a	3.6 ab
OZSL	26.5 ab	79.3 a	134.5 a	3.4 ab

Values followed by different letters in the same column are significantly different at the $P < 0.05$ level according to the LSD test

The addition of zeolite, Osmocote, or Slavol alone did not influence plant growth, as the obtained results were not significantly different compared to the control treatment. The combined treatment of Osmocote and zeolite resulted in the greatest shoot length and the highest number of leaves at the final sampling date. A significantly high mean number of leaves was also recorded in plants treated with the combination of zeolite and Slavol; however, the dry weight of shoots in this treatment remained low (Table 3). Although shoot length was lower in combined Osmocote, zeolite, and Slavol treatment, compared to Osmocote and zeolite treatment, recorded 9 weeks after sowing, the number of leaves was high (Table 3), indicating shorter internodes and a more compact plant habitus. Such a compact growth form is considered favorable in nursery production, as it facilitates handling and transplanting, while also supporting uniform development and improved ornamental quality, which is especially important for this species cultivated both as an ornamental and medicinal plant.

Table 3.
The effect of different treatments on peppermint shoot growth

	Length of shoots (mm)			No of leaves			DWS (mg)
	April 18	May 9	May 30	April 18	May 9	May 30	May 30
Control	4.7b	53.9d	122.1cd	0.8c	9.7a	16.9b	95.8bc
O	7.0a	68.6abc	148.9b	1.25abc	11.5a	17.6b	118.1b
Z	5.1b	65.3abc	130.6bc	1.0bc	10.1a	17.1b	76.1c
SL	5.0b	62.5bcd	105.1d	1.4ab	10.1a	16.2b	117.7b
OZ	4.9b	70.1ab	169.1a	1.2ab	9.7a	22.6a	101.0bc
OSL	5.4b	74.4ab	134.0bc	1.3ab	10.4a	22.5a	113.5b
ZSL	5.8b	58.1cd	105.8d	1.3ab	11.2a	16.2b	113.1b
OZSL	4.9b	73.8ab	135.7bc	1.6ab	11.5a	20.6ab	139.8a

Values followed by different letters in the same row are significantly different at the $P < 0.05$ level according to the LSD test; DWS = Dry weight of shoots

Contrary to our findings, the addition of zeolite alone proved to be favorable for maize development, with an effect similar to N fertilization (Kakabouki et al., 2025). Moreover, Mohammadi et al. (2024) reported a positive effect of zeolite use in peppermint production; however, in their experiment, zeolite was applied to enhance tolerance to salinity stress, and the growing conditions differed substantially from those in the present study. However, Kolar et al. (2010) reported that the addition of zeolite to a substrate containing slow-release and controlled-release fertilizers did not influence plant growth. Nevertheless, a positive effect of zeolite combined with microbial fertilizer was observed in improving mining arid soils where *Medicago sativa* was planted (Li et al., 2023). Likewise, zeolite combined with urea and manure proved effective in enhancing wheat productivity in degraded mountain soils (Khaliq et al., 2024).

Previous studies have also reported positive effects of zeolite application on ornamental plant growth, including improved vegetative development, photosynthetic performance, water use efficiency, and biomass accumulation, mainly attributed to enhanced cation exchange capacity and reduced nutrient leaching (Farzad et al., 2007; Dobrowolska & Żurawik, 2016). In contrast to these findings, the application of zeolite alone or in combination with Osmocote did not result in significantly improved growth parameters compared with the control in our study. However, a pronounced positive effect was observed when zeolite was combined with Osmocote and a biofertilizer (Slavol), indicating that the beneficial role of zeolite in this production system became evident only when coupled with microbial activity, which promoted its key function in increasing the supply or availability of primary nutrients. The biofertilizer used in this study contains nitrogen-fixing and phosphorus-mineralizing bacteria (*Derxia* sp., *Azotobacter chroococcum*, *A. vinelandii*, *Bacillus megaterium*, *B. licheniformis*, and *B. subtilis*), suggesting that the synergistic interaction between controlled-release fertilization, microbial nutrient transformation, and zeolite-mediated nutrient retention was crucial for achieving improved plant performance.

Conclusion

The combined treatment with Osmocote, zeolite, and Slavol showed a possible synergistic effect on peppermint growth under greenhouse conditions with daily temperature fluctuations. This combination promoted root development, higher shoot dry weight, and a more compact plant habitus. In contrast, single-component and two-component treatments did not significantly improve growth compared with the control. The results indicate that the beneficial effects of zeolite in peppermint cultivation become evident primarily when combined with controlled-release fertilization and biofertilizers. Further research is required to evaluate the effects of different application rates of biofertilizers, mineral fertilizers, and microfertilizers coupled with zeolite mixed into the substrate and optimized for *Mentha x piperita* growth for ornamental or medicinal purposes.

Acknowledgments

The Ministry of Education, Science and Technological Development financed this scientific research work of the Faculty of Forestry of the University of Belgrade in 2025 according the Agreement Ref. No.: 451-03-137/2025-03/ 200169 dated 04.02.2025

Declaration of competing interests

The authors declare that they have no conflicts of interest.

References

Aaina, H. N., Ahmed, O. H., Majid, N. M. A. (2018). Effects of clinoptilolite zeolite on phosphorus dynamics and yield of Zea mays L. cultivated on an acid soil. *PLoS ONE*, 13, e0204401. https://doi.org/10.1371/journal.pone.0204401

Aasfar, A., Bargaz, A., Yaakoubi, K., Hilali, A., Bennis, I., Zeroual, Y., Meftah Kadmiri, I. (2021). Nitrogen fixing Azotobacter species as potential soil biological enhancers for crop nutrition and yield stability. *Frontiers in Microbiology*, 12, 628379. https://doi.org/10.3389/fmicb.2021.628379

Aloo, B. N., Tripathi, V., Makumba, B. A., Mbega, E. R. (2022). Plant growth-promoting rhizobacterial biofertilizers for crop production: The past, present, and future. *Frontiers in Plant Science*, 13, 1002448. https://doi.org/10.3389/fpls.2022.1002448

Asadipoor, M., Khorgami, A., Sayahfar, M. (2026). Synergistic effects of mycorrhizal fungi and zeolite on wheat yield and soil chemical properties. *Journal of Soil Science and Plant Nutrition*, 26, 937–951. https://doi.org/10.1007/s42729-025-02905-y

Ogacheva, N., Afonina, Y., Serazetdinova, Y., Kolpakova, D., Naik, A., Faskhutdinova, E., Lyubimova, N., Lyudmila, A. (2025). Combined use of zeolite and growth-stimulating bacteria to improve wheat growth. *Food Processing: Techniques and Technology*, 3, 509–520.

Cataldo, E., Salvi, L., Paoli, F., Fucile, M., Masciandaro, G., Manzi, D., Masini, C. M., Mattii, G. B. (2021). Application of zeolites in agriculture and other potential uses: A review. *Agronomy*, 11, 1547. https://doi.org/10.3390/agronomy11081547

Dobrowolska, A., Żurawik, P. (2016). Zeolite as a component of substrate in cultivation of ornamental plants–*Catharanthus roseus* (L.) G. Don and *Gazania rigens* var. *rigens* (L.) Gaertn. *Acta Scientiarum Polonorum Hortorum Cultus*, 15(2), 13–25.

Ebrahimi Chamani, H., Fallah Amoli, H., Niknejad, Y., Barari Tari, D. (2021). Effects of zeolite and biofertilizers on yield components, yield and nutrients uptake in grains of two corn cultivars (cv. 6010 and ns71). *Journal of Plant Nutrition*, 44, 1670–1681. https://doi.org/10.1080/01904167.2021.2014876

Farzad, N., Khosh-Khui, M., Eshghi, S., Salehi, H. (2007). The effects of natural zeolite on vegetative growth, flower and physiological characteristics of African marigold (Tagetes erecta L. 'Queen'). *Horticulture, Environment, and Biotechnology*, 48, 241–245.

Golijan Pantović, J., Kulić, G., Šarčević Todosijević, L., Sečanski, M., Popović, V., Lakić, Ž., Ikanović, J., Pisinov, B. (2025). Potential of essential oils in control of pathogenic plant viruses. *Acta agriculturae Serbica*, 30(60), 97-109. https://doi.org/10.5937/AASer2560097G

Golzarian, M. R., Frick, R. A., Rajendran, K., Berger, B., Roy, S., Tester, M., Lun, D. S. (2011). Accurate inference of shoot biomass from high-throughput images of cereal plants. *Plant Methods*, 7, 2. https://doi.org/10.1186/1746-4811-7-2

Jarmila, N., Katarína, K. (2012). Evaluation of antioxidant potential of different taxa of genus Mentha L. Proceedings of the Seventh Conference on Medicinal and Aromatic Plants of Southeast European Countries (7th CMAPSEEC), Subotica, Serbia, 27–31 May 2012, 151–157.

Javaid, A., Munir, N., Abideen, Z., Siddiqui, Z. S., Yong, J. W. H. (2024). The role of natural and synthetic zeolites as soil amendments for mitigating the negative impacts of abiotic stresses to improve agricultural resilience. *Plant Stress*, 14, 100627. https://doi.org/10.1016/j.stress.2024.100627

Kakabouki, I., Roussis, I., Mavroeidis, A., Stavropoulos, P., Kanatas, P., Pantaleon, K., Folina, A., Beslemes, D., Tigka, E. (2025). Effects of zeolite application and inorganic nitrogen fertilization on growth, productivity, and nitrogen and water use efficiency of maize (*Zea mays* L.) cultivated under Mediterranean conditions. *Sustainability*, 17, 2178. https://doi.org/10.3390/su17052178

Khaliq, A., Shehzad, M., Huma, M. K., Tahir, M. M., Javeed, H. M. R., Saeed, M. F., Jamal, A., Mihoub, A., Radicetti, E., Mancinelli, R. (2024). Synergistic effects of urea, poultry manure, and zeolite on wheat growth and yield. *Soil Systems*, 8, 18. https://doi.org/10.3390/soilsystems8010018

Kolar, M., Dubský, M., Šrámek, F., Pintar, M. (2010). The effect of natural zeolite in peat-based growing media on *Pelargonium zonale* plants. *European Journal of Horticultural Science*, 75, 226–230.

Li, W., Zhang, J., Song, S., Liang, Y., Sun, B., Wu, Y., Mao, X., Lin, Y. (2023). Combination of artificial zeolite and microbial fertilizer to improve mining soils in an arid area of Inner Mongolia, China. *Journal of Arid Land*, 15, 1067–1083. https://doi.org/10.1007/s40333-023-0028-1

Mayer, J. (2025). *Das große Gemüse- und Obstgartenbuch: mit Tipps zu Pflanzen und Gartenarbeit für Anfänger und Profis – Planen, anbauen, pflegen und erfolgreich ernten für Selbstversorger*. Stiftung Warentest, 265.

Mohammadi, H., Parviz, L., Beyrami, A., Anosheh-Bonab, F., Ghorbanpour, M. (2024). Exposure to TiO_2 nanoparticles (NPs) and zeolite stimulates growth, physiology, and phytochemical characteristics and elevates *Mentha piperita* L. tolerance to salinity stress. *Industrial Crops and Products*, 211, 118228. https://doi.org/10.1016/j.indcrop.2024.118228

Moraditochaee, M., Azarpour, E., Bozorgi, H. R., Heidarian, A. R. (2013). Investigation effects zeolite and their integrated bio-fertilizer and different levels of chemical nitrogen fertilizer under irrigation management on physiological traits of peanut (*Arachis hypogaea* L.). *Annals of Biological Research*, 4, 65–69.

Tahervand, S., Jalali, M. (2017). Sorption and desorption of potentially toxic metals (Cd, Cu, Ni and Zn) by soil amended with bentonite, calcite and zeolite as a function of pH. *Journal of Geochemical Exploration*, 181, 148–159.

Wu, X., Liu, Y., Jia, B., Tao, L., Li, H., Wang, J., Yuan, Z., Sun, X., Yao, Y. (2025). Four decades of Bacillus biofertilizers: Advances and future prospects in agriculture. *Microorganisms*, 13, 187. https://doi.org/10.3390/microorganisms13010187