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# Foliar application of plant growth regulators increases the production of biomass, content, yield and chemical constituents of *Origanum vulgare* L. essential oil

Aplicação foliar de reguladores de crescimento de plantas aumenta a produção de biomassa, conteúdo, rendimento e constituintes químicos do óleo essencial de *Origanum vulgare* L.

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*Origanum vulgare* is used in the prevention of neurodegenerative diseases; for feeding poultry, fish, and cattle; and in the food industry. The objective of this study was to evaluate the influence of foliar application of plant growth regulators (PGRs) on biomass, secondary metabolites and antioxidant enzymatic activity. The experiment had a double-factorial design with a control, 3 types of regulators and 3 doses. After 90 days, the dry weight, essential oil content and photosynthetic pigments were measured. The effects of PGRs on the antioxidative system and the activities of several antioxidant enzymes, suchas superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX), were investigated. With the use of PGRs, the increase in branch length was greater than that in the control. Compared with the control, the application of 25 mg/L GA3 increased the leaf, stem, root, shoot and total dry weights. The highest chlorophyll a content was observed with the KIN25 dose. The oil content gains from the KIN25 and IBA25 doses were 30.9 and 29.6%, respectively, compared to the control. The highest thymol content was observed in the plants that received the GA350 dose (24.80%), which was 12.14% greater than that in the control. The lowest mean hydrogen peroxide ($H_2O_2$) and malondialdehyde (MDA) levels were observed in the presence of thePGRs. The PGRs stimulated enzymatic antioxidant activity, decreasing oxidative stress signaling. These findings suggest that foliar application of PGRs at low doses may contribute to increased biomass and active ingredient levels.
Keywords: medicinal plants, secondary metabolite, phytoregulator.

*Origanum vulgare* é utilizado na prevenção de doenças neurodegenerativas; para alimentação de aves, peixes e bovinos; e na indústria alimentícia. O objetivo deste estudo foi avaliar a influência da aplicação foliar de reguladores vegetais (RV) na biomassa, metabólitos secundários e atividade enzimática antioxidante. O experimento foi conduzido em um delineamento fatorial duplo com um controle, 3 RV e 3 doses. Após 90 dias, foram mensurados o peso seco, conteúdo de óleo essencial e pigmentos fotossintéticos. Os efeitos dos RV no sistema antioxidante e em algumas atividades enzimáticas antioxidantes, como superóxido dismutase, catalase e ascorbato peroxidase, foram investigados. O uso de RV aumentou o ganho no comprimento dos ramos em relação ao controle. A aplicação de GA3 na dose de25 mg $L^{-1}$ aumentou o peso seco da folha, caule, raiz, broto e peso seco total em comparação com o controle.O maior teor de clorofila a foi observado para a dose KIN25. Os ganhos de teor de óleo das doses KIN25e IBA25 foram de 30,9% e 29,6%, respectivamente, em comparação com o controle. O maior teor de timolfoi observado em plantas que receberam a dose GA350 (24,80%), o que foi 12,14% maior em comparaçãocom o controle. As menores médias de peróxido de hidrogênio e malondialdeído (MDA) foram observados na presença dos RV. Os RV estimularam a atividade antioxidante enzimática, diminuindo o sinal de estresse oxidativo. Esses achados sugerem que a aplicação foliar de RV em doses baixas pode contribuir para aumentar a biomassa e os ingredientes ativos.
Palavras-chave: plantas medicinais, metabólito secundário, fitorregulador.

## 1. INTRODUCTION

Commonly known as oregano, *Origanum vulgare* L. (Lamiaceae) originated and is widely distributed in the Mediterranean, Euro-Siberian and Iranian-Turanian regions [1]. This species has nutritional importance and is a widely appreciated natural condiment. At the industrial level, it is used in perfumery and personal hygiene, whereas in medicine, it is used in the preparation of anesthetics, sedatives, antispasmodics, antirheumatics and expectorants [2].

The global production of oregano generates an approximate revenue of US $22.5 million [3]. Oregano essential oil has great value because it has antimicrobial, antiradical, and acaricidal activities and plays a role in the prevention of phytopathogen infection [4-6]. In addition, oregano can beused as an antidiabetic and food supplement [7]; in the prevention of neurodegenerative diseases [8]; in the feed of poultry, fish, and cattle [9]; and in the food industry [10].

The characteristics of oregano are not the same year-round. The plant undergoes changes, and therefore, the concentration of active ingredients may vary seasonally. Among the various abiotic and biotic factors, such as fertilization [11], drying [12], irrigation [13], ontogeny [14] and genetics [15], that affect the production of essential oils, the use of growth regulators has been highlighted in recent years as a widely applicable tool for improving the productionof secondary metabolites in medicinal plants [16].

Plant growth regulators (PGRs), also known as plant hormones, are natural or synthetic products that affect development and metabolic processes in plants [17, 18]. Sharafzadeh and Zare (2011) [19] reported that the use of growth regulators can influence the production of essential oils through effects on plant growth (biomass of leaves or flowers), essential oil biosynthetic pathways and the number of oil storage structures. In addition, the timing of exogenous application and the type of growth regulator applied can also affect the essential oil content. According to the same authors, among the main growth regulators used to influence the production of secondary metabolites in species of the Lamiaceae family, auxins, cytokinins and gibberellins stand out.

Studies have shown that the application of indole-3-butyric acid (IBA) and gibberellic acid (GA3) improved the phytochemical characteristics of the species *Thymus vulgare* L. [20]. The use of IBA improved vegetative growth, the yield of chalices and the phytochemical parameters of *Hibiscus sabdariffa* L. [21]. Hasan-Beigi et al. (2021) [22] reported that the foliar action of gibberellic acid (GA3) along with salicylic acid increased the dry biomass and essential oil content in *Echinacea purpúrea* (L.) Moench. Khan et al. (2022) [23] reported that foliar application of kinetin (KIN) provided a relatively high yield of essential oil and active constituents in the species *Mentha arvensis* L.

In this context, the objective of this study was to observe the influence of foliar applicationof plant growth regulators on the production of biomass and secondary metabolites and antioxidant enzymatic activity in *O. vulgare* L.

## 2. MATERIAL AND METHODS

### 2.1 Study area and seedling production

The experiment was carried out in the greenhouse of the Tissue Culture Laboratory of the Department of Agriculture (DAG) of the Federal University of Lavras, Brazil, located at 21° 14'S and 45° 00'W at an altitude of 918 m. The specimen material of the species has already been deposited in the ESAL Herbarium of the Department of Biology at UFLA (nº 22.156).

Seedlings were obtained via vegetative propagation of apical cuttings of parent plants (5–7 cm). The cuttings were rooted in 128-cell Styrofoam trays with the commercial substrate Basaplant®. After the cuttings had rooted (30 days), the plants were transplanted to pots (10 L) containing a mixture of dystrophic red Latosol and sand (2:1) and 300 g of cattle manure per pot. The chemical characteristics of the substrate (2:1 soil + 300 g of cattle manure) were as follows: pH in water = 5.4; K, Na (mg $dm^{-3}$) = 217.25, 72; P-Rem (mg $L^{-1}$) = 17; $Ca^{2+}$, $Mg^{2+}$, $Al^{3+}$, H+Al (cmolc $dm^{-3}$) =

2.53, 0.72, 0.10, 1.30; base saturation index (V%) = 74.5; organic matter (dag $kg^{-1}$) = 2.28; Zn, Fe, Mn,Cu, B and S (mg $dm^{-3}$) = 2, 34, 13.50, 1.47, 0.22, 11.20.

## 2.2 Experimental design

The experimental design was completely randomized in a double factorial scheme (3 types of regulators × 3 doses + 1 control) and four replicates; each replicate contained four plants, totaling 160 experimental units distributed in 10 treatments, as shown in Table 1.

*Table 1: Description and abbreviations of each treatment used in the experiments. $GA_3$ (gibberellic acid); IBA (indole-3-butyric acid); KIN (kinetin).*

| No. | Treatment description | Abbreviationused |
|---|---|---|
| **T1** | Gibberellic acid 25 mg $L^{-1}$ | GA325 |
| **T2** | Gibberellic acid 50 mg $L^{-1}$ | GA350 |
| **T3** | Gibberellic acid 100 mg $L^{-1}$ | GA3100 |
| **T4** | Indole-3-butyric acid 25 mg $L^{-1}$ | IBA25 |
| **T5** | Indole-3-butyric acid 50 mg $L^{-1}$ | IBA50 |
| **T6** | Indole-3-butyric acid 100 mg $L^{-1}$ | IBA100 |
| **T7** | Kinetin 25 mg $L^{-1}$ | KIN25 |
| **T8** | Kinetin 50 mg $L^{-1}$ | KIN50 |
| **T9** | Kinetin 100 mg $L^{-1}$ | KIN100 |
| **T10** | Control - $H_2O$ + Tween 80 (0.05%) | Control |

On the day of application, 500 mL of stock solution was prepared for each PGR ($GA_3$, IBA and KIN); from this solution, dilutions (25, 50 and 100 mg $L^{-1}$) were prepared.After each dilution was prepared, Tween 20 (0.05%) was added to the solutions for all the treatments and the control, and 200 mL of the final solution was sprayed on the plants to the point of dripping.

Three applications were performed. The first and second applications were performed 30 and 60 days after transplanting, respectively. The third application occurred 72 hours before harvest (87 days after transplanting) during the 90-day cycle of cultivation of the species. Plant irrigation was performed two to three times a week, maintaining the field capacity between 80% and 90%. The temperature and humidity (Figure 1) were measured with a Datalogger Elitech® during the 90 days of oregano cultivation.

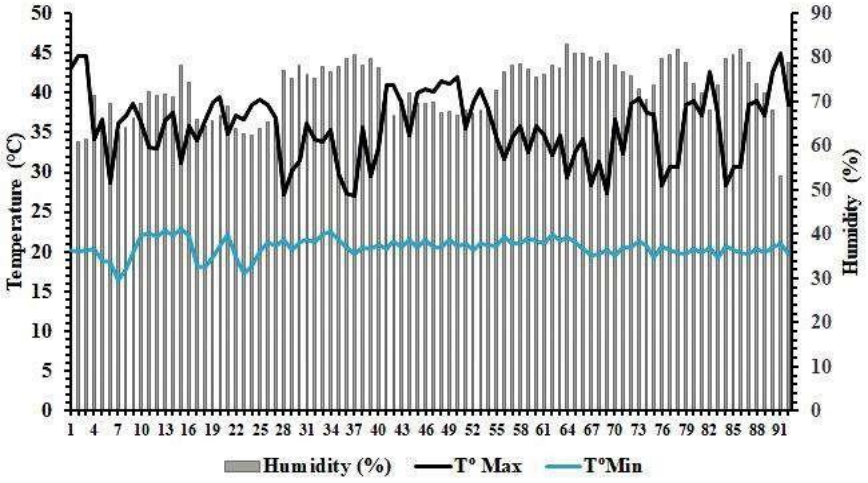


*Figure 1: Daily averages of temperature and humidity during the 90 days of oregano cultivation in a greenhouse using a Datalogger Elitech®.*

### 2.3 Growth analysis

At 90 days after transplanting, the length of the longest branch was measured via a tape measure, and the results are expressed in cm. To obtain dry matter, the leaves, stems and roots were placed on Kraft paper and kept in a forced-air oven at 40°C until a constant weight was reached. Each part of the plant was subsequently weighed on an analytical balance, and the result was expressed in grams (g). The parameters evaluated were the leaf (LDW), stem (SDW), root (RDW), shoot (ShDW) and total (TDW) dry weights and the root–shoot ratio (R/Sh).

### 2.4 Photosynthetic pigments

The photosynthetic content was extracted and analyzed via methods developed by Hiscox and Israelstam (1979) [24] and Barnes et al. (1992) [25]. Fresh leaves of oregano (50 mg)were weighed and incubated in 10 mL of dimethyl sulfoxide (DMSO) saturated with calcium carbonate ($CaCO_3$) according to methods described by Santos et al. (2015) [26]. The samples were subsequently placed in an oven at 65°C for 48 h.

The samples were prepared in quintuplicate, and three aliquots (3 mL) of each replicate were transferred to a quartz cuvette; the optical density values were read in a TECAN INFINITY M200 PRO spectrophotometer at 480, 649 and 665 nm. The spectrophotometer was operated in the I - Control® data processing system (version 3.37) against a blank sample containing DMSO. The specific optical density value recorded for each sample was calculated on the basis of the average of the readings for the aliquots.

The wavelengths and equations adopted for these calculations were based on the methodology of Wellburn (1994) [27]:

Chlorophyll *a* 649 = (12.47 × A665) - (3.62 × A649); chlorophyll *b* 665 = (25.06 × A649) - (6.5 × A665); carotenoids 480 = (1000 × A480 - 1.29 × Ca - 53.78 × Cb)/220.Total chlorophyll = chlorophyl*l a* + chlorophyll *b*

### 2.5 Extraction of essential oil

The essential oil was extracted via the hydrodistillation technique in a modified Clevenger apparatus. Forty grams of dry leaves was placed in a 2,000 mL volumetric flask that was then filled with 1,000 mL of distilled water. From the boiling point, the extraction time was fixed at 90 min. Fourextractions were performed for each treatment. The essential oil was weighed, stored in an airtight amber bottle and kept in a refrigerator at 4°C. The essential oil content was expressed in g 100 $g^{-1}$ of leaf dry mass, and the yield was expressed in g $plant^{-1}$.

### 2.6 Chemical analysis of essential oils

Qualitative and quantitative analyses of the essential oil were performed according to De Assis et al. (2020) [28]. Briefly, measurements were performed in triplicate on an Agilent® 7890A gas chromatography (GC) system operated on an MSD CHEM Station Ver. E.02.02.1431 data processing system equipped with a CombiPAL autosampler/injector autosampler system (CTC Analytic AG, Switzerland) and flame ionization detector (FID). Qualitative analyses were performed on an Agilent® MSD 5975C selective mass detector (Agilent Technologies, California, USA) operated by electronic impact ionization at 70 eV in scan mode with a 1.0 s−1 scan speed and mass acquisition interval of 40–400 $mz^{-1}$. Chemical constituents were identified by comparing their retention rates relative to the coinjection of a standard n-alkane solution (C8-C20; Sigma–Aldrich®, St. Louis, USA) via the Van Den Dool and Dec Kratz equation (1963) [29]. Oregano EO constituents were identified by comparison with mass spectra from the NIST/EPA/NHI library database [30] and based on retention rates described in the literature [31].

### 2.7 Extraction and quantification of hydrogen peroxide ($H_2O_2$) and malondialdehyde (MDA)

The extraction of hydrogen peroxide ($H_2O_2$) and malondialdehyde (MDA) (a lipid peroxidation product) was performed according to Biemelt et al. (1998) [32]. A total of 0.2 g of fresh leaves was macerated in liquid $N_2$ with polyvinylpyrrolidone (PVPP). The material was subsequently placed in an Eppendorf tube, homogenized in 1,500 µL of trichloroacetic acid (TCA) at 0.1% (w/v) and centrifuged at 12,000 × g for 15 minutes. The supernatant was separated to obtain the sample solution, which was stored at -20°C until quantitative analyses were performed in quadruplicate.

#### 2.7.1 Hydrogen peroxide ($H_2O_2$)

The mixture was incubated according to Velikova et al. (2000) [33]. A total of 45 µL of 10 mmol $L^{-1}$ potassium phosphate buffer solution (pH 7.0) and 90 µL of potassium iodide were added to each well of a microplate, along with 45 µL of the sample mixture. Readings were taken at 390 nm. The $H_2O_2$ concentration of the samples was calculated on the basis of the linear equation for the curve obtained from the densities, and the results are expressed in mmol $H_2O_2$ $g^{-1}$ FW.

#### *2.7.2 Malondialdehyde (MDA)*

The incubation was performed according to Buege and Aust (1978) [34]. A total of 250 µL of solution with 0.5% thiobarbituric acid (TBA) and 10% trichloroacetic acid (TCA) was added to an Eppendorf tube together with 125 µL of the sample solution. The solution was homogenized and placed in a water bath at 95°C for 30 min. After cooling, 200 µL was used for measurement, which was performed at 535 and 600 nm. The MDA contents were calculated using an extinction coefficient of $1.56 \times 10^{-5}$, and the results are expressed in nmol MDA $g^{-1}$ FW.

### 2.8 Extraction of antioxidant enzymes and enzymatic activity

The extracts used for the enzyme assays (SOD, CAT and APX) were prepared according to Biemelt et al. (1998) [32]. Fresh leaves (0.2 g) were macerated in liquid $N_2$ with polyvinylpyrrolidone (PVPP). The material was subsequently placed in Eppendorf tubes and homogenized with 1,500 µL of extraction buffer solution composed of 375 µL of 400 mM potassium phosphate (pH 7.8),15 µL of 10 mM EDTA, 75 µL of 200 mM ascorbic acid and 1,035 µL of distilled water. The reagent mixture was centrifuged for 10 min at 13,000 × g in an Nl 1801 benchtop microcentrifuge, and the supernatant (sample mixture) was collected and stored in Eppendorf tubes at -20°C until analysis was performed in quadruplicate.

#### *2.8.1 Enzymatic activity of superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX)*

Superoxide dismutase **(**SOD) activity is based on the ability of the enzyme to inhibit the photoreduction of nitrotetrazolium blue (NBT). Activity measurement was performed as described by Giannopolitis and Ries (1977) [35]. Briefly, the incubation buffer solution used consisted of 100 µL of 100 mM potassium phosphate (pH 7.8), 40 µL of 70 mM methionine, 3 µL of 10 µM EDTA, 15 µL of 1 mM NBT, 2 µL of 0.2 mM riboflavin and distilled water and was pipetted into a microplate along with 10 µL of the sample mixture. The plate was then illuminated for 7 min and subsequently read at 560 nm. The results are expressed as U SOD activity $min^{-1}$ $g^{-1}$ FW.

Catalase (CAT) activity measurement was performed as described by Havir and Mchale (1987) [36]. Briefly, 90 µL of buffer solution containing 200 mM potassium phosphate (pH 7.0),

previously incubatedin a water bath at 30°C, and 72 µL of distilled water were pipetted into a microplate along with 9 µL of the sample solution. Then, 9 µL of hydrogen peroxide (250 mM) was added only at the time of reading, which was performed at 240 nm every 15 s for 3 min. The activity was calculated using an extinction coefficient of 36 $M^{-1}$ $cm^{-1}$.

Ascorbate peroxidase (APX) activity measurement was performed as described by Nakano and Asada (1981) [37]. Briefly, 90 µL of 200 mM potassium phosphate buffer solution (pH 7.0), 9 µL of 10 mM ascorbic acid and 63 µL of distilled water were added to the microplate along with 9 µL of the sample solution. Then, 9 µL of hydrogen peroxide (250 mM) was added only at the time of reading, which was performed at 290 nm every 15 s for 3 min. The activity was calculated using an extinction coefficient of 2.8 $M^{-1}$ $cm^{-1}$.

### 2.9 Statistical analysis

The data were subjected to analysis of variance, and the means were compared via the Scott–Knott test ($p < 0.05$) with R software [38]. Principal component analysis (PCA) was performed via Statistica® software, version 10.0 (StatSoft - Tulsa, USA) [39].

## 3. RESULTS AND DISCUSSION

### 3.1 Plant growth analysis

Figure 2 shows the influence of the application of different doses of PGRs on the length of the largest branch of *O. vulgare* L. There was a significant interaction between the types of PGRs ($GA_3$, IBA, KIN) and the doses (25, 50, 100 mg $L^{-1}$) used in oregano cultivation. Gibberellin treatment resulted in longer branch lengths than treatment with the other regulators (IBA and KIN) and the control. The 100 mg $L^{-1}$ $GA_3$ dose yielded a length of 107.9 cm, which significantly differed from the lengths observed with the highest doses of IBA (86.7 cm) and KIN (90.8 cm) and the control (82.8 cm) (Figure 2). Compared with the IBA100 and KIN100 doses and the control, the 100 mg $L^{-1}$ $GA_3$ dose resulted in branch length gains of 19.6%, 15.8% and 23.3%, respectively.

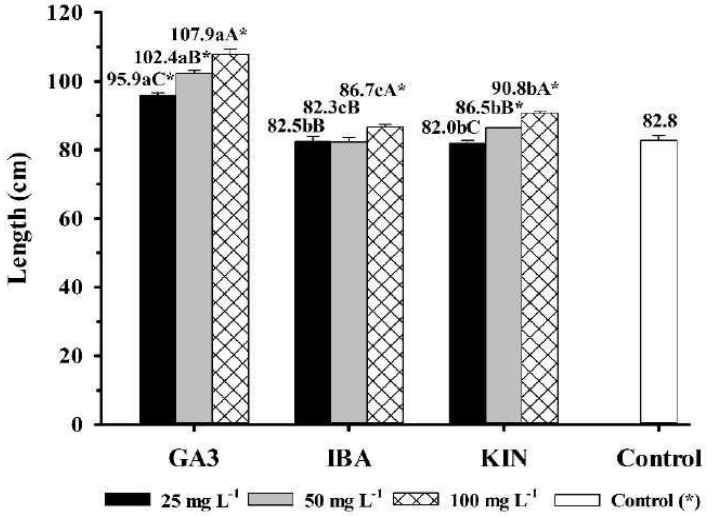


*Figure 2: Influence of foliar application of plant growth regulators (PGRs) at different doses on the length of the largest branch of Origanum vulgare L. grown in a greenhouse. The means followed by the same lowercase letters between the PGR types with the same dose and capital letters for the dose factor within the PGRs do not differ from each other according to the Scott–Knott test ($p < 0.05$). The means followed by (*) differ from those of the control treatment according to Dunnett's test ($p < 0.05$). $GA_3$ (gibberellic acid); IBA (indole-3-butyric acid); KIN (kinetin).*

The greater mean length of the oregano branches in the gibberellin treatment than in the control, IBA and KIN treatments can be explained by the effects of gibberellin on plant growth and development. One of the main roles of biologically active gibberellins is the promotion of cell elongation and the induction of internode elongation in plants [40].

Dadkhah et al. (2016) [41] reported similar results; these authors also reported that the growth of the species *Satureja hortensis* L. was greater following the exogenous application of $GA_3$ at the highest doses (100, 200 and 300 mg $L^{-1}$). The results of this study are also in agreement with those from the study by Sajid et al. (2015) [42], who reported that foliar application of PGRs (GA and BAP) promoted height increases with increasing doses (0, 25, 50, 100 mg $L^{-1}$) in the species *Gladiolus grandifloras* L. In addition, other studies have shown that, compared with a control, the exogenous application of PGRs affects plant growth [43, 44].

Figure 3 shows the influence of foliar application of different doses of PGRs on the LDW, SDW, RDW, ShDW, TDW and R:Sh of oregano. There was a significant interaction for all observed dry weight variables (LDW, SDW, RDW, ShDW, TDW, and R:Sh). Compared with the control, foliar application of the PGRs $GA_3$, IBA and KIN significantly increased the oregano dry weight. Compared with the control treatment (without the application of PGRs), the application of 25 mg/L $GA_3$ increased the LDW, SDW, RDW, ShDW and TDW by approximately 3.5%, 21.8%, 14.0%, 14.1% and 12.9%, respectively.

The lowest dry weight accumulation of oregano was observed at the highest dose of PGRs, especially with the application of $GA_3$100 and KIN100, which resulted in relatively low LDW, SDW, ShDW and TDW values. In the case of $GA_3$, importantly, the highest mean branch length (Figure 2), observed at the $GA_3$50 and $GA_3$100 doses, did not reflect the LDW gain (Figure 3A) but rather significant increases in SDW (Figure 3B), with values of 26.8 and 25.0 g, respectively.

The RDW increased as the IBA and KIN doses increased, and the highest mean RDW (17.7 g) was observed following the IBA100 dose. For $GA_3$, the opposite pattern was observed: at the highest dose ($GA_3$100), a lower RDW (7.4 g) was observed.

R:Sh is an important variable for evaluating plant growth and development because it expresses the influence of root growth in relation to that of shoots and the influence of shoot growth relative to that of roots [45, 46]. All the treatments showed R:Sh values less than one (1.0), indicating greater dry weight production among the aerial plant parts at the expense of root production.

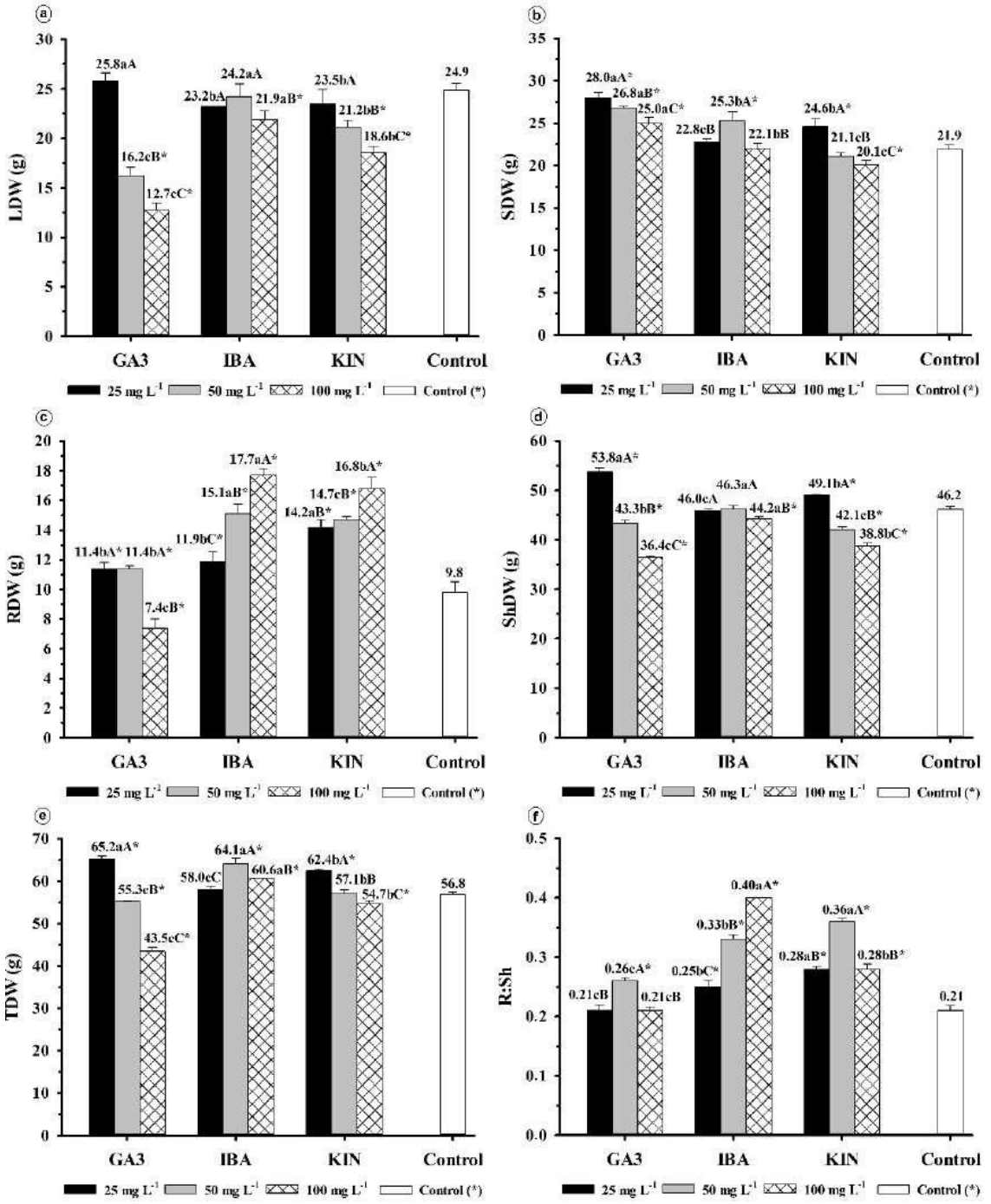


*Figure 3: Influence of foliar application of plant growth regulators (PGRs) at different doses on the dry weight of Origanum vulgare L. grown in a greenhouse. The means followed by the same lowercase letters between the PGR types with the same dose and capital letters for the dose factor within the PGRs do not differ from each other according to the Scott–Knott test ($p < 0.05$). The means followed by (*) differ from those of the control treatment according to Dunnett's test ($p < 0.05$). GA3 (gibberellic acid); IBA (indole-3-butyric acid); KIN (kinetin).*

Studies have shown that foliar application of PGRs directly influences plant growth and development. $GA_3$, for example, is responsible for controlling various aspects of plant growth and physiology, such as stem elongation, shoot growth, and the juvenile leaf stage for adults [43, 47]. Sajid et al. (2015) [42] reported that the highest $GA_3$ dose (25, 50 and 100 mg $L^{-1}$) resulted in a greater weight of the species *G. grandifloras* L. Hasan and Ismail (2018) [48] reported that spraying 150 mg $L^{-1}$ $GA_3$ on peanut plants significantly increased the plant height, total dry weight

and number of twigs; these results are in contrast with those reported for oregano, for which higher weights were observed for the lowest dose ($GA_3$25), indicating that each species is influenced differently by the PGR dose. In the present study, oregano performed better with lower doses of PGRs ($GA_3$25, IBA50, and KIN25) than with the control.

Kulkarni et al. (2013) [49] reported that the foliar application of IBA (5 µM) on mustard increased the dry weights of the aerial parts and roots of the plant, corroborating the results of the present study; foliar application of IBA stimulated the growth of both the aerial parts and roots of oregano in relation to the control. In addition, Arora et al. (2020) [50] reported that exogenous application of IBA increased the fresh and dry root weights, plant height, number of branches, shoot diameter and shoot fresh weight of the medicinal species *Punica granatum* L. relative to the control. These results confirm the role of auxin (IBA) in the process of cell growth and division as well as in plant rooting [40, 51].

With respect to KIN, which is related to the activity of the apical meristem and participates in breaking the dormancy of buds and shoots [43, 52], studies have shown that spraying KIN (0.5, 1.0 or 5 µM) increases shoot length and the fresh and dry biomasses of soybean plants [53]. El et al. (2018) [54] reported that the exogenous application of kinetin (75 mg $L^{-1}$) increased the fresh and dry weights of *Dendranthema grandiflorum* (Ramat.) Kitam.

### 3.2 Analysis of photosynthetic pigments

The influence of different doses of PGRs on the photosynthetic pigments of *O. vulgare* is shown in Figure 4. There was a significant interaction for all the observed variables (chlorophyll *a*, chlorophyll *b*, total chlorophyll, and carotenoids). For the PGRs $GA_3$ and KIN, there were differences among the doses, with the highest averages observed for KIN25 (0.69 mg $g^{-1}$ FW) and for $GA_3$50 and $GA_3$100 (both 0.68 mg $g^{-1}$ FW) (Figure 4A). However, there were no significant differences in chlorophyll *a* content among the IBA doses, and the values ranged from 0.63 to 0.66 mg $g^{-1}$ FW. In the control, the mean chlorophyll *a* content (0.50 mg $g^{-1}$ FW) was significantly lower than that in the PGR treatments. In the case of the KIN25 dose (0.69 mg $g^{-1}$ FW), the production of chlorophyll *a* was 27.54% greater than that in the control. Notably, the higher mean amount of chlorophyll *a* present at the KIN25 dose (0.69 mg $g^{-1}$ FW) may have contributed to the increases in the LDW, SDW, ShDW and TDW, as shown in Figure 3.

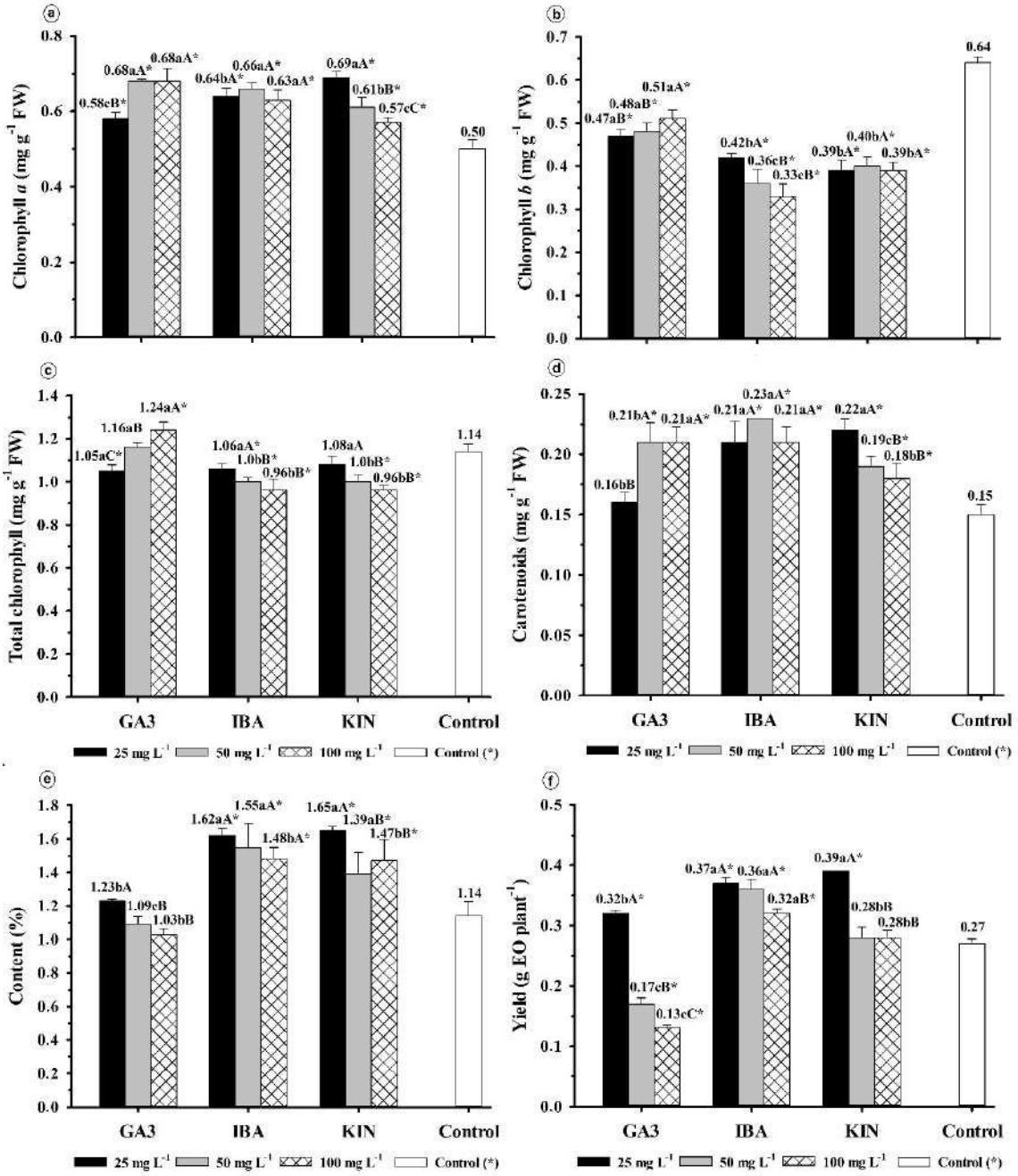


*Figure 4: Effects of foliar application of plant growth regulators (PGRs) at different doses on the photosynthetic pigment contents and essential oil yield of Origanum vulgare L. grown in a greenhouse. The means followed by the same lowercase letters between the PGR types with the same dose and capital letters for the dose factor within the PGRs do not differ from each other according to the Scott–Knott test ($p < 0.05$).The means followed by (*) differ from those of the control treatment according to Dunnett's test ($p < 0.05$). GA3 (gibberellic acid); IBA (indole-3-butyric acid); KIN (kinetin).*

Compared with the control, the application of PGRs led to increased production of carotenoids at all doses (Figure 4D). The highest average was recorded at the IBA50 dose, with a value of 0.23 mgg$^{-1}$ FW, which was 34.8% greater than that in the control (0.15 mg g$^{-1}$ FW). There was no significant difference among the IBA doses, with values ranging from 0.21 to 0.23 mg g$^{-1}$ FW.

According to Cheng and Liu (2010) [55], among the photosynthetic pigments, chlorophyll is one of the most important because it is related to the absorption, transmission and transformation of light energy in the photosynthetic process. With respect to the higher chlorophyll content provided by the application of PGRs, similar results were found for $GA_3$, which afforded an

increase in chlorophyll content with increasing dose. The higher chlorophyll content at higher doses may be due to the inhibition of pigment degradation mediated by $GA_3$ [56]. Saleem et al. (2020) [57] reported the same behavior in *Corchorus capsularis* L. under copper stress, applied at doses of 10, 50 and 100 mg $L^{-1}$. However, the highest levels of chlorophyll in *C. capsularis* resulted in a relatively high dry weight per plant, unlike in oregano, in which a decrease in dry weight per plant was observed as the $GA_3$dose increased. This result shows that high doses of $GA_3$ can have different effects depending on the species.

In the case of the lowest dose (25 mg $L^{-1}$) of the PGRs IBA and KIN, for which higher pigment levels and, consequently, higher dry weights were observed (Figure 3), studies showed that both PGRs stimulated the production of chlorophyll. Mansouri and Talebizadeh (2017) [58] reported similar results, wherein low concentrations of IBA (0.002, 0.020 and 0.203 mg $L^{-1}$) had a stimulatory effect on chlorophyll *a* and the accumulation of carotenoids in the species *Nostoc linckia*. Bashri et al. (2021) [59] reported that the lowest doses of KIN (2.15, 10.74 mg $L^{-1}$) significantly improved the growth and photosynthetic activity of *Trigonella foenum-graecum* L.

The higher chlorophyll *a* content in the presence of PGRs can be explained by the fact that auxins (IBAs) promote pigment synthesis and/or delay its degradation [21]. Exogenous application of cytokinins (KIN) can increase the activity of the enzyme ribulose 1,5-diphosphate carboxylase (RuBisCO), promoting greater efficiency of carboxylation andphotosynthesis [60]. The lower levels of chlorophyll in the IBA100 treatment may have occurred because of the high concentration of auxin (IBA), which induces the synthesis of 1-aminocyclopropane-1-carboxylic acid, the key regulatory enzyme in ethylene biosynthesis, because at high levels, this enzyme may lead to the degradation of photosynthetic pigments [61]. The greater production of carotenoids in oregano treated with PGRs than in the control may have contributed to greater photosynthetic activity and consequently to dry weight gain, since carotenoids are a group of isoprenoid compounds with a wide range of structures and can act as accessory pigments in photosynthesis, increasing light uptake and conferring photoprotective properties to plants [62]. In addition, because it is one of the most appreciated condiments in the world, the high content of carotenoids in oregano may be of interest and add market value. Studies have shown that among plant pigments, carotenoids have bioactive properties and can be beneficial for human health [63], with several therapeutic effects, such as anticancer, immunomodulatory, anti-inflammatory, antibacterial, antidiabetic and neuroprotective effects [64]. According to this last author, this pigment is also widely appreciated for its industrial applications and can be used as an additive for food, feed, supplements and natural dyes.

### 3.3 Essential oil content, yield and constituents

Figure 4 shows the influence of foliar application of different doses of PGRs on the contentand yield of oregano essential oil. There was a significant interaction effect between the PGRs and the doses on both the content (Figure 4E) and yield (Figure 4F) of essential oil. The highest mean essential oil content occurred in the plants treated with the lowest doses of PGRs (KIN25: 1.65%; IBA25: 1.62%; and $GA_3$25: 1.23%). The KIN25 and IBA25 doses resulted in increases of 30.9 and 29.6%, respectively, relative to the mean essential oil content in the control (1.14%).

The highest doses of the PGRs ($GA_3$100, IBA100 and KIN100) resulted in the lowest average oregano essential oil content; these results demonstrated the greater sensitivity of the plants to the $GA_3$100 dose, which resulted in the lowest essential oil content (1.03%). These results show that the oil content of the species *O. vulgare* L. can vary by both the type of PGR and the foliar dose applied.

The highest yields of oregano oil also occurred at the lowest doses, KIN25, IBA25 and $GA_3$25, with values of 0.39, 0.37 and 0.32 g EO $plant^{-1}$, respectively, whereas the control yielded 0.27 g EO $plant^{-1}$. The essential oil content in the control decreased by 30.8, 27 and 15.6% relative to that in plantsthat received the KIN25, IBA25 and $GA_3$25 doses, respectively. In addition to stimulating plant growth and development, growth regulators, or plant hormones, also influence the biosynthesis of terpenes in many aromatic plant species, affecting the content and quality of essential oils [65, 66]. This phenomenon was observed in the results of this study, as the KIN25,

IBA25 and $GA_3$25 doses resulted in higher contents and yields than did the control (without the application of PGRs).

Singh et al. (2016) [67] reported that the application of auxins (IAA and NAA) and $GA_3$ increased the essential oil content of *Matricaria chamomilla* at doses of 25, 50, and 100 $mgL^{-1}$. Mahmoudi et al. (2022) [68] reported that, compared with the control, the application of $GA_3$ (43.3, 86.6, 129.9 mg $L^{-1}$) increased the essential oil content in *Ocimum basilicum*. When treated with $GA_3$ (0.35, 3.46, 34.6 mg $L^{-1}$), *Mentha arvensis* presented a greater oil yield than did the untreated plants, and the increase due to the 1 µM dose was 44.60% [69]. In this study, increased oil yield in oregano was observed in plants treated with $GA_3$25, with contents 15.6% greater than those in the control.

Abbas and EL-Saeid (2012) [70] reported that, for lemongrass, plants in the control group had a lower oil content than did plants that received IBA (25, 50, 100 mg $L^{-1}$). However, with respect to the oil yield per lemongrass plant, the control stood out relative to the IBA treatments. This is different from the results presented in this study, which revealed that compared with the control, the IBA25 dose increased the oil yield by 27% in oregano.

Conversely, KIN application increased the oil content and yield in *Mentha arvensis* by 46.6% and 50.80%, respectively, compared to the control [23], corroborating the data reported inthis study, since the KIN25 dose also increased the content (30.9%) and yield (30.8%) of oil in oregano compared with the control.

The chemical constituents of the essential oil of *O. vulgare* are shown in Table 2. The application of PGRs influenced the synthesis of chemical compounds present in the essential oil of oregano. The main major constituents found were o-cymene, γ-terpinene, trans-sabinene hydrate, terpinen-4-ol and thymol, accounting for more than 67.23% of the oil compounds. The highest averages of trans-sabinene hydrate were observed in the presence of PGRs, where the KIN100 (31.56%) and $GA_3$100 (31.22%) doses increased the contents by approximately 25.2 and 24.4%, respectively, compared with those in the control, for which the lowest mean (23.61%) value was observed. The same pattern occurred for thymol, since the highest mean content was observed for the $GA_3$50 dose (24.80%), which was 12.14% higher than that for the control (21.79%). Higher levels of terpinen-4-ol and γ-terpinene were also detected in the presence of PGRs than in the control.

*Table 1: Effects of foliar application of plant growth regulators (PGRs) at different doses on the chemical constituents of the essential oil of Origanum vulgare L. grown in a greenhouse.*

| % Área | IR | Control | GA3 (mg $L^{-1}$) | | | IBA (mg $L^{-1}$) | | | KIN (mg $L^{-1}$) | | | SD% |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Compound | | | 25 | 50 | 100 | 25 | 50 | 100 | 25 | 50 | 100 | |
| **Monoterpenes** | | **52.4d** | **57.1a** | **49.2f** | **51.3e** | **52.2d** | **54.8c** | **56.08a** | **57.6a** | **56.8a** | **56.0b** | 1.0 |
| **Hydrocarbons** | | | | | | | | | | | | |
| α-Thujene | 925 | 0.33e | 0.35d | 0.08i | 0.10h | 0.17g | 0.19f | 0.45b | 0.38c | 0.48a | 0.19f | 2.7 |
| α-Pinene* | 932 | 0.18 | 0.19 | nd | nd | 0.07 | 0.09 | 0.25 | 0.20 | 0.27 | 0.08 | |
| 1-octen-3-ol | 972 | 2.04d | 2.03d | 0.63i | 0.76h | 1.32g | 1.47e | 2.60b | 2.28c | 2.68a | 1.41f | 1.8 |
| β-Pinene* | 976 | 0.12 | 0.12 | nd | nd | 0.07 | 0.08 | 0.15 | 0.13 | 0.15 | 0.07 | |
| Myrcene | 990 | 1.50c | 1.52c | 0.67g | 0.76f | 1.13e | 1.19d | 1.76a | 1.60b | 1.74a | 1.11e | 1.5 |
| α-Phellandrene | 1005 | 0.27c | 0.30c | 0.24d | 0.26d | 0.30c | 0.30c | 0.39a | 0.37a | 0.33b | 0.29c | 9.5 |
| α-Terpinene | 1016 | 2.62c | 2.48c | 1.33f | 1.43f | 2.01d | 1.71e | 2.0d | 2.79b | 2.98a | 2.05d | 3.9 |
| **o-Cymene** | **1023** | **4.52d** | **3.23f** | **6.25b** | **5.55c** | **6.20b** | **7.8a** | **3.69e** | **3.27f** | **3.20f** | **2.69g** | 1.5 |
| Sylvestrene | 1027 | 1.76c | 1.75c | 1.18f | 1.28e | 1.60d | 1.66d | 2.10a | 2.03a | 1.95b | 1.56d | 3.5 |
| 1,8-Cineole | 1030 | 0.20b | 0.20b | 0.18c | 0.15d | 0.22a | 0.19c | 0.22a | 0.18c | 0.20b | 0.18c | 2.3 |
| (Z)-β-ocimene | 1036 | 3.41c | 3.61b | 2.22h | 2.44g | 2.58f | 2.69e | 3.90a | 3.56b | 3.87a | 3.11d | 1.3 |
| (E)-β-ocimene | 1046 | 0.40f | 0.45d | 0.26j | 0.29i | 0.33g | 0.31h | 0.52a | 0.46c | 0.49b | 0.41e | 1.4 |
| **γ- terpinene** | **1057** | **8.51c** | **9.65a** | **2.88g** | **3.52f** | **5.09e** | **3.49f** | **9.60a** | **9.33b** | **9.75a** | **7.65d** | 1.6 |
| Sabinene hydrate-cis | 1065 | 2.35e | 2.77c | 3.03a | 2.91b | 2.90b | 2.91b | 2.63d | 2.66d | 2.65d | 2.99a | 1.3 |
| Terpinolene | 1087 | 0.92a | 0.81c | 0.59d | 0.60d | 0.78c | 0.64d | 0.87b | 0.93a | 0.98a | 0.80c | 4.0 |
| **Sabinene hydrate-trans** | **1101** | **23.61e** | **27.91c** | **29.69b** | **31.22a** | **27.58c** | **30.22b** | **26.09d** | **27.73c** | **25.53d** | **31.56a** | 1.4 |
| **Oxygenated monoterpenes** | | **16.9b** | **13.9g** | **18.3a** | **15.7d** | **16.4c** | **15.8d** | **14.3f** | **13.7g** | **14.8e** | **14.5f** | 1.0 |
| **Terpinen-4-ol** | **1177** | **10.38b** | **7.42f** | **10.83a** | **8.17d** | **9.42c** | **8.29d** | **7.74e** | **7.11g** | **8.39d** | **7.27f** | 1.2 |
| α -Terpineol | 1190 | 3.13c | 2.95e | 3.47a | 3.16c | 3.39b | 3.356b | 3.01d | 2.94e | 2.95e | 3.04d | 1.1 |
| Thymol methyl ether | 1234 | 0.61a | 0.22g | 0.31d | 0.34d | 0.26f | 0.32d | 0.31d | 0.31d | 0.49b | 0.39c | 3.3 |
| Carvone* | 1237 | nd | nd | nd | nd | nd | nd | 0.26 | nd | nd | nd | |
| Carvacrol methyl ether | 1243 | 1.08a | 0.91e | 1.08a | 1.02c | 1.0c | 1.06b | 0.93e | 0.93e | 0.96d | 1.05b | 1.5 |
| Linalyl acetate* | 1253 | nd | nd | 0.13 | nd | nd | 0.13 | nd | nd | nd | nd | |
| Linalool acetate | 1257 | 1.70h | 2.40e | 2.63d | 3.07a | 2.39e | 2.77c | 2.32f | 2.42e | 2.0g | 2.71b | 1.1 |
| **Phenolic monoterpenes** | | **22.1d** | **22.2d** | **25.3a** | **24.2b** | **24.5b** | **23.8c** | **21.7e** | **21.6e** | **20.7f** | **21.8e** | 1.3 |
| **Thymol** | **1295** | **21.79d** | **21.75d** | **24.80a** | **23.70b** | **23.94b** | **23.29c** | **21.28e** | **21.20e** | **20.36f** | **21.42e** | 1.3 |
| Carvacrol | 1302 | 0.34e | 0.46c | 0.55a | 0.51b | 0.59a | 0.50b | 0.39d | 0.44c | 0.37d | 0.35e | 4.5 |
| Sesquiterpene hydrocarbons | | 4.7a | 4.2c | 3.6f | 4.4b | 3.1i | 2.5j | 3.3h | 3.5g | 3.8e | 4.1d | 1.2 |
| Elixene* | 1366 | nd | nd | 0.11 | 0.10 | nd | 0.10 | nd | nd | nd | nd | |
| β-Bourbonene* | 1382 | nd | nd | nd | 0.07 | nd | nd | nd | nd | nd | nd | |
| (E)-Cariophyllene | 1417 | 2.04b | 1.78d | 1.93c | 2.17a | 1.54g | 1.46i | 1.45i | 1.51h | 1.58f | 1.73e | 1.0 |
| α -Humulene | 1450 | 0.21b | 0.19c | 0.20b | 0.23a | 0.17d | 0.15e | 0.15e | 0.17d | 0.17d | 0.18c | 3.7 |
| Germacrene D | 1479 | 2.30a | 2.10b | 1.31h | 1.79e | 1.26i | 0.79j | 1.53g | 1.74f | 1.92d | 2.04c | 1.4 |
| Byciclogermacrene* | 1494 | 1.10 | 1.28 | 0.26 | 0.51 | 0.40 | nd | 0.92 | 1.13 | 1.13 | 1.36 | |
| δ-Cadinene | **1522** | 0.18a | 0.15b | 0.18a | 0.18a | 0.13c | 0.13c | 0.13c | 0.13c | 0.13c | 0.13c | 3.5 |
| **Oxygenated sesquiterpene** | | **0.97e** | **0.65f** | **1.93a** | **1.81b** | **1.30d** | **1.72c** | **0.57g** | **0.55g** | **0.51** | **0.62f** | 2.3 |
| Spathulenol | 1575 | 0.78e | 0.52f | 1.52a | 1.45b | 1.04d | 1.30c | 0.44g | 0.42g | 0.37h | 0.44g | 2.1 |
| Caryophyllene oxide | 1580 | 0.20d | 0.13f | 0.41a | 0.36b | 0.27c | 0.42a | 0.12f | 0.13f | 0.14f | 0.18e | 4.1 |
| Total % of compounds | | 98.59 | 99.61 | 98.92 | 98.11 | 98.12 | 99.04 | 98.19 | 98.51 | 98.36 | 98.44 | |
| Number of compounds | | 30 | 30 | 30 | 30 | 30 | 31 | 31 | 30 | 30 | 30 | |

RI: retention index of the alkane series (C8–C20); the HP-5MS columns are presented in the order of elution. Means followed by the same letter within the same line belong to the same group according to the Scott–Knott test ($p \leq .05$). GM (green manure); R (root); Sh (leaf + stem). *Compound for which statistical analysis was not performed because it was not identified in all treatments, SD (n = 3).

Five classes of constituents were observed: monoterpene hydrocarbons, oxygenated monoterpenes, phenolic monoterpenes, sesquiterpene hydrocarbons and oxygenated sesquiterpenes. The highest percentage of monoterpene hydrocarbons was observed at the KIN25 dose, at 57.6%. However, higher doses of gibberellin ($GA_3$100 and $GA_3$50) caused a decrease in the production of monoterpene hydrocarbons, with values of 51.3 and 49.2%, respectively, which may be associated with the absence of the constituents α-pinene and β-pinene in both treatments. For the levels of oxygenated monoterpenes, phenolic monoterpenes and oxygenated sesquiterpenes, the highest means were observed at the $GA_3$50 dose.

The variation in the chemical composition of oregano essential oil may be related to the foliar application of PGRs, which can affect the enzymatic pathways of terpenoid biosynthesis [71]. According to Assaf et al. (2022) [72], at optimal concentrations, PGRs may favor the accumulation of secondary metabolites in species of the Lamiaceae family. Elsayed et al. (2021) [73] reported that the application of $GA_3$ (25 mg $L^{-1}$) to chamomile increased the concentration of the main component of the oil. El-Kinany et al. (2020) [21] demonstrated that the application of IBA (100 mg $L^{-1}$) improved the phytochemical parameters of *Hibiscus sabdariffa* L. Thakur and Kumar (2020) [74] reported a relatively high β-myrcene content in *Rosa damascena* treated with KIN (35 mg $L^{-1}$). These findings corroborate the data presented in this study because, in the case of oregano, greater production of major constituents (trans-sabinene hydrate, thymol, terpinen-4-ol and γ-terpinene) was observed in the presence of PGRs.

The decrease in the mean amounts of monoterpene hydrocarbons present under the $GA_3$100 (51.3%) and $GA_3$50 (49.2%) doses may have occurred because of the influence of gibberellin on the metabolic pathway of terpenoids. Mansouri et al. (2011) [75] reported that $GA_3$ caused a decrease in the activity of one of the key enzymes involved in the synthesis of terpenes (DXS: 1-deoxy-D-xylulose-5-phosphate synthase) compared with that in control plants. In addition, $GA_3$ treatment decreased the number and percentage of monoterpenes in treated plants (Mansouri et al. 2011). Hazzoumi et al. (2014) [76] noted that the use of $GA_3$ (70 mg $L^{-1}$) suppressed β-cedrene and azulene production in *Ocimum gratissimum* L. Like Ghassemi-Golezani et al. (2022) [77], these authors reported that $GA_3$ (0,346 g $L^{-1}$) reduced the contents of most of the constituents in *Anethum graveolens* L. Similarly, the essential oil constituents α-pinene and β-pinene were not found in oregano treated with the $GA_3$100 and $GA_3$50 doses.

### 3.4 Oxidative stress and enzymatic antioxidant activity

Figure 5 shows the influence of foliar application of different doses of PGRs on the oxidative stress response of oregano. Significant interactions were observed for all the variables ($H_2O_2$, MDA,SOD, CAT and APX). The highest mean hydrogen peroxide ($H_2O_2$) content was observed for the $GA_3$100 dose, with a value of 7.9 μmol $H_2O_2$ $g^{-1}$ FW (Figure 5A). However, the lowest mean amounts of $H_2O_2$ also occurred in the presence of PGRs, namely, at the $GA_3$25, $GA_3$50 and IBA100 doses, with values of 3.2, 3.3 and 3.4 μmol $H_2O_2$ $g^{-1}$ FW, respectively, differing significantly from the control, which had an average of 5.5 μmol $H_2O_2$ $g^{-1}$ FW.

In the absence of PGRs, an increase in lipid peroxidation, measured as the malondialdehyde (MDA) content, was observed. The control treatment had the highest MDA content of 6.2 nmol MDA $g^{-1}$ FW, which was significantly greater than that of the oregano plants treated with PGRs. The MDAproduction in the control was 95.8% and 95.3% greater than that in the IBA50 (2.6 nmol MDA $g^{-1}$ FW) and KIN100 (2.9 nmol MDA $g^{-1}$ FW) treatments, respectively (Figure 5B).

With respect to the activity of antioxidant enzymes, the presence of PGRs generally stimulates the antioxidant defense system of oregano. For SOD, the highest activity occurred in the control, with a value of 131 U SOD $min^{-1}$ $g^{-1}$ FW, but this value did not differ statistically from the SOD values observed for the IBA25 and KIN25 doses, which also presented high activity,with values of 128 and 125 U SOD $min^{-1}$ $g^{-1}$ FW, respectively (Figure 5C). Conversely, for CAT, greater activity was observed at the KIN100, $GA_3$50 and $GA_3$25 doses, with values of 179, 119 and 116 mmol $H_2O_2$ $min^{-1}$ $g^{-1}$ FW, respectively; these values were significantly greater than those observed in the control (79 mmol $H_2O_2$ $min^{-1}$ $g^{-1}$ FW) (Figure 5D).

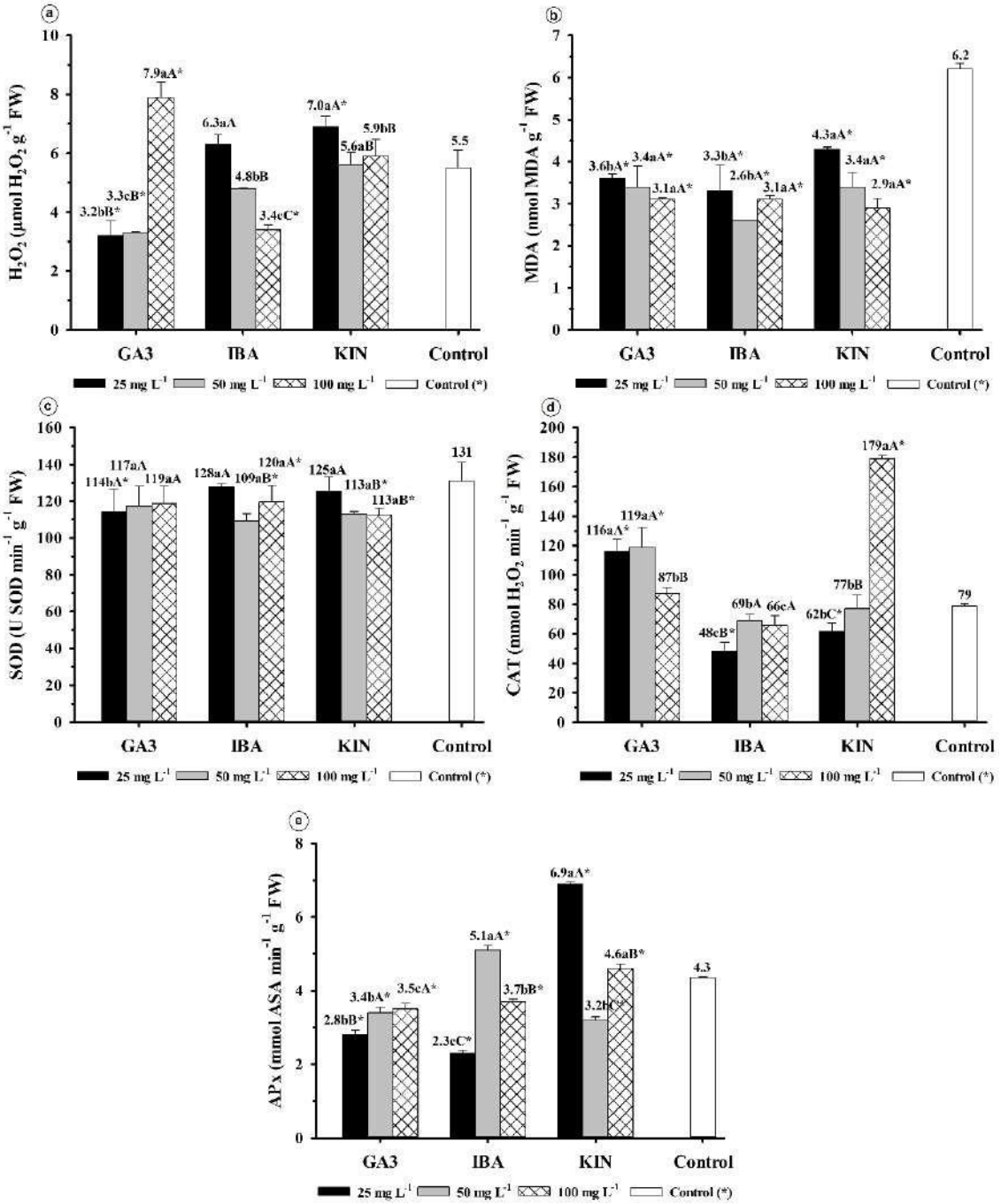


*Figure 5: Influence of foliar application of plant growth regulators (PGRs) at different doses on the oxidative stress response of Origanum vulgare L. grown in a greenhouse. The means followed by the same lowercase letters between the PGR types with the same dose and capital letters for the dose factor withinthe PGRs do not differ from each other according to the Scott–Knott test ($p < 0.05$). The means followed by (*) differ from those of the control treatment according to Dunnett's test ($p < 0.05$). GA3 (gibberellic acid); IBA (indole-3-butyric acid); KIN (kinetin)*

For the antioxidant enzyme APX, the highest activity was observed at the KIN25 and IBA50 doses, with values of 6.9 and 5.1 mmol ASA $min^{-1}$ $g^{-1}$ FW, respectively, and the activity levels were 37.7 and 15.7% higher than that in the control (4.3 mmol ASA $min^{-1}$ $g^{-1}$ FW) (Figure 5E).

The finding that the SOD activity level was greater than the CAT and APX activity levels can be explained by the fact that the SOD enzyme is the first to act in the detoxification process, i.e., the first barrier against oxidative damage; the main function of SOD is the conversion or dismutation of toxic radicals (superoxide, $O_2$ •-) in $H_2O_2$ and molecular oxygen ($O_2$). The enzymes

CAT and APX act in the dismutation of $H_2O_2$ in water and oxygen, minimizing oxidative stress [78, 79].

The foliar application of the different doses of PGRs stimulated the activation of the enzymatic antioxidant system; this occurred because there was a lower level of oxidative stress in the oreganoplants treated with PGRs, which presented lower MDA contents than did the control plants. Studies haveshown that PGRs play an important role in the adaptation and minimization of oxidative stress inplants by activating the enzymatic antioxidant system [80]. Jan et al. (2021) [81] reported that foliar application of $GA_3$ and auxin (indole acetic acid-IAA) combined with EDTA significantly increased antioxidant activities (CAT and SOD) and reduced MDA levels. Ahanger et al. (2018)

[82] and Kaya et al. (2018) [83] also demonstrated that foliar application of KIN activated the antioxidant defense system and reduced oxidative stress in *Zea mays* L.

Although the control had high antioxidant activity (SOD), there were still relatively high levels of$H_2O_2$ and MDA. The higher enzymatic antioxidant activity of SOD in the control may have occurred in an attempt to mitigate cellular oxidative stress due to the high levels of $H_2O_2$ and MDA, as studies have shown that high levels of $H_2O_2$ and MDA are among the main responses to stress conditions. To stabilize this process, it is necessary to activate the enzymatic antioxidant system [78].

Another important point is the high levels of carotenoids present in plants treated with PGRs (Figure 4D), which may have contributed to a lower level of oxidative stress; these results indicate that, among the various functions of carotenoids in plants, carotenoids can eliminate free radicals and protect against lipid peroxidation of the membrane [84-87]. The lower carotenoid content in the control (Figure 4D) may have increased the likelihood of stress and consequently oxidative damage.

These results indicate that the application of PGRs may have minimized the production of reactive oxygen species (ROS) and consequently decreased the effects of stress conditions, such as temperature and humidity variation, during the growth and development cycle of oregano (Figure 1).

### 3.5 Principal component analysis

Principal component analysis (PCA) was used to evaluate the effects of the interactions between growth regulator application and the evaluated parameters so that more information could be extracted from the results (Figure 6). PCA was performed for a correlation matrix includingthe variables dry weight, essential oil content and essential oil yield, and the two major components of the essential oil explained 74.38% of the total variation.

The graph of scores in Figure 3 shows the separation of the PCA results into three groups: the first group includes GA50 and GA100; the second group includes Kin50 and IBA100; and the third group includes IBA25, IBA50, Kin25 and GA25. On the basis of these separations, each treatment wasin a different quadrant of the graph, indicating that there was a difference between the treatments.The GA50 and GA100 treatments (Group 1) positively influenced the contents of the two major compounds thymol and trans-sabinene hydrate and the SDW. There was a positive correlation between the two major compounds of the essential oil and SDW. In addition, the analysis of the vectors revealed that the two major compounds of the essential oil and SDW were negatively correlated with the vegetative growth variables of the plant (LDW, TDW and ShDW), corroborating the hypothesis of environmental stress caused by the application of growth regulators at the GA50 and GA100 doses.

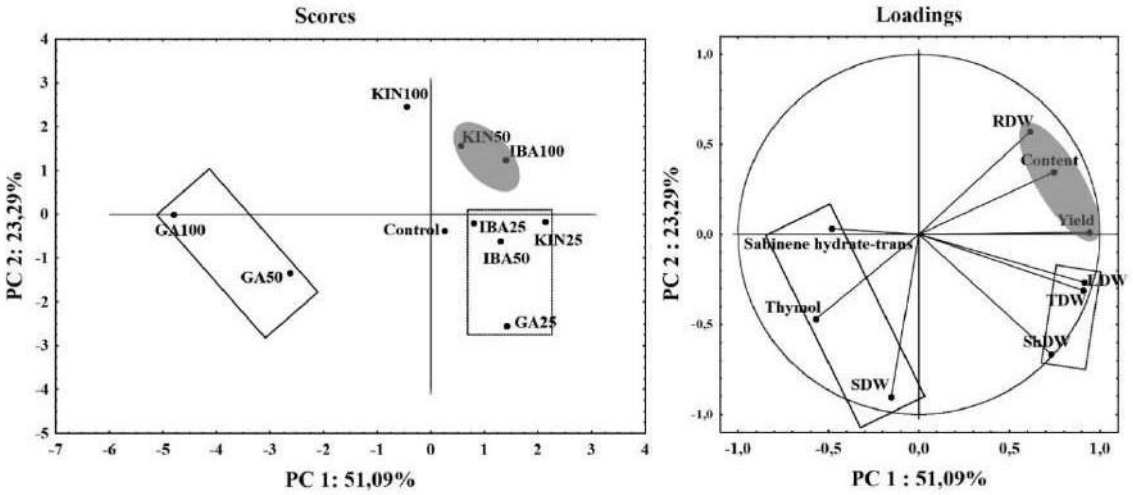


*Figure 6: Principal component analysis (PCA) of the constructed matrix correlation using data for leaf (LDW), stem (SDW), shoot (ShDW), root (RDW), total (TDW) dry weight; essential oil content; essential oil yield; and thymol and trans-sabinene hydrate levels at different doses of GA3 (gibberellic acid), IBA (indole-3-butyric acid), and KIN (kinetin).*

Positive correlations were observed between the KIN50 and IBA100 treatments and between the content and yield of essential oil and RDW (Figure 3). With the application of lower doses of KIN, IBA and GA, the weight gain (LDW, TDW and ShDW) increased. These treatments were negatively correlated with the contents of thymol and trans-sabinene hydrate.

This analysis provides additional important information about the results. PCA revealed that there was a difference between the types and doses of PGRs and between the development ofplants and the volatile compounds of *O. vulgare*.

## 4. CONCLUSION

Foliar application of PGRs at the lowest doses ($GA_3$25, IBA25 and KIN25) resulted in greater dry weights in *O. vulgare*, contributing to significant gains in essential oil content and yield. The use of PGRs also increased the concentrations of carotenoids and major constituents of the essential oil, especially trans-sabinene hydrate and thymol. In addition, the presence of PGRs decreased oxidative stress signaling in this species, which may have contributed to the better performance of oregano. Therefore, the results of this study suggest that foliar application of PGRs at low doses may contribute to increased production of biomass and active ingredients in oregano.

## 5. ACKNOWLEDGMENTS

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