Exogenous salicylic acid modifies gas exchange and biomass production of Mentha x piperita L.

Fabrício Custódio de Moura Gonçalves1,2, Nathália de Souza Parreiras1, Felipe Girotto Campos2, Luís Paulo Benetti Mantoan2, Carmen Silvia Fernandes Boaro3,4

1Universidade Estadual Paulista, Faculdade de Ciências Agronômicas, Botucatu, SP, Brasil
2Universidade Estadual Paulista, Instituto de Biociências, Botucatu, SP, Brasil

*Corresponding author: fabricio-moura-07@hotmail.com

Abstract

Peppermint (Mentha x piperita L.) is a medicinal plant that produces an essential oil used as an additive in food and pharmaceutical formulations. Salicylic acid is a phenolic compound widely distributed throughout the plant kingdom, being able to modify plant physiological and metabolic responses. The objective of this study was to evaluate the effects of the application of salicylic acid on the production of biomass, pigments, as well as gas exchange of peppermint plants. For this purpose, a completely randomized design composed of four replications was applied. Pigment evaluations were carried out in a 4x4 factorial scheme, consisting of the application treatments: 0 (control), 75, 150, and 225 mg L⁻¹ of salicylic acid and four harvest periods. Gas exchange and physiological growth rates were evaluated in a 4x3 factorial scheme. Data were submitted to analysis of variance and regression. The application of salicylic acid increased CO₂ assimilation and use, stomatal conductance, water use efficiency, and transpiration, which in turn, resulted in increased height, and dry stem and root mass production. It is concluded that the application of salicylic acid influences the primary metabolism, increasing gas exchange and biomass production of peppermint plants.

Keywords: Medicinal plants, Biological elicitor, Vegetative productivity.

Introduction

Salicylic acid is considered a widely distributed hormone in leaves and flowers (Misra and Saxena, 2009; Colli, 2008). It presents an important regulatory role in growth and development (Amanullah et al., 2010; Hegazi and El-Shrayi, 2007; Belt et al., 2017; Khoshbakht and Sgharei, 2015). Among the effects of salicylic acid on plant physiology, the most remarkable involves transpiration and stomatal conductance (Lu et al., 2011; Abdullahi et al., 2011; Eraslan et al., 2007; Amanullah et al., 2010). Furthermore, this hormone elevates net photosynthesis (Hosseini et al., 2015) due to its influence in the activity and content of the rubisco enzyme (Vazirimehr and Rigi, 2014). However, the exact mechanism of increased photosynthesis as a function of the exogenous application of salicylic acid is not fully understood (Janda et al., 2014).

In addition, salicylic acid plays a key role in plant productivity (Hayat et al., 2010; Hesami et al., 2012), affecting stem formation and root development (Carvalho Junior, 2013; Gorni and Pacheco 2016), influencing nutrient uptake, such as magnesium and iron, which in turn stimulate the biosynthesis of chlorophylls (Parashar et al., 2014; Abdollahi et al., 2011; Shakirova and Sakhabutdinova, 2003). Moreover, depending on the species and its sensitivity, concentration, and growing conditions, this phytohormone might cause leaf abscission (Nourafkan et al., 2012; Horváth et al., 2007; Kovacik et al., 2009). Furthermore, contingent on the concentration, salicylic acid may act as a promoter or inhibitor of growth (Rivas San Vicente and Plasencia, 2011). At low concentrations, salicylic acid may favor photosynthetic activity while high levels might lead to a reduction (Sahu et al., 2002). However, the response to salicylic acid may result from an induction of oxidative stress (Perez et al., 2014). Thus, despite applications at low concentrations, salicylic acid interacts with stress-signaling mechanisms. Consequently, in order to avoid harmful effects, it is necessary to consider applied concentrations and treatment duration (Rodrigues Brandão et al., 2014).
Salicylic acid might also act in isolation or in association with other hormones to control their effects (Cobucci et al., 2008). As a consequence of its antagonistic effect on auxins, salicylic acid may reduce their concentration and cellular elongation (Rodrigues-Brandao et al., 2016). Nevertheless and Hinojosa (2005) and Mukherjee and Kumar (2007) reinforced that the interaction of salicylic acid with auxins influences the synthesis of gibberellins, which in turn induces plant growth, especially in height. Salicylic acid alters the carbon partitioning and consequently metabolite production (Antoniuzzi and Deschamps, 2006). Studies with Silybum marianum (Khalli et al., 2009), Taraxacum officinale (Kim et al., 2009), Lippia citriodora (Nourafcan et al., 2014), and Calendula officinalis (Bayat et al., 2012) demonstrated that the application of salicylic acid influences the growth and synthesis of photosynthetic pigments, resulting in increased gas exchange. Misra et al. (2014) and Dianat et al. (2016) found positive effects on the metabolism of Catharanthus roseus with the application of 500 mg L⁻¹ and 300 mg L⁻¹ salicylic acid under saline and dry stress conditions, respectively. Despite the widespread use of salicylic acid, especially as a defense inducer under abiotic stress conditions, salicylic acid can also be used under normal conditions because of its beneficial effects on physiological processes related to growth and development (Pál et al., 2013; Hegazi and El-Shrify, 2007). Bayat et al. (2012) verified that the exogenous application of 1 and 2 mM salicylic acid favored the dry mass production of the shoot, root, and total plant in C. officinalis grown under normal conditions. Similar results were observed by Gorni and Pacheco (2016), who verified an increase of 83.11% in the root dry mass of Achillea millefolium. L. sprayed with 500 mg L⁻¹ salicylic acid, which contributed to increase of 61.93% in total dry mass. Therefore, the application of plant regulators such as salicylic acid has been used in medicinal plants with the objective of increasing biomass, providing medicinal and commercial value (Pacheco et al., 2013), including yield enhancement of essential oils in species of the genus Mentha (Sangwan, 2001).

For this reason, the exogenous application of salicylic acid may act as a signal in increments of gas exchange, biomass, and metabolites from the specialized metabolism of Mentha piperita. However, little is known about the action of plant regulators, especially the group of biological elicitors, such as salicylic acid, on the productivity of medicinal species. Thus, the present study aimed to evaluate the effects of the exogenous application of salicylic acid on the production of biomass, pigments, and gas exchange of peppermint plants.

Results

Plant productivity

Statistical analyses revealed that salicylic acid (SA) application affected the stem, root, and total mass production, besides increasing plant height and gas exchange, determined by the concentration applied and the plant phenological phase (Figs. 1C, B and D; Fig. 1A and Fig. 2). However, leaf number, leaf area, leaf dry mass and pigment production of mint plants were not affected (Table 1; Figs. 3A and C).

At 60 days after transplantation (DAT), the plants cultivated with the highest concentration of SA (225 mg L⁻¹) presented lower height (Fig. 1A). Although the application of this concentration increased assimilation and carboxylation rates (Figs. 2A and C), no increased leaf area was observed (Table 1). It is important to emphasize that, although the higher concentration decreased plant height, this did not reflect in reduction of leaf area, stem, and root dry mass (Fig. 1A; Table 1; Figs.1C and B). The plants sprayed with the different SA concentrations showed a total dry mass elevation, especially the plants sprayed with 225 mg L⁻¹ SA (Fig. 1D). These results are consistent with those of CO₂ assimilation and use (Figs. 2A and C).

SA application did not affect leaf area ratio (LAR), specific leaf area (SLA), net assimilation rate (NAR) and relative growth rate (RGR) (Figs. 4A, C, E and G). However, the mint plants cultivated with 225 mg L⁻¹ SA tended to raise NAR and RGR (Figs. 4E and G). At 45 DAT, the plants that received this concentration also experienced an increase in the rate of assimilation mediated by rubisco activity (RubP), which resulted in a general elevation in water use efficiency, indicating adjustments of possible losses that resulted from increased transpiration (Figs. 2A, C and D; Fig. 5C).

Sprayed and non-sprayed plants showed higher values of LAR at the beginning of the cycle at 45 and 60 DAT (Fig. 4A). The plants sprayed with 150 mg L⁻¹ presented a slower decrease of LAR in comparing to the other treatments, which translates into leaf area useful for high photosynthesis (Fig. 2A). This may justify the increase in the efficiency of CO₂ use, which did not result in an increment of leaf area, despite elevating total dry mass (Fig. 2C; Table 1; Fig. 1D). The plants submitted to SA application showed no variation of SLA throughout their development, a behavior that is in accordance with leaf area, which also did not vary among plants treated or not with SA (Fig. 4C; Table 1). These SLA results corroborate with LAR, which, controversially, did not translate into higher leaf mass increment (Figs. 4C and A; Table 1). A decrease in SLA can be seen at 60 and 75 DAT (Fig. 4D).

At 45 and 60 DAT, the NAR of the plants cultivated with 225 mg L⁻¹ exhibited a smaller decrease. Interestingly, plants cultivated with 225 mg L⁻¹ presented a noteworthy regrowth pattern at the end of the cycle (Fig. 4E and F; Fig. 1D). This behavior matches the observed for RGR (Fig. 4G).

Photosynthetic pigments

Although the application of 75, 150, and 225 mg L⁻¹ SA elevated net photosynthesis values (Fig. 2A), the plants sprayed with SA did not show significant differences in chlorophyll a and b (μg g fresh matter⁻¹) (Figs. 3A and C). Probably, these chlorophyll results explain the absence of leaf number, leaf area, and leaf dry mass variation (Table 1). However, it did not reflect in gas exchange since plants sprayed with SA generally revealed higher rates of assimilation, carboxylation, water use efficiency, stomatal conductance, and transpiration (Figs. 2A, C and D; Figs. 5A and C). At 45 and 75 DAT, a higher production of chlorophyll a and b was verified whereas at 90 DAT, no decrease in pigment concentration was detected, regardless of SA application (Figs. 3B and D). This pattern of chlorophyll
Table 1. Leaf number, leaf area, and leaf dry mass in plants of *Mentha x piperita* L. grown under different concentrations of salicylic acid and at different harvest periods.

<table>
<thead>
<tr>
<th>Salicylic acid (mg L⁻¹)</th>
<th>Days after transplantation (DAT)</th>
<th>Leaf number (Unit)</th>
<th>Leaf area (dm²)</th>
<th>Leaf dry mass (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>30</td>
<td>45</td>
<td>60</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>157.00a±3.09</td>
<td>173.75a±81.13</td>
<td>219.00a±38.73</td>
<td></td>
</tr>
<tr>
<td>75</td>
<td>147.75a±26.58</td>
<td>193.75a±35.59</td>
<td>147.25a±30.83</td>
<td></td>
</tr>
<tr>
<td>150</td>
<td>188.50a±31.51</td>
<td>209.75a±84.77</td>
<td>170.00a±35.88</td>
<td></td>
</tr>
<tr>
<td>225</td>
<td>160.25a±16.76</td>
<td>165.50a±17.99</td>
<td>243.00a±50.76</td>
<td></td>
</tr>
<tr>
<td>CV (%)</td>
<td>24.36</td>
<td></td>
<td></td>
<td></td>
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</tbody>
</table>

|                        | 0                               | 75                 | 150             | 225              |
| 0                      | 28.63a±7.82                     | 32.92a±23.65       | 28.11a±6.89     |                  |
| 75                     | 28.01a±10.21                    | 40.23a±14.55       | 24.85a±4.14     |                  |
| 150                    | 34.97a±9.73                     | 45.62a±24.03       | 21.99a±3.67     |                  |
| 225                    | 25.91a±6.25                     | 26.64a±4.11        | 32.14a±1.17     |                  |
| CV (%)                 | 39.10                           |                    |                 |                  |

|                        | 0                               | 75                 | 150             | 225              |
| 0                      | 0.82a±0.20                      | 1.16a±0.68         | 1.14a±0.25      |                  |
| 75                     | 0.88a±0.35                      | 1.40a±0.42         | 0.83a±0.13      |                  |
| 150                    | 0.89a±0.17                      | 1.45a±0.69         | 0.88a±0.10      |                  |
| 225                    | 0.74a±0.20                      | 1.00a±0.12         | 1.48a±0.35      |                  |
| CV (%)                 | 34.50                           |                    |                 |                  |

Means followed by the same letter in the column do not differ at p < 0.05 (Tukey test). Values represent mean ± standard error of four replicates ± error of mean.

Fig 1. Height (cm) (A); root (B), stem (C), and total (D) biomass, in grams; in plants of *Mentha x piperita* L. grown under different concentrations of salicylic acid and at different harvest periods. Values represent mean ± standard error of four replicates.
Fig 2. CO₂ assimilation (A, μmol m⁻² s⁻¹) (A), internal CO₂ concentration (Ci, μmol mol⁻¹) (B), carboxylation efficiency (A/Ci), (C) and water use efficiency (A/E, μmol CO₂ (mmol H₂O)⁻¹) (D) in plants of Mentha x piperita L. grown under different concentrations of salicylic acid and at different harvest periods. Values represent mean ± standard error of four replicates.

Fig 3. Chlorophyll a (A and B) and b (C and D) in plants of Mentha x piperita L. grown under different concentrations of salicylic acid and at different harvest periods. Values represent mean ± standard error of four replicates; values followed by different letters differ significantly by the Tukey’s test (0.05).
Fig 4. Leaf area ratio (A and B), specific leaf area (C and D), net assimilation rate (E and F), and relative growth rate (g, h) in Mentha x piperita L. plants grown under different concentrations of salicylic acid and different harvest periods. Values represent mean ± standard error of four replicates; values followed by different letters differ significantly by the Tukey's test (0.05)
Fig 5. Stomatal conductance (gs, mol m$^{-2}$ s$^{-1}$) (A and B) and transpiration ($E$, mmol m$^{-2}$ s$^{-1}$) (C and D) in Mentha x piperita L. plants grown under different concentrations of salicylic acid and different harvest periods. Values represent mean ± standard error of four replicates; values followed by different letters differ significantly by the Tukey's test (0.05).

Gas exchanges

At 45 DAT, the plants sprayed with SA showed a higher rate of CO$_2$ assimilation, exhibiting a reduction in the internal CO$_2$ concentration, which indicates that the CO$_2$ present in the substomatal chamber was used to assimilate CO$_2$ via ribulose 1,5-diphosphate carboxylase (Fig. 2A, B and C). At 60 DAT, internal CO$_2$ concentration increased when the highest SA concentration was applied, due to lower CO$_2$ efficiency use (Figs. 2B and C). At 75 DAT, the concentration of 150 mg L$^{-1}$ favored the activity of the ribulose enzyme 1,5-diphosphate carboxylase (Fig. 2C).

The exogenous application of 75 and 225 mg L$^{-1}$ increased, for the most part, the transpiration rate, in the same way as observed for stomatal conductance (Figs. 5C and A). In addition, at 45 and 60 DAT, the treatments of 75 and 225 mg L$^{-1}$ increased CO$_2$ assimilation rate mediated by the RuBP activity, which resulted in a higher water efficiency (Figs. 2A, C and D). Moreover, at 75 DAT, the plants sprayed with 150 mg L$^{-1}$ showed an increase in water use efficiency (Fig. 2D).

Discussion

The application of SA did not affect leaf dry matter yield (Table 1). In contrast, it favored the translocation of photoassimilates produced for both root and stem (Figs. 1B and C). Gorni et al. (2017) verified that the application of SA at 0.50 mM in fennel (Foeniculum vulgare) promoted a significant increase in leaf dry mass and that of 0.25 mM increased root dry mass. However, these authors did not verify differences in leaf number, dry mass, total leaves, as well as leaf area and specific leaf area after the application of 0.25, 0.50, and 1.00 mM salicylic acid. The application of SA did not influence the leaf number (Table 1). Nonetheless, it was efficient in increasing total dry mass (Fig. 1D) with elevation of the carboxylation activity (Fig. 2C). El-Tarawy et al. (2012) verified in fennel plants that SA significantly increased the photosynthetic rate and the vegetative growth variables, including height and total dry mass production. Pacheco et al. (2013) observed accumulation of total
biomass in calendula (*Calendula officinalis* L) sprayed with 0.25, 0.50, and 1.00 mM SA. However, changes in the photosynthetic rates were not found. These authors observed that the application of 1.00 mM SA increased the number and dry mass of leaves, and leaf area. The exogenous application of SA did not modify leaf area (Table 1). Nevertheless, an increase in the LAR at the beginning of the vegetative cycle was observed (Fig. 4B). This fact is justified since at this period the plants invest in leaf area for the capture of light and CO$_2$, which decreases over plant development. The NAR is elevated at the beginning of the vegetative cycle due to the lower leaf area and the high photosynthetic capacity of the leaves, expressing the efficiency of the plants in producing dry mass, which tends to decrease due to the shading of lower leaves and a decrease in the relative capacity of the plant to produce new material. In addition, biomass allocation is directly related to the RGR (Antoniazzi and Deschamps, 2006; Li et al., 2016), with the reduction in the allocation of biomass to the aerial part resulting in a reduction in the RGR. It is important to note that RGR reflects growth as a function of preexisting plant material and, in general, coincides with NAR (Figs. 4G, H, E and F).

The application of SA did not affect pigment production (Figs. 3A and C), which reflected in the leaf area values (Table 1). However, these results did not influence the variables of height, dry mass of stem, and dry mass of root (Figs. 1A, C and B). Interestingly, research results differ as to the effects of the application of SA on the production of photosynthetic pigments (Figs. 3A and C). Grzeszcuzk et al. (2018) found that the application of 1.00 mM SA significantly increased the content of chlorophyll a and total chlorophylls in *Salvia coccinea*. Ghasemi et al. (2016) observed that application of 1, 10, 25, and 100 mg L$^{-1}$ SA in chamomile plants did not affect chlorophyll content. The last authors attributed these results to the different responses of chamomile cultivars regarding specific concentrations of SA exogenous application.

Borsatti et al. (2015) state that SA reduces the activity of the primary metabolism, leading to a reduction in pigment synthesis. On the other hand, Idrées et al. (2010) report that application of SA may increase photosynthetic pigments by virtue of its stimulatory effect on the activity of the ribulose 1,5-diphosphate carboxylase enzyme. The absence of SA effects on the pigment values, even with elevation in CO$_2$ assimilation and use (Figs. 3A and C; Figs. 2A and C), may be justified since the positive effects of SA on the photosynthetic activity is not always due to the increase in the level of pigments. In fact, the increase or decrease of the pigment content depends on the genotype, applied concentration, mode of application, duration of the treatment, and environmental conditions (Cag et al., 2009; Mateo et al., 2006).

The increase of net photosynthesis after the application of SA and due to the different seasons was not always accompanied by an increase in stomatal conductance and transpiration rate (Figs. 2A; Figs. 5A and C). It is important to emphasize that CO$_2$ access to mesophilic photosynthetic cells occurs through the stomatal opening; therefore, variations in stomatal conductance may affect CO$_2$ assimilation and transpiration. The application of SA raises the efficiency of the enzymatic activity related to CO$_2$ absorption and use (Khan et al., 2003). On the other hand, it is important to emphasize that the exogenous application of SA can induce stomatal closure and lead to a decrease in photosynthetic activity, with consequent reduction of productivity (Wu et al., 2013).

The high efficiency of carboxylation demonstrates the importance of RuBP fixing CO$_2$ to the process of photosynthesis, which together with the adequate stomatal opening provides high CO$_2$ assimilation, as well as an increase in water use efficiency (Fig. 2A; Fig. 5A; Fig. 2A and D). The efficiency of water use reveals the plant’s ability to open its stomata to assimilate CO$_2$, losing less water during this process and resulting in greater water savings (Amaro et al., 2009). It is important to emphasize that from a productive point of view, information about CO$_2$ assimilation and water use efficiency is of great importance, with CO$_2$ assimilation being one of the factors that influence growth and development (Brandão Filho et al., 2003).

That being said, when the treatment provides conditions of high stomatal conductance, the CO$_2$ assimilation rate is high but depends on the use of internal CO$_2$ for organic synthesis, which maintains the variation of the CO$_2$ chemical gradient, ensuring its acquisition by the leaf. The plants receiving SA increased the carboxylation rate and reduced internal CO$_2$ values, resulting in higher organic synthesis (Figs. 2C and B; Fig. 1D).

### Materials and methods

#### Experimental area

The experiment was conducted at the Botany Department of the Institute of Biosciences, UNESP, Campus Botucatu/SP, in a greenhouse, with controlled humidity and temperature.

#### Plant material and treatments

Plant stems were selected, and from them 10-cm cuttings with the first four pairs of leaves were excised and subsequently placed in expanded polystyrene trays containing commercial Carolina® substrate. The cuttings were kept in intermittent nebulization chamber until rooting.

After rooting, the cuttings were transferred to a nutrient solution No. 2 by Hoagland and Arnon (1950) with ¾ ionic strength for four days and then ½ for another four days in order to acclimatize seedlings in solution. Acclimatized plants were transferred to a nutrient solution with ¾ ionic strength, where they remained until the end of the experiment.

During this period, solutions were continuously aerated and renewed every two weeks (Dantas et al., 1979). Whenever necessary, the volume of the solution was supplemented with deionized water, maintaining the volume of six liters. Daily pH control of the nutrient solution was carried out adjusting it to 5.5 - 6.5, with the aid of a pH meter. The electrical conductivity of the solution was controlled and maintained between 1.5 - 2.5 mS cm$^{-1}$ using a conductivity meter.

The first application of salicylic acid (SA) was performed 30 days after rooting and transfer of the cuttings to a nutrient solution with ¾ ionic strength. The concentrations of SA
(ortho-hydroxybenzoic acid) used were 0, 75, 150, and 225 mg L\(^{-1}\). The solutions were previously diluted in 10 mL of 90% ethanol and then dissolved in one liter of deionized water. Folic spraying of the four solutions was carried out using a pressurized CO\(_2\) sprayer.

**Experimental design**

The experimental design was completely randomized, with four replicates. Pigment evaluations were carried out in a 4x4 factorial scheme, with four SA concentrations: 0 (control), 75, 150, and 225 mg L\(^{-1}\) and four harvest periods, with evaluation intervals of 15 days. Gas exchange and physiological growth indices were evaluated in a 4x3 factorial scheme.

**Growth rates**

Plants were dissected into leaves, stems, and roots. Leaves were immediately submitted to the determination of leaf area in dm\(^2\), with the aid of Li-Cor 3100 leaf area meter. Subsequently, all the material was packed in paper bags, labeled, and placed to dry in a forced circulation oven at 40 °C until constant dry matter was obtained. After drying, the material was weighed in an Ohaus Analytical Standard analytical balance with a sensitivity of up to 0.1 mg for dry mass determination.

Plant functional growth analyses were performed using dry masses, according to Portes and Castro Júnior (1991), evaluating leaf area ratio, specific leaf area, net assimilation rate, and relative growth rate.

**Pigment determination**

Chlorophyll \(a\) and \(b\) were determined according to the methodology described by Sims and Gamon (2002).

**Gas exchange**

The gas exchange evaluations were determined using a portable open system of photosynthesis with water vapor and CO\(_2\) analyzer through infrared radiation ("Infra-Red Gas Analysr – IRGA", model GFS-3000 - Walz). Gas exchange evaluations were performed between nine and eleven o’clock in the morning, selecting one plant per replicate of each treatment, in which the 2\(^{nd}\) or 3\(^{rd}\) leaves with the limb fully expanded and photosynthetically active were selected and standardized.

The gas exchange variables evaluated were: CO\(_2\) assimilation rate (\(A\), \(\mu\)mol CO\(_2\) m\(^{-2}\) s\(^{-1}\)), transpiration rate (\(E\), mmol H\(_2\)O m\(^{-2}\) s\(^{-1}\)), stomatal conductance (\(g_s\), mol H\(_2\)O m\(^{-2}\) s\(^{-1}\)), and CO\(_2\) internal concentration in the leaf (\(C_i\), \(\mu\)mol CO\(_2\) mol\(^{-1}\)), adopting 1700 \(\mu\)mol m\(^{-2}\) s\(^{-1}\) (photoresist photon flux density (DFFFA)) of luminosity.

Water use efficiency (USA, \(\mu\)mol CO\(_2\) (mmol H\(_2\)O)) was determined by the relationship between CO\(_2\) assimilation and transpiration rate (\(A/E\)) and calculated according to Berry and Downton (1983). The instantaneous efficiency of carboxylation of the ribulose 1,5 - diphosphate carboxylase (RuBP) enzyme was calculated by the ratio of assimilation rate and internal CO\(_2\) concentration in the leaf (A/Ci), according to Zhang et al. (2001).

**Statistical analysis**

Data were submitted to analysis of variance and regression using the statistical software SAS University (R Core Team, 2017).

**Conclusions**

Exogenous application of salicylic acid in of peppermint raises gas exchanges, which in turn increases the height, as well as stem, root, and total dry mass yield. However, it does not result in changes in pigment production, even though we identified assimilation rates of CO\(_2\) and of relative growth compatible with faster growth over time.

The responses plants of peppermint to salicylic acid depend on the concentration of this elicitor and the development period. In general, the concentration of 225 mg L\(^{-1}\) is recommended for biomass increase and gas exchange. Thus, although salicylic acid is widely used in plants when subjected to either abiotic or biotic stress conditions, under normal conditions, this elicitor can be used to increase the biomass of medicinal plants and the consequent increase of metabolites of interest for the pharmaceutical and perfume industries.

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