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Influence of exogenous salicylic acid on phytochemical improvement and antioxidant activity in *Cannabis sativa* L.

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Abstract The impact of salicylic acid (SA) foliar spray at varied concentrations (0 and 1 M) and preharvest periods (24, 36, and 42 hours) on secondary metabolite in inflorescences of cannabis (*Cannabis sativa* L.) was investigated. Result was significantly differed in secondary compound composition. Application of 1 M SA at 24 hours preharvest increased total phenolics, total flavonoids, and total chlorophyll. Moreover, 1 M SA at 24 and 42 hours preharvest showed higher chlorophyll a and b contents than 1 M SA at 36 hours preharvest. While antioxidant activity was not significantly differed among 1 M SA treatments at different preharvest periods, it surpassed non-SA-treated plants. The total pigment was not significantly differed among SA treatments. 1 M SA spray reduced carotenoid content in cannabis inflorescences, with the highest carotenoid observed in non-SA treated plants.

Keywords: Cannabis, Salicylic acid, Secondary compound, Plant hormone

Introduction

Cannabis (*Cannabis sativa* L.) referred to as marijuana, is an annual herbaceous plant (Jiang *et al*., 2006), celebrated for its extensive history of use in both traditional and modern medicine (Kalant, 2001). This versatile botanical specimen has recently become the focal point of global attention due to its multifaceted applications in commerce, medicine, and industry. This surge of interest is primarily fueled by its therapeutic potential within contemporary medical practice and the burgeoning popularity of recreational utilization (Shiponi and Bernstein, 2021). Female cannabis flowers contain trichomes that are rich in cannabinoid terpenes and various phytochemicals (Jin *et al.,* 2020). Within cannabis, there are hundreds of known chemicals, with over 100 falling under the cannabinoid category (Rock and Parker, 2021). The primary

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cannabinoids encompass tetrahydrocannabinol (THC), cannabidiol (CBD), cannabinol (CBN), cannabigerol (CBG), tetrahydrocannabivarin (THCV), and cannabichromene (CBC) (Berman *et al.,* 2018). The efficacy of cannabis and its cannabinoids in alleviating nausea and vomiting induced by anticancer and anti-HIV chemotherapy (Abrams, 2022) has been widely recognized. Moreover, these secondary metabolites found in the cannabis plant hold substantial significance as sources of active pharmaceuticals, flavors, and nutraceuticals. They are known for their anti-inflammatory, hepatoprotective, hypoglycemic, antiallergic, and antioxidant properties (Kandar, 2020).

Enhancing the production of these valuable secondary metabolites is of great interest. Elicitor application, a highly effective method for boosting the production of secondary substances (Rani *et al.,* 2023), has gained attention. Elicitors can be categorized into two groups (Thakur *et al.,* 2019). biotic, which originate from biological sources like pathogens or the plant itself, and abiotic, which consists of physical factors and chemical compounds (Halder *et al.,* 2019). Elicitation in plants has been shown to increase the accumulation of secondary compounds (Ramakrishna and Ravishankar, 2011).

One promising elicitor is salicylic acid (SA), a plant hormone classified within the category of phenolic acids (López *et al.,* 2019). Extensive research has unveiled SA is role in a wide range of physiological and developmental responses in plants, including flowering, membrane permeability, and enzyme activities (Yusuf *et al.,* 2013). SA application has been found to stimulate the biosynthesis and accumulation of secondary metabolites in plants (Ali, 2021). Additionally, SA enhances antioxidant activity in plants by regulating enzymes like catalase (CAT), peroxidase (POX), and superoxide dismutase (SOD), impacting various physiological and biochemical processes (Arif *et al*., 2020) depending on factors such as concentrations, plant type, growth stage, and environmental conditions. The use of SA to enhance the accumulation of secondary metabolites has been reported in various plant species, including *Musa acuminata* L. (Jirakiattikul *et al*., 2021), *Mentha piperita* L. (Abdi and Karami, 2020), *Centella asiatica* L. (Ibrahim *et al*., 2017), *Zingiber officinale* Roscoe. (Ghasemzadeh and Jaafar, 2013), *Rosmarinus officinalis* L. (Najafian *et al*., 2009) and *Cannabis sativa* L. (Mirzamohammad *et al*., 2021).

Therefore, the study aimed to evaluate the influence of different concentrations of SA and various preharvest periods on the content of secondary compounds in cannabis inflorescence, shedding light on a potential method to enhance the medicinal and commercial value of this remarkable plant.

Materials and methods

Plant preparation

Shoots were obtained from 6 months old mother plants of *Cannabis sativa* L. 'Killer A5 Haze' The selected shoots, measuring 10-15 cm in height and having 5-6 nodes were used for cutting, with an 80:20 (v:v) mix of peat moss and perlite serving as the cutting substrate. After three weeks, the shoots developed roots and were subsequently transplanted into 11 L (3 gallons) airflow pots, which were filled with a living soil mixture. Uniform seedlings of *Cannabis sativa* L. 'Killer A5 Haze' were chosen for the experiment, and they were organized in rows with a spacing of 1.2 meters between them and 2.0 meters within the rows.

Growing conditions

The experiment was carried out in the greenhouse at The Education Nine Co., Ltd., Nang Lae Nai, Mueang Chiang Rai District, Chiang Rai, Thailand. All cultural practices, such as the application of nutrient solutions, as well as insect and weed control, were conducted by the established standard commercial practices in Thailand.

Preparation of SA

Salicylic acid was dissolved in 95% ethanol and adjusted to a final volume of 1 L with distilled water, resulting in the preparation of a 4 M stock solution. From this stock solution, a 1 M working solution was prepared by dilution with distilled water, which was subsequently sprayed onto the plants. Control plants were sprayed exclusively with distilled water. The application of these solutions to all experimental trees was carried out using a sprayer.

Treatments and experimental design

Two concentrations of salicylic acid (SA), 0 M and 1 M were applied, at three varied preharvest periods: 24, 36, and 42 hours. The experimental design employed a 2x3 factorial in a completely randomized design, with four replicates, and each replication comprising five plants. The experiment was repeated twice. Following the application of treatments, the cannabis inflorescences were meticulously harvested, trimmed, and then hung in a curing room. This curing process spanned 14 days, maintaining a controlled environment at 24-25°C with a relative humidity of 45%-50%. For each

replication, a total of three plants were sampled for harvesting and subsequent extraction.

Extraction of plant samples

In each inflorescence extraction process, a randomized selection of samples was taken from the upper, middle, and lower sections of the cannabis inflorescence. These chosen inflorescences were trimmed and cured for 2 weeks before being dried in a hot air oven at 40°C for 3 hours. Subsequently, the dried samples were blended, and 0.3 g of the dried powder was subjected to extraction with 10 mL of 95% ethanol under a low frequency ultrasonic at 40°C for 30 minutes (conducted in triplicate). The resulting 30 mL of ethanol extract solution was filtered through the Whatman No. 1 filter paper. This ethanol extract solution was employed for the analysis and monitoring of secondary compounds.

Determination of total phenolic content

The total phenolic content was determined using the Folin-Ciocalteu method, as described by Velioglu *et al.* (1988). Plant extract solution (67 µL) was combined with 500 µL of 10% Folin-Ciocalteu reagent. The mixture was well mixed, and after 5 minutes 500 µL of 6% (w/v) sodium carbonate solution was added. This solution was incubated for 90 minutes in a dark room at room temperature. The absorbance of the solution was then measured at 765 nm using a microplate reader (SpectraMax i3x). The determination of total phenolic content was based on a calibration curve using gallic acid as the standard. Total phenolic content was reported as milligrams of gallic acid equivalent per gram of dry weight (mg GAE/g DW).

Determination of total flavonoid content

The determination of total flavonoid content followed a modified method based on Basker *et al.* (2010). A 25 µL plant extract solution was combined with 475 µL of distilled water, and after 5 minutes 250 µL of 2% (w/v) aluminium chloride ($AlCl_3$). After 5 minutes, it was mixed with 250 µL of distilled water. The solution was left to stand for 30 minutes in a dark place at room temperature. The absorbance of the solution was then measured at a wavelength of 415 nm using a microplate reader. The determination of total flavonoid content was based on a calibration curve using quercetin as the standard. Total flavonoid content was reported as milligrams of quercetin equivalent per gram of dry weight (mg QE/g DW).

Determination of chlorophyll a, chlorophyll b, total chlorophyll, carotenoids, and total pigments

To determination of chlorophyll a, chlorophyll b, total chlorophyll, carotenoids, and total pigments the absorbance of the solution were measured at three specific wavelengths of 666 nm, 653 nm, and 470 nm using a microplate reader and these values were compared to those obtained from an ethanol control. Ethanol was employed as the control in this study. The content of chlorophyll a, chlorophyll b, total chlorophyll, carotenoids, and total pigments for each extract was determined using the following formulas (Thambavani and Sabitha, 2011):

Chlorophyll a (Chl a) = 15.65 A666 – 7.340 A653
Chlorophyll b (Chl b) = 27.05 A653 – 11.21 A666
Total chlorophyll = Chlorophyll a + Chlorophyll b
Carotenoids = [1000 A470 – 2.860 (Chl a) – 85.9 (Chl b)] /245
Total pigments = Total chlorophyll + Chlorophyll a + Chlorophyll b

Determination of antioxidant activity

The antioxidant capacity was assessed using the DPPH method following Khattak *et al*. (2018) using 100 µL of plant extract solution diluted with ethanol 95% 1:10. The mixture was well mixed with the extract, poured into a microcentrifuge tube, and 900 µL of DPPH solution was added. The solution was mixed and rested in a dark environment at room temperature for 15 minutes. The absorbance of the solution was then measured at a wavelength of 517 nm using a microplate reader. For the control, the same procedure was followed but instead of the plant extract, 100 µL of 95% methanol was used.

Statistical design and analysis

Data were analyzed using Statistrix v.8 software with a 2x3 Factorial in Completely Randomized Design. An analysis of variance (ANOVA) was applied. Post hoc comparisons for assessing mean differences among treatments were executed using Tukey's Honest Significant Difference (HSD) method.

Results

Total phenolic content

Findings demonstrated that the total phenolic content in inflorescences was influenced by the application of varying concentrations of salicylic acid as a foliar treatment. The total phenolic content increased from 49.088 to 53.584 mg

GAE/g DW after a foliar spray of SA. The highest total phenolic content was obtained in 1 M of SA treatment, whereas the lowest was obtained in the non-SA treated plants. Total phenolic contents in inflorescences were also affected by various periods of preharvest foliar spraying. Application of SA at 24, 36, and 42 hours at preharvest had a positive effect on the accumulation of total phenolic contents. The 24 and 36 hours of preharvest treatments had higher contents of total phenolic when compared with 42 hours of preharvest treatment. The total phenolic contents at 52.691 and 52.292 mg GAE/g DW were obtained from 24 hours and 36 hours of SA preharvest treatment, respectively, whereas the lowest phenolic content at 45.141 mg GAE/g DW was obtained in 0 M SA at 24 hours of preharvest treatment. Interaction between different SA concentrations and preharvest periods produced different total phenolic contents. The highest total phenolic content at 60.241 mg GAE/g DW was achieved in 1 M SA at 24 hours of preharvest treatment. (Table 1).

Total flavonoid content

Results indicated that the application of salicylic acid at varying concentrations had a significant impact on the total flavonoid content in inflorescences. The highest total flavonoid content was obtained in 1 M of SA treatment, whereas the lowest was obtained in the non-SA treated plants. Total flavonoid content in inflorescences was also affected by various periods of preharvest foliar spraying. Application of SA at 24 and 36 hours preharvest had a higher total flavonoid content when compared with 42 hours of preharvest treatment. The total flavonoid contents at 68.667 and 66.217 mg QE/g DW were obtained from 24 hours and 36 hours of SA preharvest treatment, respectively. Whereas the lowest total flavonoid content at 58.367 mg QE/g DW was obtained for the 42 hours of SA preharvest treatment. The combination of various concentrations of SA and preharvest periods had a significant effect on total flavonoid contents. The highest total flavonoid contents at 78.767 mg QE/g DW was achieved in 1 M SA at 24 hours preharvest treatment, whereas the lowest flavonoid content at 52.166 mg QE/g DW was obtained in 1 M SA at 42 hours preharvest treatment (Table 2).

Antioxidants

Results showed that the percentage of DPPH inhibition in inflorescences was affected by various foliar spraying concentrations of salicylic acid. The highest percentage of DPPH inhibition at 24.550 % was obtained from 1 M SA treatment, whereas the lowest percentage of DPPH inhibition at 14.852 % was

found in the non-SA treated plants. The percentage of DPPH inhibition in inflorescences was also affected by various periods of preharvest foliar spraying. The highest percentage of DPPH inhibition in inflorescences at 21.615% was found in the 42 hours preharvest treatment, whereas the percentage of DPPH inhibition was lower in the 24- and 36-hours SA preharvest treatments. The combination of various concentrations of SA and preharvest periods had a significant effect on the percentage of DPPH inhibition. Application of 1 M SA at 24, 36, and 42 hours of preharvest treatments had a higher percentage of DPPH inhibition when compared with non-SA treated (0 M) at 24, 36, and 42 hours of preharvest treatments (Table 3).

Table 1. Total phenolic contents in *Cannabis sativa* L. 'Killer A5 Haze' after spraying with various concentrations of salicylic acid at different preharvest periods

Concentrations (M)	Total phenolic contents (mg GAE/g DW)			
	Preharvest periods (hours)			
	24	36	42	Mean
0 M	45.141c	53.204b	48.919bc	49.088b
1 M	60.241a	51.380b	49.132bc	53.584a
Mean	52.691a	52.292a	49.025b	
C.V. %	4.26			

Note: Numbers followed by the same letter in the same column are not significantly different at $p \leq 0.05$ level

Table 2. Total flavonoid contents in *Cannabis sativa* L. 'Killer A5 Haze' after spraying with various concentrations of salicylic acid at different preharvest periods

Concentrations (M)	Total flavonoid contents (mg QE/g DW)			
	Preharvest periods (hours)			
	24	36	42	Mean
0 M	58.567d	61.684cd	64.567c	61.606b
1 M	78.767a	70.750b	52.166e	67.228a
Mean	68.667a	66.217a	58.367b	
C.V. %	3.80			

Note: Numbers followed by the same letter in the same column are not significantly different at $p \leq 0.05$ level

Table 3. DPPH inhibition percentage in *Cannabis sativa* L. 'Killer A5 Haze' after spraying with various concentrations of salicylic acid at different preharvest periods

Concentrations (M)	% DPPH inhibition			
	Preharvest periods (hours)			
	24	36	42	Mean
0 M	14.870bc	12.836c	16.850b	14.852b
1 M	23.823a	23.448a	26.380a	24.550a
Mean	19.346b	18.142b	21.615a	
C.V. %	8.42			

Note: Numbers followed by the same letter in the same column are not significantly different at $p \leq 0.05$ level

Chlorophyll a

The results indicated that chlorophyll a content in inflorescences was significantly influenced by the application of different concentrations of salicylic acid, as well as the combined effect of various SA concentrations and the preharvest periods. In contrast, the different preharvest periods of SA had no significant impact on the observed outcomes.

The highest chlorophyll a content was obtained at 0.905 mg/g DW in 1 M of SA treatment, whereas the lowest content at 0.739 mg/g DW was obtained in the non-SA treated plants. The content of chlorophyll a was not significant among 24, 36, and 42 hours of SA preharvest treatment. The 1 M SA at 24 hours preharvest had chlorophyll a content at 1.032 mg/g DW and significantly higher than 0 M SA at 24 hours preharvest treatment, 0 M SA at 36 hours preharvest treatment, and 1 M SA at 36 hours preharvest treatment but not significant with 0 M SA at 42 hours preharvest treatment and 1 M SA preharvest treatment. The 1 M SA treatment applied 24 hours preharvest resulted in chlorophyll a content of 1.032 mg/g DW, which was significantly higher than that of the 0 M SA treatment at 24 hours preharvest, the 0 M SA treatment at 36 hours preharvest, and the 1 M SA treatment at 36 hours preharvest. However, it was not significantly different from the 0 M SA treatment at 42 hours preharvest and the 1 M SA treatment at 42 hours preharvest (Table 4).

Chlorophyll b

The findings from this study underscore the substantial impact of salicylic acid concentrations, preharvest periods, and their combined influence on the levels of chlorophyll b in inflorescences. Specifically, the highest chlorophyll b content, measuring 1.669 mg/g DW, was achieved with a 1 M SA treatment, while the lowest content, at 0.977 mg/g DW, was observed in the non-SA treated plants. Regarding chlorophyll b, no significant differences were observed between the 24 hours and 42 hours preharvest treatments. However, a significant difference was found with the 36 hours of SA preharvest treatment. Notably, the 1 M SA treatment at 24 hours and 42 hours before preharvest resulted in chlorophyll b contents of 1.903 mg/g DW and 1.793 mg/g DW, respectively, both of which were significantly higher than the content observed with the 36 hours preharvest treatment. Among the various combinations of salicylic acid concentrations and preharvest periods, the treatment involving 1 M SA at 24 and 42 hours before preharvest demonstrated significantly higher concentrations of chlorophyll b compared to the other treatment combinations (Table 5).

Total chlorophyll

The findings is demonstrated the substantial influence of varying concentrations of salicylic acid, preharvest periods, and their interactive effects on the concentrations of total chlorophyll in inflorescences. Remarkably, the highest total chlorophyll content, measuring 2.591 mg/g DW, was achieved through the application of a 1 M SA treatment, while the lowest concentration, at 1.725 mg/g DW, was observed in the non-SA treated plants. Significant differences in total chlorophyll content were observed between the 24 hours, 36 hours, and 42 hours SA preharvest treatments. Notably, the application of SA 24 hours preharvest resulted in the highest total chlorophyll content, measuring 2.465 mg/g DW. Among the various combinations of salicylic acid concentrations and preharvest periods, it is important to highlight that the treatment involving 1 M SA at 24 hours preharvest showed the highest and significantly elevated concentrations of total chlorophyll when compared to the other treatment combinations (Table 6).

Carotenoids

The study findings revealed the impact of different salicylic acid concentrations as foliar treatments on carotenoid levels in inflorescences. Notably, carotenoid concentrations exhibited a reduction following the

application of salicylic acid and the highest carotenoid content was observed in the non-SA treated plants. Moreover, the carotenoid levels in inflorescences were influenced by the preharvest periods. Applying salicylic acid 36 hours preharvest resulted in a significantly higher carotenoid content in inflorescences when compared to the 24 hours and 42 hours preharvest foliar treatments. The combination of varying salicylic acid concentrations and preharvest timing also led to significant differences in carotenoid content among the treatments. Specifically, the highest carotenoid content was achieved in non-SA treated (0 M SA) at 36 hours preharvest, 0.766 mg/g DW (Table 7).

Table 4. Chlorophyll a content in *Cannabis sativa* L. 'Killer A5 Haze' after foliar spraying with various concentrations of salicylic acid at different preharvest periods

Concentrations (M)	Chlorophyll a (mg/g DW)			
	Preharvest periods (hours)			
	24	36	42	Mean
0 M	0.700b	0.731b	0.787ab	0.739b
1 M	1.032a	0.711b	0.972ab	0.905a
Mean	0.866a	0.721a	0.879a	
C.V. %	15.62			

Note: Numbers followed by the same letter in the same column are not significantly different at $p \leq 0.05$ level

Table 5. Chlorophyll b contents in *Cannabis sativa* L. 'Killer A5 Haze' after foliar spraying with various concentrations of salicylic acid at different preharvest periods

Concentrations (M)	Chlorophyll b (mg/g DW)			
	Preharvest periods (hours)			
	24	36	42	Mean
0 M	1.088bc	0.705c	1.137bc	0.977b
1 M	1.903a	1.312b	1.793a	1.669a
Mean	1.495a	1.008b	1.465a	
C.V. %	15.60			

Note: Numbers followed by the same letter in the same column are not significantly different at $p \leq 0.05$ level

Table 6. Total chlorophyll contents in *Cannabis sativa* L. 'Killer A5 Haze' after foliar spraying with various concentrations of salicylic acid at different preharvest periods

Concentrations (M)	Total chlorophyll (mg/g DW)			
	Preharvest periods (hours)			
	24	36	42	Mean
0 M	1.921cd	1.481e	1.774de	1.725b
1 M	3.010a	2.222bc	2.541b	2.591a
Mean	2.465a	1.852c	2.157b	
C.V. %	8.09			

Note: Numbers followed by the same letter in the same column are not significantly different at $p \leq 0.05$ level

Table 7. Carotenoid contents in *Cannabis sativa* L. 'Killer A5 Haze' after foliar spraying with various concentrations of salicylic acid at different preharvest periods

Concentrations (M)	Carotenoids (mg/g DW)			
	Preharvest periods (hours)			
	24	36	42	Mean
0 M	0.222b	0.766a	0.049b	0.346a
1 M	0.171b	0.122b	0.219b	0.171b
Mean	0.196b	0.444a	0.135b	
C.V. %	64.32			

Note: Numbers followed by the same letter in the same column are not significantly different at $p \leq 0.05$ level

Table 8. Total pigment contents in *Cannabis sativa* L. 'Killer A5 Haze' after foliar spraying with various concentrations of salicylic acid at different preharvest periods

Concentrations (M)	Total pigment (mg/g DW)			
	Preharvest periods (hours)			
	24	36	42	Mean
0 M	2.142a	2.248a	2.724a	2.372a
1 M	3.106a	2.145a	2.985a	2.746a
Mean	2.624ab	2.196b	2.855a	
C.V. %	18.94			

Note: Numbers followed by the same letter in the same column are not significantly different based on Tukey's HSD at $p \leq 0.05$ level

Total pigments

The shed light on the impact of preharvest periods of salicylic acid was applied as a foliar treatment on the total pigments in inflorescences. In contrast, the concentrations in combination of various concentrations and preharvest periods of SA had no significant impact on the total pigments. The total pigment values reached 2.855 mg/g DW were achieved at 42 hours of preharvest foliar

spraying and significantly higher than 36 hours but not significant with 24 hours treatment (Table 8).

Discussion

In our examination, we observed that the concentrations of salicylic acid (SA) played a pivotal role in enhancing several essential components in the flowers of *Cannabis sativa* L. 'Killer A5 Haze' and the resulting percentage of DPPH inhibition. Particularly, preharvest foliar spraying of SA at a concentration of 1 M exhibited the most notable effects, leading to increased levels of total phenolics, total flavonoids, total chlorophyll, chlorophyll a, chlorophyll b, and total pigments. These findings align with previous studies, such as the work of Ahmed *et al*. (2022), which reported a similar increase in total phenolics, total flavonoids, and enhanced antioxidant activity in date palm fruits following SA treatment. Moreover, the influence of SA on enzymatic and photosynthetic activities, coupled with its role in maintaining the balance between ROS elimination and inhibition, was consistent with other studies (Silva *et al*., 2022). These results are indicative of SA is ability to induce the accumulation of phenylalanine and flavanone, thereby enhancing the production of phenolic acids and flavonoids. This enhancement has been linked to an increased antioxidant capacity, which helps plants cope with various stress factors. The relationship between antioxidant activity and total phenolic content has been emphasized, further highlighting the significant role of phenolic compounds in a plant's antioxidant capacity (Blanch *et al*., 2020). This reaffirms SA is potential to promote the production of secondary compounds in plants, as demonstrated by the elevated levels of total phenolics, total flavonoids, total chlorophyll, and antioxidant capacity in spinach leaves subjected to SA preharvest spraying (Singh, 2023).

Nonetheless, it is essential to acknowledge that not all outcomes are universally favorable. In the case of inflorescences, preharvest foliar spraying at a concentration of 1 M resulted in a decrease in carotenoid levels following SA treatment. It indicated that the impact of SA on secondary compound production can vary among different plant species. For instance, *Jacobaea vulgaris* treated with 5 mM SA exhibited lower levels of chlorogenic acid, fumaric acid, glucose, and glutamate compared to control plants, underlining the variability of SA is effects (Wei *et al*., 2021).

The various periods of preharvest SA treatment were also crucial factors in determining the observed results. In our study, the 24 hours preharvest foliar spraying of SA proved to be the most effective for enhancing total phenolics, total flavonoids, chlorophyll a, chlorophyll b, and total pigments in inflorescences. Interestingly, for total chlorophyll and the percentage of DPPH

inhibition, the 42 hours of preharvest spraying yielded higher values, whereas the 36 hours of preharvest spraying led to the highest carotenoid levels.

It is important to note that SA plays a significant role as a plant growth regulator. It rapidly accumulates during hypersensitive reactions in plants and triggers various defense responses. Additionally, SA activates genes associated with secondary metabolite synthesis, and it acts as an elicitor that binds G-protein receptors, prompting rapid secondary compound production (Mahady *et al.,* 1998). SA, being a phenolic compound, can activate enzymes like phenylalanine ammonia lyase, which is responsible for the production of key phenolic compounds (Raskin, 1992). Furthermore, SA influences chalcone synthase activity, leading to the production of major flavonoid constituents, and it regulates the photosynthesis system in plants by affecting carotenoid and chlorophyll composition (Gao *et al*., 2012).

Our findings strongly suggested that preharvest foliar SA application holds significant promise for enhancing the production of valuable secondary compounds in plants. For instance, the application of sodium salicylate solution (NaSa) during watercress tissue culture, at different periods ranging from 24 to 48 hours for 4, 6, and 8 days before harvest, resulted in increased levels of total flavonoids, chlorophyll a, chlorophyll b, total chlorophyll, and total pigments. Notably, the highest values were achieved when spraying occurred 24 hours before harvest, showcasing the versatility and effectiveness of SA application (Szczykutowicz *et al*., 2022).

The combination of various concentrations and the preharvest spraying period of SA had a notable impact on secondary compounds and pigments in cannabis plants. In our study, spraying 1 M SA at a 24 hours preharvest treatment was the most effective in enhancing total phenolics, total flavonoids, total chlorophyll, chlorophyll a, chlorophyll b, and total pigments in inflorescences of *Cannabis sativa* L. 'Killer A5 Haze' Furthermore, this combination resulted in the highest percentage of DPPH inhibition. It is essential to highlight that the optimal concentrations of SA may vary depending on the plant species and application method. SA is the ability to induce the accumulation of secondary metabolites and its overall positive effects on plants are documented in various studies (Wang *et al.*, 2017). For example, Gacnik *et al.* (2021). found that SA treated apple peels exhibited higher levels of total phenolics and flavonoids, with the peak values recorded 24 hours after spraying. Conversely, lower levels of these compounds were observed 48 hours and 7 days after application. However, it's essential to acknowledge that the impact of SA can vary among different plant species. In the case of inflorescences, preharvest foliar spraying at a concentration of 1 M, regardless of the various preharvest periods (24, 36, or 42 hours), resulted in a reduction in carotenoid levels. This is consistent with

findings suggesting that SA may have an inhibitory effect on the production of some secondary compounds in plants. For example, Jamun fruit (*Syzygium cumini* Skeels.) treated with 1 and 1.5 mM SA after a 2 days coating exhibited lower levels of total anthocyanins and total flavonoids compared to control plants (Saurabh *et al.*, 2019). These outcomes emphasize the need to consider the specific plant species, the concentrations of SA, and the timing of application when utilizing SA to enhance secondary compounds.

Research finding demonstrated that the application of 1 M of salicylic acid (SA) at the 24 hours preharvest treatment significantly enhanced the production of secondary metabolites in *Cannabis sativa* L. 'Killer A5 Haze' flower. This treatment led to the highest levels of total flavonoids, total phenolics, chlorophyll a, chlorophyll b, total chlorophyll, total pigments, and antioxidant activity, thereby highlighting the potential for SA to positively impact the chemical composition and antioxidant properties of these flowers. However, we observed a decrease in carotenoid levels. Further research is needed to explore the broader implications of SA treatment on different plant species and compounds. Understanding the specific mechanisms governing these effects will be instrumental in harnessing the full potential of SA for the enhancement of secondary metabolites in plants.

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