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Influence of Zeatin, Kinetin, and Gibberellic Acid Doses on Growth and Biochemical Responses of *Melissa officinalis* L. (Lemon Balm) Seedlings

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Abstract: This study aimed to assess the impact of Zeatin, Kinetin, and gibberellic acid biostimulant doses key regulators of plant growth and development on the growth and biochemical properties of *Melissa officinalis* (lemon balm). Conducted under greenhouse conditions using a Completely Randomized Experimental Design with three replicates, the research measured parameters such as seedling and root lengths, fresh and dry weights of seedlings and roots, total phenolic content, and antioxidant activities (CUPRAC and FRAP). The findings revealed that biostimulant treatments significantly improved growth parameters compared to the control, with the exception of root length. The 200 ppm gibberellic acid treatment yielded the highest fresh and dry weights for both seedlings and roots. For biochemical effects, the highest antioxidant activity was recorded in Zeatin40 treatments, while the greatest phenolic content was observed in Zeatin20, highlighting Zeatin's effectiveness in enhancing total secondary metabolite content.

Keywords: Antioxidant, Biostimulant, Seedling Development, Lemon Balm, Total Phenolic Content

INTRODUCTION

Lemon balm, scientifically known as *Melissa officinalis* L., is a member of the Lamiaceae family. It is a perennial plant that stands erect or semi-erect, with an average height of 100 cm (Ceylan 1997). This medicinal plant is widely used in traditional medicine practices around the world. It naturally grows in the Mediterranean region and Western Asia (Abdel-Naime et al. 2019; Ghiulai et al. 2020), while it is also cultivated in Bulgaria, Germany, Romania, Italy, France, and some regions of North America (Katar 2004). This plant is also known by various common names such as bee balm, lemon balm, *melissa*, and balm mint (Petrisor et al. 2022).

In Turkey, there are three different subspecies of the lemon balm plant: ssp. officinalis, ssp. altissima, and ssp. inodora. Among these subspecies, only *Melissa officinalis ssp. officinalis* is of medical significance, as the other two are not preferred due to being odorless or emitting an unpleasant odor. The subspecies used for medicinal purposes can be seen in four different forms naturally occurring in various regions of Turkey (Baytop 1984).

Lemon balm cultivation in Turkey is quite limited. The main reasons for this include the plant's low essential oil content and the scarcity of distillation facilities. Chemical research on *Melissa officinalis* has shown that the plant contains mainly flavonoids, terpenoids, phenolic acids, tannins, and essential oil (Zarei et al. 2014). The primary active components of *Melissa officinalis* are volatile compounds (geranial, neral, citronellal, geraniol), triterpenes (ursolic acid and oleanolic acid), phenolic compounds (rosmarinic acid, caffeic acid, and protocatechuic acid), and flavonoids (quercetin,

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rhamnocitrin, luteolin). Although the essential oil is generally considered the therapeutic principle responsible for most biological activities, polyphenols are also effective (Shakeri et al. 2016; Miraj et al. 2017).

Lemon balm is used in many industrial areas; especially leading in pharmaceuticals, perfumery, food, and cosmetic industries. Fundamentally, the essential oil of the plant is concentrated in the leaves, while the stems and flowers have a lower rate. From the dry lemon balm plant, volatile oil can be extracted in amounts ranging between approximately 0.01% and 0.3% (Zeybek 1387; Akgül 1983). In traditional medicine, it is commonly preferred for various purposes such as fever reduction, sleep regulation, headache, and common cold treatment (Katar and Gürbüz 2008).

Studies on *Melissa officinalis* leaf extracts have identified phenolic profiles associated with antiproliferative, anti-angiogenic, antiviral, antioxidant, anti-anxiety, antidepressant, anti-Alzheimer's, neuroprotective, cardioprotective, antifungal, and antibacterial effects (Petrisor et al. 2022).

Plant biostimulants can be described as both synthetic and natural substances or microorganisms applied at various times and by various methods, with the purpose of increasing plant nutrient uptake, enhancing the capacity to cope with abiotic stress, and/or improving product quality (Patrick 2015).

Zeatin, as a type of cytokinin plant biostimulant, plays significant roles in plant growth and development. This biostimulant is reported to promote the development of new shoots in plants, aid in leaf growth, accelerate chloroplast formation, slow down aging, facilitate seed germination, and regulate the cyclic activity of cells (Havlicek et al., 1997; Mok and Mok, 2001). Kinetin, belonging to the cytokinin family, is known as a biostimulant in plants that slows down the aging process, promotes cell division, thereby contributing to growth and development, and also increases chlorophyll production (Toprak, 2019). Gibberellic acid (GA) is a biostimulant produced by plants themselves and acts as a signal in vital functions such as seed germination, water absorption, flowering initiation, fruit development, shoot elongation, and regulation of metabolic processes. During these functions, GA moves in synchronization with the plant's other biostimulants to direct these processes (Zhu et al., 2019; Khan et al., 2020).

This study was conducted to determine the effects of different doses of foliar applications of zeatin, kinetin, and gibberellic acid biostimulants on the growth and biochemical parameters of *Melissa officinalis* plants during the seedling development phase.

MATERIALS and METHODS

Material

The study was carried out in the greenhouse of the Agricultural Sciences and Technologies Education Application and Research Center at Sakarya University of Applied Sciences' Faculty of Agriculture. The lemon balm seedlings used were sourced from a commercial supplier.

Method

The trial followed a Completely Randomized Experimental Design with three replicates. Lemon balm seedlings were treated with biostimulants—zeatin (20 and 40 mg/l), kinetin (50 and 100 mg/l), and gibberellic acid (100 and 200 mg/l)—to assess their effects on plant growth and development. The experiment utilized 21 pots, each with a 2-liter capacity, filled with a uniform mix of finely sifted garden soil (3/4) and Klassman TS1 peat (1/4). The greenhouse ground was first leveled and compacted, and then the pots were arranged with 20 cm spacing within rows and 30 cm between rows.

After placing the pots in the greenhouse, five randomly chosen pots were each watered with 500 ml. Collection plates were set under each pot to capture the runoff. Once drainage was complete, an average of 215 ml of water per pot was collected, indicating a water retention capacity of 285 ml. The seedlings were then planted on November 1, 2023, at a depth of approximately 3 cm. To minimize fungal contamination, the lower two leaves were manually removed if they were in contact with the soil. Each pot received around 100 ml of water weekly for the duration of the experiment.

Kinetin and gibberellic acid were dissolved in 96% ethanol, while zeatin was dissolved in NaOH before being diluted with distilled water to a total volume of 1 liter. These biostimulant solutions were stored in 1-liter spray bottles wrapped in aluminum foil to prevent light exposure and kept in the refrigerator. The initial foliar applications occurred on January 5, 2024 (about two months post-planting). Since the study took place during the winter, plant growth was slower than in summer, resulting in a delayed start for biostimulant applications. Each application involved spraying approximately 10 ml onto the leaves in each pot, repeated three times at four-day intervals. Final measurements, including plant height, were taken on January 19, 2024, marking the end of the 2.5-month experiment. The average daytime and nighttime temperatures during the study period were recorded as 15°C and 4°C, respectively (Anonymous 2024). To measure root lengths, plants were carefully washed to loosen soil, then measured with a ruler. Fresh weights of shoots and roots were taken using a precision scale. Seedlings and roots were then placed on drying paper and dried in an oven at 35°C for 108 hours, after which their dry weights were recorded.

The total phenolic content was assessed using the Folin–Ciocalteu method according to Waterhouse (2002). The FRAP reducing capacity was determined according to the method of Sachett et al. (2021). Determination of CUPRAC Reducing Capacity was assessed using according to Ak and Gülçin 2008.

Statistical Analysis

Statistical analyses of the data obtained were performed using the COSTAT (version 6.03) package program, and multiple comparison tests were performed according to the Least Significant Difference (LSD = 0.05) test.

RESULTS and DISCUSSION

The effect of synthetic biostimulants on all growth parameters of lemon balm plants, except for seedling length, was found to be statistically significant at the 5% level. The highest value for root length was 33.25 cm, obtained from the control treatments. The highest values for seedling fresh weight, root fresh weight, seedling dry weight, and root dry weight were obtained from gibberellic acid 200 treatments, with values of 6.06 g, 12.29 g, 1.86 g, and 1.25 g, respectively, as shown in Table 1.

The significant effect of GA3 application on the fresh and dry weight of Coleus amboinicus leaves was reported by Morales-Payan (2005). Gul et al. (2006) demonstrated that it increased the height and quality of Araucaria heterophylla plants. Similarly, Yousef and Gomma (2008) stated that spraying Dahlia pinnata plants with 100 or 200 ppm GA3 increased the plant growth parameters. The application of GA3 in onion and garlic plants (Ouzounidou et al., 2011) and in fenugreek plants (Dar et al., 2015) was reported to cause significant increases in both fresh and dry weights of the plants. Further support for these findings comes from studies on fenugreek (Dar et al., 2015). Previous studies, including those by Santos et al. (1998) and Srivastava & Srivastava (2007), have demonstrated that GA3 treatment positively influences growth, chlorophyll content, and essential oil production in plants, as seen in thyme (Dadkhah et al., 2016).

Table 1. *Effects of Some Synthetic Biostimulants on Growth Parameters of Lemon Balm Plants*

Biostimulants	**Seedling length (cm)**	**Seedling fresh weight (g)**	**Root length (cm)**	**Root fresh weight (g)**	**Seedling dry weight (g)**	**Root dry weight (g)**
Control	7.60	3.38 b	33.25 a	7.82 c	1.19 b	0.73 cd
Zeatin20	7.50	3.99 b	28.40 ab	7.46 c	1.17 b	0.84 cd
Zeatin40	7.47	2.82 b	26.50 b	5.69 d	0.77 c	0.59 d
Kinetin50	8.03	3.24 b	26.90 b	7.10 c	1.08 bc	0.69 cd
Kinetin100	7.93	4.03 b	25.55 b	8.13 c	1.27 b	0.87 bc
Gibberellic acid100	8.93	5.82 a	30.20 ab	10.69 b	1.29 b	1.14 ab
Gibberellic acid200	8.70	6.06 a	30.15 ab	12.29 a	1.86 a	1.25 a
LSD (0.05)	ns	1.28	5.24	1.33	0.31	0.27
CV (%)	10.91	17.53	10.43	9.02	14.64	17.91

Research has also linked GA3 application to various beneficial effects on plant physiology, morphology, and biochemistry (Taiz & Zeiger, 2010). Increased growth following GA3 use has been attributed to enhanced activity of enzymes like carbonic anhydrase, nitrate reductase, and ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBPCO) (Yuan & Xu, 2001; Afroz et al., 2005; Aftab et al., 2010). Additionally, GA3 is known to promote growth through its roles in cell expansion and division (Taiz & Zeiger, 2010). These findings align with our results, affirming the observed growth responses.

Table 2. *Effects of Some Synthetic Biostimulants on Biochemical Parameters of Melissa officinalis*

Biostimulants	**CUPRAC (mM/g TE)**	**FRAP (mM/g AAE)**	**Total Phenolics (mg/g GAE)**
Control	2,09 b	1,08 ab	0,24 bc
Zeatin20	4,21 a	1,08 ab	0,34 a
Zeatin40	3,85 a	1,35 a	0,31 ab
Kinetin50	4,27 a	1,01 bc	0,33 a
Kinetin100	2,11 b	0,76 c	0,16 d
Gibberellic acid100	2,47 b	1,01 bc	0,28 ab
Gibberellic acid200	2,33 b	0,77 c	0,20 cd
LSD (0.05)	0,68	0,29	0,07
CV (%)	12,79	16,85	16,34

Table 2 shows that the biostimulants applied during the seedling development stage of Lemon Balm plants have a statistically significant effect at the 5% level on antioxidant activities measured by CUPRAC and FRAP methods and on the total phenolic content. In the CUPRAC antioxidant activity method, the Kinetin50 application gave higher results than other applications with 4.27 mM/g TE. According to the FRAP antioxidant method, the highest value was obtained from the Zeatin40 applications with 1.35 mM/g AAE. In terms of total phenolic content, the highest value was determined in Zeatin20 applications with 0.34 mg/g GAE (Table 2).

It has been reported that zeatin and kinetin biostimulants doses applied to medicinal sage increased both antioxidant activity and total phenolics compared to the control (Santos-Gomes et al., 2003), that zeatin biostimulant applied to red cabbage increased total phenolics and antioxidant activity compared to the control (Ravanfar et al., 2020), that zeatin biostimulant applied to corn plants increased antioxidant activity compared to the control (Yousaf et al., 2024), that kinetin biostimulant application increased total phenolics, flavonoids, and antioxidant parameters in thyme plants compared to the control (Günaydın et al., 2017), and that kinetin biostimulant in coffee plants was effective in scavenging free radicals and increased both total phenolics and flavonoids as well as phenolic compounds compared to the control (Acidri et al., 2020).

The effects of cytokinins, including zeatin and kinetin, on plant development, particularly on phenolic compounds and related antioxidant defense systems, are reported to be due to the regulation of the expression of genes related to secondary metabolism under normal or stress conditions. Among these genes are those involved in flavonoid and phenylpropanoid biosynthesis, as well as glutaredoxin, peroxidase, glutathione transferase, and antioxidant enzyme genes (Brenner and Schmulling, 2012; Bhargava et al., 2013; Kocsy et al., 2013; Reguera et al., 2013).

Table 3. *Correlation Table Among the Parameters Investigated for Melissa officinalis with Some Synthetic Biostimulants*

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
1	1								
2	0.890**	1							
3	0.220	0.331	1						
4	0.878**	0.968**	0.436	1					
5	0.680	0.839*	0.379	0.924**	1				
6	0.865*	0.995**	0.327	0.978**	0.886**	1			
7	-0.440	-0.500	-0.469	-0.595	-0.559	-0.529	1		
8	-0.555	-0.614	-0.003	-0.690	-0.818*	-0.678	0.565	1	
9	-0.308	-0.382	-0.085	-0.489	-0.584	-0.443	0.877**	0.732	1

* Correlation is significant at the 5% level
** Correlation is significant at the 1% level
1: Seedling Length, 2: Seedling Fresh Weight, 3: Root Length, 4: Root Fresh Weight, 5: Root Dry Weight, 6: Seedling Dry Weight, 7: CUPRAC, 8: FRAP, 9: Total Phenolic Content

Table 3.3 shows that plant height has a positive correlation at the 1% level with seedling fresh weight and root fresh weight, and at the 5% level with seedling dry weight. Seedling fresh weight has a positive correlation at the 1% level with root fresh weight and seedling dry weight, and at the 5% level with root dry weight. Root fresh weight has a positive correlation at the 1% level with root dry weight and seedling dry weight. Root dry weight has a positive correlation at the 1% level with seedling dry weight, but a negative correlation at the 5% level with FRAP antioxidant activity. CUPRAC antioxidant activity shows a positive correlation at the 1% level with total phenolic content.

CONCLUSION

Synthetic biostimulants have shown significant effects on the growth performance of lemon balm plants. In particular, the foliar application of 200 ppm gibberellic acid is recommended as it provided the highest values for certain growth parameters. Cytokinins, specifically zeatin and kinetin biostimulants, were found to be more effective than the control and other biostimulants in terms of antioxidant activities and total phenolic content. Future studies should focus on optimizing the dosage and application frequency of these biostimulants under field conditions to maximize their benefits for sustainable cultivation practices.

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AUTHOR CONTRIBUTIONS

The authors contributed equally to this study.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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