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## **Effect of foliar application of biostimulants on the yield and quality of Greek oregano herb (*Origanum vulgare* L. subsp. *hirtum* (Link.) Ietswaart)**

Wpływ dolistnego stosowania biostymulatorów na plon i jakość ziela greckiego  
oregano (*Origanum vulgare* L. subsp. *hirtum* (Link.) Ietswaart)

**Abstract.** The field experiment was carried out on a private farm in Trębanów, Świętokrzyskie Province, in 2020–2022. The aim of the study was to determine the effect of selected biopreparations on the morphological traits of plants, the size and structure of yields, and the content and yield of Greek oregano essential oil. Natural biostimulants Stimplex (2 dm<sup>3</sup> ha<sup>-1</sup>), Tecamin Max (1.5 dm<sup>3</sup> ha<sup>-1</sup>), and Kendal (1 dm<sup>3</sup> ha<sup>-1</sup>) were used in the study. The results demonstrated positive effects of the foliar application of the biostimulants on the morphological, performance, and quality parameters of Greek oregano. The most effective treatment was the application of the biostimulant containing extracts of *Ascophyllum nodosum algae* (Stimplex), followed by the preparation containing amino acids (Tecamin Max). They increased the plant growth rate, the number of lateral branches, and the raw material yield and had a positive effect on the content of essential oil. The size and quality of the Greek oregano yield also depended on the age of the plants and varied between the study years. Higher raw material and oil yields were obtained from two-year plantations. The drought in 2022 limited plant growth and resulted in a lower yield; nevertheless, the essential oil content in the herb increased in these conditions.

**Keywords:** yield, essential oil, seaweed, *Ascophyllum nodosum*, amino acids

## INTRODUCTION

The genus *Origanum* (family Lamiaceae) comprises 43 species widely distributed in the Mediterranean, Euro-Siberian, and South-Asian regions [Skoula and Harborne 2002]. The *Origanum vulgare* L. species includes six subspecies (subsp. *vulgare*, *glandulosum*, *gracile*, *hirtum*, *virens*, and *viridulum*), which differ in both their morphological features and the chemical composition [Morshedloo et al. 2018, Euro+Med 2024]. In Poland, oregano raw material is mainly obtained from *Origanum vulgare* L. subsp. *vulgare* (common oregano), which grows in natural sites or is cultivated [Nurzyńska et al. 2012, Kosakowska and Czupa 2018]. In turn, *Origanum vulgare* L. subsp. *hirtum* (Link) Ietswaart, so-called Greek oregano, is used most frequently as a spice and medicinal plant worldwide [Sarrou et al. 2017, Kosakowska et al. 2019, Alekseeva et al. 2020]. Greek oregano is a perennial herbaceous plant growing in the Mediterranean basin and North Africa. In these regions, the raw material is obtained from natural sites and plantations [Vokou et al. 1993, Skoufogianni et al. 2019]. Recent studies indicate the possibility of cultivation of this subspecies in the moderate climate of Central Europe [Grevsen et al. 2009, Baranauskiene et al. 2013, Kosakowska et al. 2019, Król et al. 2020, Węglarz et al. 2020].

Greek oregano is rich in essential oil (approximately 5%), with carvacrol and thymol as its main components. Additionally, it contains non-volatile phenolic compounds, e.g. flavonoids (luteolin and apigenin), phenolic acids (dominated by rosmarinic and lithospermic acids), tannins, resins, bitter substances, and sterols [Grevsen et al. 2009, Lukas et al. 2015, Gutiérrez-Grijalva et al. 2017, Król et al. 2019, Panagiotidou et al. 2024]. This raw material exhibits various pharmacological activities, e.g. antimicrobial, antioxidant, expectorant, choleric, antispasmodic, and anticancer effects [Milos et al. 2000, Kosakowska et al. 2021, Jafari Khorsand et al. 2022]. Oregano extracts have been used in medicine in the treatment of gastrointestinal disorders, to stimulate bile secretion, and externally in skin and mucous membrane inflammations [Singletary 2010, Chishti et al. 2013]. It is widely used not only in the pharmaceutical industry but also as a culinary spice, food preservative, and flavoring agent [Rodriguez-Garcia et al. 2016, Veenstra and Johnson 2019, Jaiswal et al. 2024].

Healthy and sustainable food production and environmental protection have become the most important priorities in modern agriculture. In this context, new technological solutions are sought to ensure high yields of good quality crops and limit the use of pesticides and mineral fertilizers that have a negative impact on the ecosystem and human health. One of such actions is the use of biostimulant preparations in plant cultivation [Le Mire et al. 2016, Jiang et al. 2024]. As specified by the European Biostimulants Industry Council (EBIC), a biostimulant is a product containing substances and/or microorganisms whose function upon application to plants or the rhizosphere is to stimulate natural processes that enhance the efficiency of nutrient uptake and utilization, tolerance to abiotic stress, and/or crop quality, regardless of the nutrient content in the product [Ricci et al. 2019]. Preparations containing algal extracts, free amino acids, effective microorganisms, and humin and humic acids are being increasingly applied in agriculture [du Jardin 2015]. The action of active ingredients present in these preparations takes place at different metabolic levels, which has a beneficial effect on the absorption, translocation, and utilization of nutrients, thus stimulating plant development and growth. They can also increase plant tolerance to abiotic (drought, frost, salinity) and biotic (pest and pathogen activity) stress conditions

and facilitate regeneration upon the action of stress factors [Calvo et al. 2014, Wozniak et al. 2020, Bell et al. 2022, Mandal et al. 2023, Alvarez et al. 2024, Johnson et al. 2024].

Biostimulants are gaining interest and are increasingly often used in plant cultivation, as evidenced by the doubling of the global biostimulant market in 2015–2023 (1.4 billion dollars and 2.8 billion dollars, respectively). The value of this market is predicted to increase further to 4.6 billion dollars in 2030 [Market Analysis Report 2023]. The effectiveness of biostimulants depends on their type, concentration, application methods (foliar or soil), and crop species and variety [Calvo et al. 2014, Bulgari et al. 2015, Paradiković et al. 2019, Kumari et al. 2023, Sun et al. 2024].

The few available reports on the effect of biostimulants on Greek oregano are focused on the Mediterranean region [Abdali et al. 2023, Amato et al. 2024, Farruggia et al. 2024a]. In turn, there is no information on the use of biostimulants in the cultivation of this species in the moderate climate conditions of Poland. Therefore, the study was undertaken to determine the effect of selected biostimulants on the morphological traits, yield size and structure, and content and yield of essential oil in Greek oregano. Literature data indicate that the use of biopreparations has a positive effect on the yield of herbal plants [Mehrafarin et al. 2015, Elansary et al. 2016, Nassar et al. 2020, Shafie et al. 2021, Rahimi et al. 2022, Velička et al. 2022, Farruggia et al. 2024b, 2024c]; hence, the research hypothesis assumes a positive effect of the preparations used on the yield and quality of the Greek oregano raw material. Based on available reports on the application of biostimulants on Greek oregano [Abdali et al. 2023, Amato et al. 2024, Farruggia et al. 2024a], it was hypothesized that seaweed extract would have the best effect on this species, especially under water stress conditions.

#### MATERIAL AND METHODS

The field study was conducted in 2020–2022 in Trębanów (Świętokrzyskie Province; 50°51'06"N, 21°29'10"E) on a lessive soil developed from loess (good wheat complex, quality class III). The soil in the experimental plots was characterized by high potassium content (175–191 mg K kg<sup>-1</sup> soil), moderate phosphorus (119–134 mg P kg<sup>-1</sup> soil), magnesium (56–68 mg kg<sup>-1</sup> soil), and humus (17–21 g kg<sup>-1</sup>) levels, and neutral reaction (pH in 1 mol KCl 6.9–7.1). The experiment was conducted using the randomized block method in four replications in 6 m<sup>2</sup> plots established on a production plantation managed using the integrated method. In the third ten-day period of April, Greek oregano seeds (obtained from Hild Samen GmbH Marbach am Neckar, Germany) were sown (3 kg ha<sup>-1</sup>) directly into the plot soil using a garden seeder at a row spacing of 40 cm. Root crops or vegetables were the forecrops. All the experimental plots received mineral fertilization: N – 70 kg ha<sup>-1</sup>, P – 60 kg ha<sup>-1</sup>, and K – 50 kg ha<sup>-1</sup>. In the first year of plant growth, nitrogen was applied twice (½ dose before sowing the seeds and ½ three weeks after the plants emerged). In the second year, half of the fertilizer dose was applied in April and the rest was used after the first harvest of the herb. Phosphorus and potassium fertilization was applied at full doses before the establishment of the plantation and in early spring in the second year. Since there are no registered herbicides approved for use in oregano cultivation, the protection against weeds consisted in manual and mechanical removal thereof (hoe, burner). To reduce the risk of fungal diseases, Miedzian 50 WP was used as part of prophylaxis. In 2021, the plants were infected with fungal diseases; therefore, they were sprayed with the Caspara 400 SC fungicide (1 dm<sup>3</sup> ha<sup>-1</sup>).

The use of the biostimulants was the experimental factor (dose per one spray): Stimplex ( $2 \text{ dm}^3 \text{ ha}^{-1}$ ), Tecamin Max ( $1.5 \text{ dm}^3 \text{ ha}^{-1}$ ), and Kendal ( $1 \text{ dm}^3 \text{ ha}^{-1}$ ). The detailed composition of the preparations used in the experiment is presented in Table 1. All the biostimulants were applied as foliar sprays. One-year-old plants were sprayed twice: in the phase of intensive vegetative growth (third ten days of June) and after another three weeks. Three spray treatments were applied in the two-year plantations: in the phase of vegetative growth (third ten days of April), after another three weeks, and after the first harvest (first-second ten days of July). The control plants were sprayed with pure water at the same time points.

Table 1. Components of the biostimulants used in the experiment ( $\text{g } 100 \text{ g}^{-1}$ )

Name of biostimulants	Composition of preparations
Stimplex (Arramara Teoranta; Irlanda) *	extract of seaweed <i>Ascophyllum nodosum</i> ; amino acids 3–6; lipid – 1; Alginic acid 12–18; Mannitol 5–6; carbohydrates 10–20; cytokinin 0.01; mikroelements (Fe, Zn)
Tecamin Max (Agri Tecno Co, Spain.)	total amino acids 14.4, free L – amino acids 12, organic substances 60 (phytohormones, organic acids, oligosaccharides, enzymes), nitrogen 7
Kendal (Valagro SpA, Italy)	total nitrogen 3.5; potassium oxide ( $\text{K}_2\text{O}$ ) – 15.5; organic carbon (C) 3.0; oligosaccharides; glutathione; saponins

\* Maker

The herb from the one-year plantation was harvested once (third ten days of September) when approximately 40–50% of plants were in the flowering phase (the flowering was incomplete due to the short vegetation period). The two-year-old plants were harvested twice when 80–90% of the plants were flowering (second half of June and first-second ten days of September). Before harvesting, 24 plants underwent biometric measurements of the height and the number of lateral shoots. The harvested herb was dried at a temperature of  $35^\circ\text{C}$  in a dryer with forced air circulation. Subsequently, the yield of the air-dried herb and the percentage of the herbal raw material (leaves and inflorescences) and stems were determined (morphological plant parts were separated from 3-kg samples of dried herb taken from each object and their proportion was calculated). Next, samples of the herbal raw material were taken to determine the content of essential oil using the pharmacopoeial method [Farmakopea Polska XI 2017]. The numerical data were processed statistically using the variance analysis method and verified with the Tukey test at a significance level of  $\alpha = 0.05$ .

## RESULTS AND DISCUSSION

The weather conditions in the study years were determined from data on the precipitation sum and average air temperatures (Fig. 1). The humidity and thermal conditions in the study years varied and had an impact on the Greek oregano growth and yield. The most

favorable weather conditions were recorded in the 2020 growing season, with the precipitation sum (377 mm) and average temperature (15.5°C) close to long-term averages (LTAs). Moreover, during the intensive growth period (June–August), the air temperature was optimal for the oregano growth and development, i.e. in the range of 18–22°C, as reported by Kyriakos et al. [2020]. Such conditions promoted the intensive oregano growth and contributed to the high yields of the raw material. In 2021, the highest precipitation sum (435 mm) was recorded during the growing season, whereas the temperature had the lowest values (average 13.9°C), compared to the other vegetation seasons. The low temperatures in April and May 2021 delayed the emergence and initial development of Greek oregano plants. In August, the particularly high precipitation together with low air temperatures promoted the development of fungal infections of the plants. The 2022 growing season was characterized by low precipitation levels (precipitation sum 258 mm); combined with high temperatures (average 16.7°C), they had an adverse effect on the growth and yield of the plants.

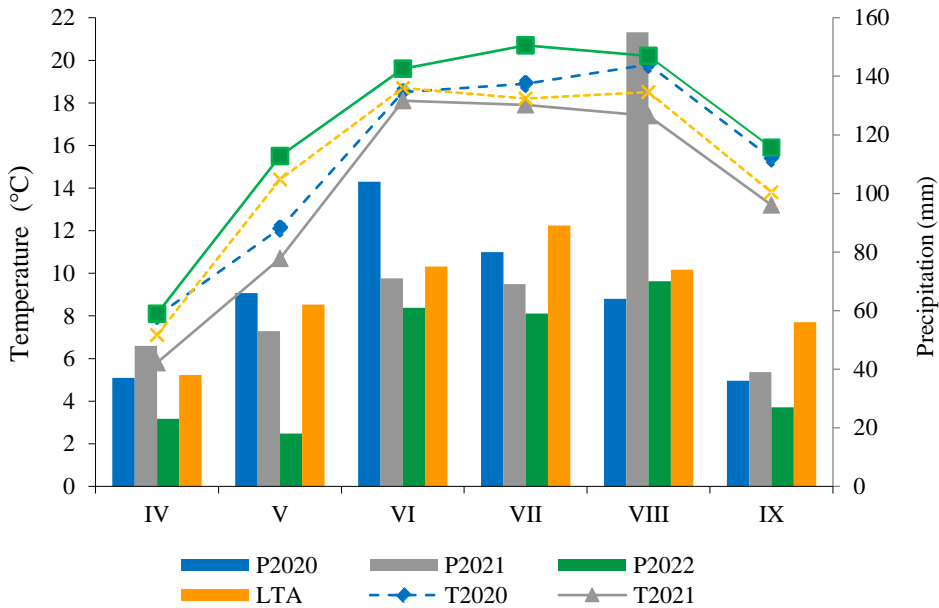


Fig. 1. Comparison of average air temperature and precipitation sums in 2020–2022 with the 1981–2010 long-term averages (LTAs)

Regardless of the biostimulants used, the Greek oregano plants reached a height of 30.8 cm in the first year of growth and produced on average 13.2 lateral shoots. In the second year, the plants were higher (35.4 cm) and produced a greater number of lateral shoots (18.3) – Table 2. These values were comparable to those reported by Król et al. [2020] and Węglarz et al. [2020], who conducted their research in the climatic conditions of Poland. Oregano plants growing in the Mediterranean region were higher and more intensely branched [Dordas 2009, Sarrou et al. 2017, Gonceariuc et al. 2021].

Table 2. Greek oregano plant height and number of shoots

Biostimulants	1-year old plants			2-years old plants		
	2020	2021	mean	2021	2022	mean
plant height (cm)						
Stimplex	35.7	31.5	33.6 <sup>a</sup>	40.9	36.5	38.7 <sup>a</sup>
Tecamin Max	34.9	30.9	32.9 <sup>a</sup>	39.7	35.1	37.4 <sup>a</sup>
Kendal	31.3	27.2	29.3 <sup>b</sup>	35.9	31.5	33.7 <sup>b</sup>
Control	29.2	25.5	27.4 <sup>b</sup>	34.1	29.4	31.8 <sup>b</sup>
Mean	32.8 <sup>A</sup>	28.8 <sup>B</sup>	30.8	37.7 <sup>A</sup>	33.1 <sup>B</sup>	35.4
HSD <sub>(0.05)</sub> for:	BS – 2.81 Y – 2.75 BS × Y – 2.27			BS – 2.12 Y – 2.31 BS × Y – 2.38		
number of shoots (unit per plant)						
Stimplex	15.7	12.9	14.3 <sup>a</sup>	22.3	17.3	19.8 <sup>a</sup>
Tecamin Max	15.4	12.3	13.9 <sup>a</sup>	21.8	16.6	19.2 <sup>a</sup>
Kendal	14.1	11.1	12.6 <sup>b</sup>	20.5	14.7	17.6 <sup>b</sup>
Control	13.5	10.5	12.0 <sup>b</sup>	19.5	13.8	16.7 <sup>b</sup>
Mean	14.7 <sup>A</sup>	11.7 <sup>B</sup>	13.2	21.0 <sup>A</sup>	15.6 <sup>B</sup>	18.3
HSD <sub>(0.05)</sub> for:	BS – 0.84 Y – 0.82 BS × Y – 0.93			BS – 0.99 Y – 1.09 BS × Y – 0.96		

BS – biostimulants; Y – years; BS × Y – interaction

Mean values indicated by the same letters are not statistically significant at the 0.05 level, according to Tukey's test

The application of the biostimulants had a beneficial effect on the morphometric traits of Greek oregano (Tab. 2). The greatest plant height and number of lateral shoots were recorded after the use of the biostimulant containing algal extracts (Stimplex). This was associated with the presence of natural growth hormones (auxin and cytokinin), which stimulate cell division and contribute to an increase in plant height [Marhoon and Abbas 2015]. An increase in the number of lateral shoots may also be caused by the presence of cytokinins, which stimulate cell division and contribute to the growth of lateral shoots and plant branching [Wu and Lin 2000]. As shown by the results of the study, the biostimulant

containing amino acids (Tecamin Max) had a significant effect on the morphological parameters of Greek oregano. Amino acids can directly or indirectly influence plant growth and development, e.g. they are involved in the biosynthesis of hormones [Kawade et al. 2023]. The amino acid tryptophan is a precursor of the synthesis of auxin, which regulates the process of cell division and elongation [Zhao 2012]. Phenylalanine participates in the synthesis of gibberellins, which play an important role in the elongation of internodes and support the initiation and emergence of lateral buds, thereby increasing the height of the plant and the number of shoots [Bajguz and Piotrowska-Niczyporuk 2023]. Similarly, an increase in the height and number of shoots was found in the Kendal-treated objects; however, these differences were statistically insignificant.

The average yield of dry Greek oregano herb in the present study was  $1494 \text{ kg ha}^{-1}$  in the first year and  $2461 \text{ kg ha}^{-1}$  in the second year (Tab. 3). Similar yields were reported by Baranauskiene et al. [2013], Król et al. [2020], and Abdali et al. [2023]. In turn, higher yields were obtained by Grevsen et al. [2009], Karamanos and Sotiropoulou [2013], Goncariuc et al. 2021], and Farruggia et al. [2024a]. It is worth emphasizing, however, that the yields of dry Greek oregano herb in other studies [Król et al. 2019] were at the level of  $2198\text{--}4401 \text{ kg ha}^{-1}$  depending on developmental phase of plants. The lower yields in the present study were related to the unfavorable weather conditions prevailing in 2021–2022.

The beneficial effect of the biostimulants on the morphological traits of the plants was manifested in an increase in the dry mass yield (Tab. 3, Fig. 2). A similar effect of the biostimulants used was noted in both growing seasons. The highest dry herb yield was achieved in the Stimplex spraying variant. It was slightly lower after the application of Tecamin Max, and the lowest and statistically insignificant increase in the dry mass of the plants (compared to the control object) was found after the Kendal treatment, with the exception of 2021, when a significant increase was caused by the application of the biostimulant to the two-year-old plants.

The raw material (leaves and inflorescences) and waste stems, in which the lowest amounts of essential oil accumulate [Morshedloo et al. 2017], were obtained after rubbing and cleaning the herb. In the first year of the Greek oregano growth, the average raw material yield was  $953 \text{ kg ha}^{-1}$ . In the second year, the average yield was  $1422 \text{ kg ha}^{-1}$  (Tab. 3, Fig. 2), which was comparable to the results reported by Król et al. [2020], Węglarz et al. [2020], and Amato et al. [2024] but lower than in the studies conducted by Grevsen et al. [2009], Giannoulis et al. [2020], and Kyriakos et al. [2020]. The most beneficial results were obtained after the application of Stimplex (an average yield increase of  $320 \text{ kg ha}^{-1}$  compared to the control), a slightly weaker effect was exerted by the Tecamin Max biostimulant (an increase of  $212 \text{ kg ha}^{-1}$ ), and the lowest effect was found in the objects sprayed with Kendal (an increase of  $111 \text{ kg ha}^{-1}$ ).

Many authors have confirmed the beneficial effect of biostimulants containing *Asco-phyllum nodosum* algal extracts on the morphological traits and yields of herbal plants. In studies on Greek oregano [Abdali et al. 2023, Amato et al. 2024, Farruggia et al. 2024a], the application of a biostimulant had a beneficial effect on the morphological parameters of plants and increased their mass. A similar plant response was reported in studies on yarrow [Shafie et al. 2021], thyme [Rahimi et al. 2022], mint and basil [Elansary et al. 2016], rosemary and sage [Farruggia et al. 2024b, 2024c], marjoram [Nassar et al. 2020, Król 2023], and hyssop [Pirani et al. 2020]. Such a beneficial effect is attributed to compounds present in *Asco-phyllum nodosum* algae, which increase photosynthetic activity and chlorophyll content in plants [De Saeger et al. 2020, Baltazar et al. 2021, Kumari et al. 2023].

Table 3. Yield of Greek oregano dry herb and raw material (leaves and inflorescences)

Biostimulants	1-year old plants			2-years old plants		
	2020	2021	mean	2021	2022	mean
yield of dry herb (kg ha <sup>-1</sup> )						
Stimplex	1922	1291	1607 <sup>a</sup>	3012*	2292	2652 <sup>a</sup>
Tecamin Max	1913	1257	1585 <sup>a</sup>	2924	2256	2590 <sup>a</sup>
Kendal	1675	1154	1415 <sup>b</sup>	2953	1813	2383 <sup>b</sup>
Control	1647	1095	1371 <sup>b</sup>	2646	1794	2220 <sup>b</sup>
Mean	1789 <sup>A</sup>	1199 <sup>B</sup>	1494	2884 <sup>A</sup>	2039 <sup>B</sup>	2461
HSD <sub>(0.05)</sub> for:	BS – 85.9 Y – 97.4 BS × Y – 102.6			BS – 163.7 Y – 168.5 BS × Y – 176.4		
yield of raw material (leaves and inflorescences) (kg ha <sup>-1</sup> )						
Stimplex	1252	952	1102 <sup>a</sup>	1791*	1391	1591 <sup>a</sup>
Tecamin Max	1193	793	993 <sup>b</sup>	1618	1350	1484 <sup>b</sup>
Kendal	1034	754	894 <sup>c</sup>	1652	1112	1382 <sup>c</sup>
Control	955	692	824 <sup>c</sup>	1411	1051	1231 <sup>d</sup>
Mean	1109 <sup>A</sup>	798 <sup>B</sup>	953	1618 <sup>A</sup>	1226 <sup>B</sup>	1422
HSD <sub>(0.05)</sub> for:	BS – 89.5 Y – 93.7 BS × Y – 101.2			BS – 96.2 Y – 101.2 BS × Y – 122.6		

\* Data represents means of two harvest

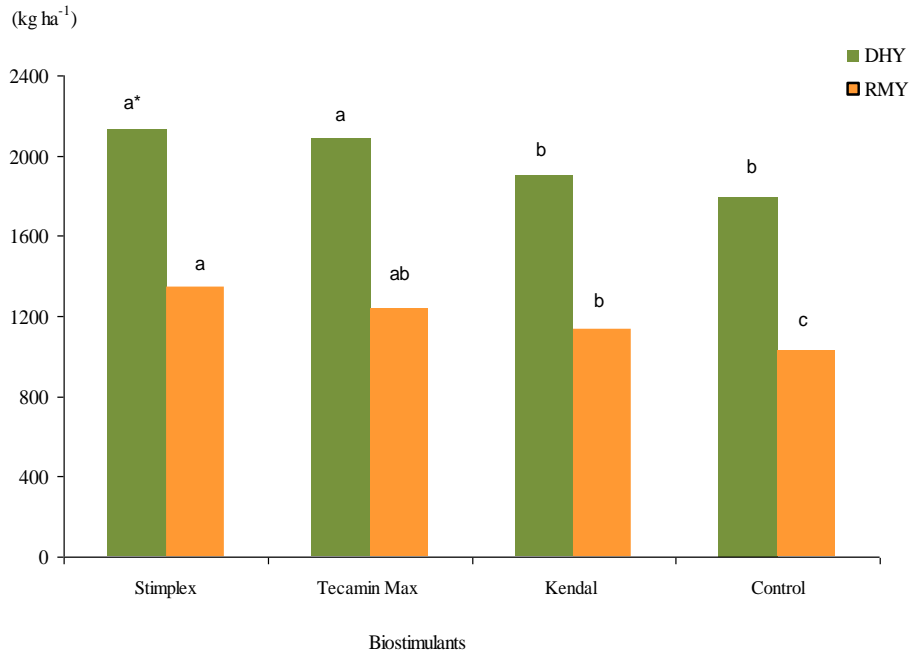
BS – biostimulants; Y – years; BS × Y – interaction

Mean values indicated by the same letters are not statistically significant at the 0.05 level, according to Tukey's test

Plant response to amino acid-containing biostimulants depends on the species and variety. Their beneficial effect on morphological traits and yields has been demonstrated in studies on Greek oregano [Amato et al. 2024], yarrow [Shafie et al. 2023], lemon balm [Mehrafarin et al. 2015], sage [Farruggia et al. 2024c], mint [Velička et al. 2022], rosemary [Farruggia et al. 2024b], and thyme [Rahimi et al. 2022]. As reported by Mezeyova et al. [2022], the effect of the Tecamin Max biostimulant on basil depended on the plant variety. Similar results were obtained in a study on chamomile [Omrani et al. 2023]. In contrast, Majkowska-Gadomska et al. [2022] did not observe an increase in the growth



and yield of savory, marjoram, and lemon balm plants after the application of biostimulants containing amino acids.

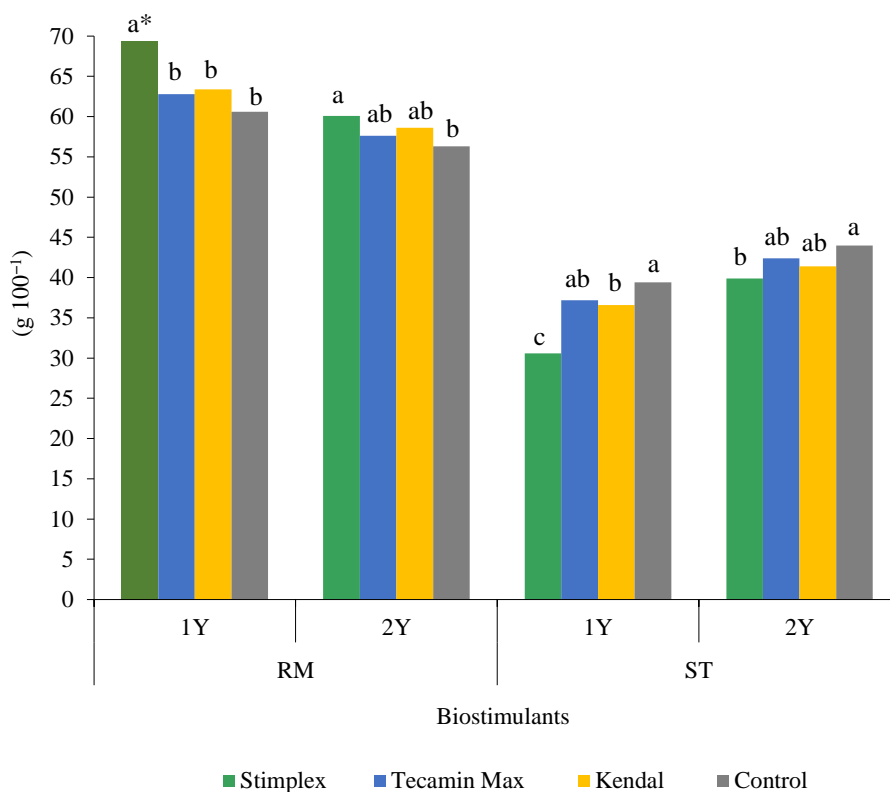


\* the same letters indicate no significant differentiation

Fig. 2. Dry herb yields (DHY) and raw material yields (leaves and inflorescences – RMY) of Greek oregano depending on the biostimulants (irrespective of year of vegetation)

The Kendal preparation stimulated the growth and yield of thyme [Król and Kiełtyka-Dadasiewicz 2019], blueberry [Chitu et al. 2012], and peppers [Paradiković et al. 2012]. In turn, Król [2023] reported no significant increase in the yield of marjoram herb after the application of the Kendal biostimulant.

The analysis of the parameter that directly determines the raw material yield, i.e. the share of rubbed herb in the yield, showed a significant increase in the value of this trait only in the Stimplex biostimulant application variant, compared to the control, which may indicate that this biostimulant improved plant foliage (Fig. 3). This was also confirmed in other studies [Amato et al. 2024, Farruggia et al. 2024a], which demonstrated higher proportions of oregano leaves and inflorescences after treatment with a biostimulant containing extracts from *Ascophyllum nodosum* algae. The analysis of the amounts of leaves and stems depending on plant age showed a greater share of leaves in the case of the one-year-old plants. The amount of leaves decreased and the share of stems increased with the age of the plants, which is consistent with the results reported by Farruggia et al. [2024a].



\* the same letters indicate no significant differentiation

RM – raw material; ST – stems; 1Y – 1-year-old plants; 2Y – 2-year-old plants

Fig. 3. Effect of biostimulants on the share of Greek oregano raw material (leaves and inflorescences) and stems

In the present study, the oregano plant growth and biomass production depended on the atmospheric conditions prevailing during the vegetation period. The smallest and least intensely branched plants were observed in 2022 (Tab. 2), hence the decrease in the plant dry mass and the raw material yield, while the highest yields were recorded in 2020 (Tabs 2 and 3). There was also an interaction between the study years and the preparations used. In the dry and hot season of 2022 when the plants were exposed to water shortage stress, the best results (compared to the control object) were obtained using the biostimulants containing extracts of seaweed and amino acids, as the bioactive compounds present in these formulations improved plant performance in stress conditions [Drobek et al. 2019, Shukla et al. 2019, Rahimi et al. 2022, Abdali et al. 2023]. As reported by Greek oregano growers and shown in this study, one of the problems of cultivation of this plant in colder climate conditions is the occurrence of fungal diseases, as recorded in 2021. The application of the Kendal and Stimplex biostimulants in the 2021 growing season reduced the prevalence of fungal diseases and increased the biomass yield. As demonstrated in various

studies, both preparations increase plant resistance to pathogen attack [Jayaraman et al. 2011, Paradiković et al. 2012, Sidhu and Nandwani 2017].

Table 4. Essential oil content and yield in Greek oregano raw material

Biostimulants	1-year old plants			2-years old plants		
	2020	2021	mean	2021	2022	mean
essential oil content (ml 100g <sup>-1</sup> DW)						
Stimplex	2.61	2.42	2.52 <sup>a</sup>	3.54	3.93	3.74 <sup>a</sup>
Tecamin Max	2.55	2.39	2.47 <sup>a</sup>	3.46	3.98	3.72 <sup>a</sup>
Kendal	2.29	2.15	2.22 <sup>b</sup>	3.02	3.43	3.23 <sup>b</sup>
Control	2.31	2.16	2.24 <sup>b</sup>	3.14	3.54	3.34 <sup>b</sup>
Mean	2.44 <sup>A</sup>	2.28 <sup>B</sup>	2.36	3.29 <sup>B</sup>	3.72 <sup>A</sup>	3.51
HSD <sub>(0.05)</sub> for:	BS – 0.154 Y – 0.182 BS × Y – 0.193			BS – 0.226 Y – 0.263 BS × Y – 0.287		
essential oil yield (dm <sup>3</sup> ha <sup>-1</sup> )						
Stimplex	32.7	23.0	27.9 <sup>a</sup>	63.4	54.7	59.0 <sup>a</sup>
Tecamin Max	30.4	19.0	24.7 <sup>b</sup>	55.7	53.7	54.7 <sup>b</sup>
Kendal	23.7	16.2	19.9 <sup>c</sup>	50.5	38.1	44.3 <sup>c</sup>
Control	22.1	14.9	18.5 <sup>c</sup>	44.3	37.2	40.8 <sup>d</sup>
Mean	27.2 <sup>A</sup>	18.3 <sup>B</sup>	22.7	53.5 <sup>A</sup>	45.9 <sup>B</sup>	49.7
HSD <sub>(0.05)</sub> for:	BS – 1.49 Y – 1.36 BS × b – 1.54			a – 2.85 b – 3.37 a × b – 3.64		

BS – biostimulants; Y – years; BS × Y – interaction

Mean values indicated by the same letters are not statistically significant at the 0.05 level, according to Tukey's test

Essential oil is the main active substance in the Greek oregano raw material. Previous studies demonstrated that its content depended on environmental and genetic factors, phenological growth phases, and agronomic practices [Vokou et al. 1993, Kokkini et al. 1994, Tibaldi et al. 2011, Baranauskiene et al. 2013, Sarrou et al. 2017, Król et al. 2019, Ninou et al. 2021]. The raw material obtained in the present study contained different amounts of essential oil, depending on the age of the plants, the biostimulant used, and the year of the study (Tab. 4). The one-year-old plants accumulated lower amounts of essential oil (on average  $2.36 \text{ ml } 100 \text{ g}^{-1}$ ) than those from the two-year cultivation (on average  $3.51 \text{ ml } 100 \text{ g}^{-1}$ ). This can be explained by the lower share of flowers, which accumulate greater amounts of essential oil than leaves, in the raw material [Król et al. 2019, Kyriakos et al. 2020]. It was found that the application of Stimplex and Tecamin Max increased the content of essential oil, compared to the control object (by  $0.34$  and  $0.31 \text{ ml } 100 \text{ g}^{-1}$ , respectively). Similarly, other authors reported a positive effect of biostimulants containing brown algae and amino acids on the content of essential oil in Greek oregano herb [Abdali et al. 2023, Farruggia et al. 2024a]. In studies on other herbal plants, the use of biostimulants increased its concentration in mint and basil [Elansary et al. 2016], rosemary [Tawfeeq et al. 2016], savory [Mehrabi et al. 2013], and thyme [Rahimi et al. 2022]. Different results were obtained by Amato et al. [2024], who reported a decrease in the content of essential oil in Greek oregano raw material upon the application of a biostimulant containing *Ascophyllum nodosum* algae. In turn, there was slight decrease in the content of essential oil in the Greek oregano herb in the Kendal-sprayed objects, which is consistent with studies on thyme and marjoram [Król and Kiełtyka-Dadasiewicz 2019, Król 2023]

The accumulation of essential oil also depended on the weather prevailing in the study years. Its lowest content was found in the cold year 2021, while the highest accumulation was recorded in the dry and hot season in 2022 when the plants were exposed to water shortage stress (tab. 4). Greater amounts of metabolites are produced in plants in response to water shortage stress, and these substances prevent oxidation in cells [Laftouhi et al. 2023, Skrypnik et al. 2024]. This was confirmed in other studies [Azizi et al. 2009, Ninou et al. 2017, Abdali et al. 2023], which showed that water deficiency during vegetation caused an increase in the content of essential oil in Greek oregano herb. A significantly higher yield of essential oil was obtained in the objects treated with the tested preparations, compared to the control, which was associated with both its higher content in the raw material and the higher raw material yields.

## CONCLUSIONS

1. The results of this study revealed positive effects of the foliar application of the biostimulants on the morphological, performance, and quality parameters of Greek oregano.
2. All the biostimulants increased the growth of the plants, number of lateral branches, and raw material yields, compared to the control object. The application of the biostimulant containing algal extracts (Stimplex) and amino acids (Tecamin Max) was the most effective approach. In the water stress condition and fungal diseases, the best results were obtained using the biostimulant containing extracts of seaweed.
3. The biostimulants used (except for Kendal) had a positive effect on the content of essential oil in the raw material and, consequently, on its yield per unit area.

4. The size and quality of the Greek oregano yield also depended on the age of the plants and varied between the study years. Higher raw material yields and essential oil content and yields were obtained from the two-year plantations. The water deficiency observed during the vegetation period in 2022 limited plant growth, which resulted in lower raw material yields but increased the essential oil content.
5. The research results suggest that biostimulants can be part of a good practice used to maximize the efficiency of Greek oregano cultivation, especially in the event of abiotic (drought) and biotic (pathogens) stresses.

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