



Influence of Cycocel and Salicylic acid on phytochemical traits and photosynthetic pigments of *Salvia officinalis* in two distinct agroecological zone

Mehrdad Ataie Kachoie*

Department of Medicinal plants, ShK.C., Islamic Azad University, Shahrekord, Iran;

*Email: Drataie@iaushk.ac.ir

ARTICLE INFO

Type: Original Research

Topic: Medicinal Plants

Received March 22th2024

Accepted June 25th2024

Key words:

- ✓ *Salvia officinalis*
- ✓ Cycocel
- ✓ Salicylic acid
- ✓ Phytochemicals
- ✓ Photosynthetic pigments
- ✓ Environmental interaction

ABSTRACT

Background & Aim: *Salvia officinalis* L., a medicinal plant from the Lamiaceae family, is widely valued for its essential oils and bioactive compounds. Environmental conditions and growth regulators significantly affect plant performance; however, limited information exists on their combined effects on *S. officinalis*. This study aimed to evaluate the impact of cycocel (CCC) and salicylic acid (SA) on phytochemical characteristics and photosynthetic pigments of *S. officinalis* grown in two distinct agroecological zones: Zob-Ahan and Shahrekord.

Experimental: This study was conducted in two regions with distinct climatic conditions in Iran (Shahrekord and ZobAhan). A total of 108 pots (54 pots for each region) were prepared for cultivation of *Salvia officinalis*. A factorial experiment was conducted under controlled pot conditions using a completely randomized design. Cycocel and salicylic acid were applied at three concentrations (0, 1, and 1.5 mg/L).

Results: The interaction between location and treatment significantly affected ($P < 0.01$) photosynthetic pigments and phytochemical traits. Application of 1 mg/L CCC increased 1,8-cineole content by 19.8% compared to the control. Higher concentrations of SA, however, reduced camphene and 1,8-cineole levels. The effect of SA on increasing chlorophyll a, chlorophyll b, and total chlorophyll content was more pronounced than that of CCC, indicating its role in promoting pigment biosynthesis. Combined application of CCC and SA under different regional conditions significantly affect the important phytochemical and physiological attributes of *S. officinalis*. Optimizing their application according to regional characteristics can improve the yield and quality of bioactive compounds in this species.

Recommended applications/industries: The observed synergistic effects of salicylic acid and cycocel indicated that the combined application of these growth regulators can serve as an effective strategy to optimize the yield and resilience of *S. officinalis* across diverse environmental condition.

1. Introduction

In recent years, extensive research has focused on the phytochemical characteristics and the effects of plant growth regulators on the performance of medicinal plants, particularly *Salvia officinalis* (common sage). *S. officinalis* is well known for its pharmacological

properties, primarily attributed to its rich content of bioactive compounds, including phenolic acids (such as rosmarinic and caffeic acid), flavonoids, terpenoids (e.g., camphor, borneol, and caryophyllene), and other polyphenolic constituents (Zhao *et al.*, 2024). These

compounds play vital roles in the plant's antioxidant, anti-inflammatory, and antimicrobial activities.

Environmental factors such as light intensity, temperature, water availability, and altitude significantly influence the growth, essential oil composition, and overall phytochemical profile of *S. officinalis* (Farhat *et al.*, 2013; Senbali *et al.*, 2015). Additionally, the application of exogenous elicitors like plant growth regulators has emerged as a promising strategy to modulate secondary metabolism and enhance the adaptive responses of medicinal plants under stress conditions.

Salicylic acid (SA), a phenolic phytohormone, plays a critical role in plant defense mechanisms and regulation of physiological processes, especially under abiotic stresses. Several studies have shown that foliar application of SA enhances photosynthetic efficiency by improving photosystem II (PSII) performance and increasing chlorophyll content, particularly under drought or salinity stress (Nasrollahi *et al.*, 2023; Mohammadi *et al.*, 2023). Moreover, SA has been reported to stimulate the biosynthesis of secondary metabolites, including phenolic compounds and terpenoids, in various aromatic and medicinal plants.

Chlormequat chloride, commonly known as cycocel (CCC), is a widely used growth retardant that modulates plant architecture by inhibiting gibberellin biosynthesis. Its application results in reduced stem elongation, increased leaf density, and enhanced photosynthetic pigment content (Rademacher, 2020). Furthermore, cycocel has been reported to influence

essential oil composition and improve overall plant quality in various ornamental and medicinal species.

Considering the increasing interest in improving the yield and quality of medicinal plants through agronomic and physiological interventions, the present study aimed to evaluate the effects of cycocel and salicylic acid on the phytochemical characteristics and photosynthetic pigments of *Salvia officinalis* cultivated in two distinct agro-climatic regions. Although the separate effects of these elicitors have been studied, limited information is available about their combined impact under different environmental conditions. Therefore, this research addresses this knowledge gap and provides insights for optimizing treatment strategies to enhance the medicinal value of *S. officinalis*.

2. Materials and Methods

2.1. Study area

This study was conducted in two regions with distinct climatic conditions in Iran: Shahrekord, located in Chaharmahal and Bakhtiari Province, characterized by a cold climate at an altitude of 2061 meters above sea level; and ZobAhan, located in Isfahan Province, with a hot and dry climate at an altitude of 1730 meters above sea level. The physicochemical properties of the pot soil used in the experiment are presented in Table 1.

Table 1. Soil characteristics used in the experiment.

Cu mg. kg ⁻¹	Fe. mg. kg ⁻¹	Mn. mg. kg ⁻¹	Zn. mg. kg ⁻¹	N %	K ava. mg. kg ⁻¹	P ava. mg. kg ⁻¹	T. N. V %	O. C %	pH of past	E. C. ds. m ⁻¹	Clay %	Silt %	Sand %
2.18	6.23	9.66	1.48	0.116	415	71.9	34.5	1.482	7.56	6.370	0.37	0.46	0.17

2.2. Experimental design and plant cultivation

In May 2023, a total of 108 pots of the same soil were prepared for cultivation of *Salvia officinalis*. For each region, 54 pots were used in a factorial experiment with three concentrations of salicylic acid (0, 1, and 1.5 mg/L) and three concentrations of cycocel (0, 1, and 1.5 mg/L), arranged in a completely randomized design with three replications per treatment.

2.3. Essential oil extraction

After harvesting, the aerial parts of *S. officinalis* were shade-dried at ambient temperature (approximately 25–27 °C) for 10–14 days in a well-ventilated area away from direct sunlight. Essential oils were extracted from the dried material using the hydrodistillation method using a Clevenger-type apparatus.

2.4. Gas chromatography–Mass spectrometry (GC-MS) analysis

Essential oil composition was analyzed using a GC-MS system (Agilent 7890 GC coupled with 5975C MS detector). Separation was performed on an HP-5MS capillary column (30 m × 0.25 mm i.d., 0.25 µm film thickness). The oven temperature was programmed from 60 °C (held for 3 min) to 240 °C at 4 °C/min and held for 10 min. Helium was used as carrier gas at 1.0 mL/min. Injector and detector temperatures were set at 250 °C. The mass spectrometer operated in electron ionization mode at 70 eV with a scanning range of m/z 40–550. Identification of compounds was based on mass spectra comparison with the NIST library and calculation of retention indices relative to n-alkanes (C8–C20) under the same conditions.

2.5. Measurement of photosynthetic pigments

Chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids were determined according to Arnon (1967). Fresh leaf samples (0.1 g) were ground in liquid nitrogen and extracted with 20 mL of 80% acetone. After centrifugation at 6000 rpm for 10 min, absorbance of the supernatant was recorded at 663, 645, and 470 nm using a spectrophotometer. Concentrations (mg/g fresh weight) were calculated as follows:

$$\text{Chlorophyll a} = (19.3 \times A_{663} - 0.86 \times A_{645}) \times V / (100 \times W)$$

$$\text{Chlorophyll b} = (19.3 \times A_{645} - 3.6 \times A_{663}) \times V / (100 \times W)$$

$$\text{Carotenoids} = [100 \times A_{470} - 3.27 \times (\text{Chl a}) - 104 \times (\text{Chl b})] / 227$$

Where A: absorbance at respective wavelength, V: extract volume (mL), and W: fresh weight (g).

2.6. Proline determination

Proline content was measured following Bates et al. (1973) with slight modifications. Fresh leaf tissue (0.1 g) was homogenized in 10 mL of 3% sulfosalicylic acid and filtered. Two mL of the filtrate was mixed with 2 mL acid ninhydrin reagent and 2 mL glacial acetic acid, then incubated at 100 °C for 1 hour. After cooling in ice, 4 mL of toluene was added, and the chromophore-containing toluene phase was separated. Absorbance was measured at 515 nm. Proline concentration (µmol/g FW) was calculated by:

$$\text{Proline } (\mu\text{mol/g FW}) = \{A_{515} \times V \times 115.5\} / \{W \times 1000\}$$

Where A_{515} : absorbance at 515 nm, V: extract volume (mL), W: fresh weight (g).

2.7. Total phenol content

Total phenolics were determined using the Folin–Ciocalteu method (Singleton et al., 1999) with minor modifications. Fresh leaf tissue (0.05 g) was homogenized in 2 mL 80% methanol and incubated at 30 °C for 15 min. After centrifugation at 5000 rpm for 15 min, 1 mL of extract was mixed with 1.8 mL distilled water and 0.2 mL Folin–Ciocalteu reagent. After 5 min incubation at 25 °C, 1 mL 12% sodium carbonate was added. Following 2 hours incubation, absorbance was measured at 765 nm. Total phenol content was expressed as mg gallic acid equivalents (GAE) per gram fresh weight using:

$$\text{Total Phenol (mg GAE/g FW)} = (C \times V) / W$$

Where: C = concentration from standard curve (mg/mL), V = extract volume (mL), W = fresh weight (g).

2.8. Statistical analysis

Data were analyzed using a factorial design with three replications. Treatments included three concentrations of salicylic acid (0, 1, and 1.5 mg/L) and cycocel (0, 1, and 1.5 mg/L). Statistical analysis was performed using Minitab 19 software. Mean comparisons were made by the least significant difference (LSD) test at a 5% probability level ($P < 0.05$).

3. Results and discussion

3.1. Phytochemical traits

The analysis of variance showed that both plant growth regulators (cycocel and salicylic acid) as well as the growing region had a significant effect ($P < 0.01$) on all measured phytochemical traits of *Salvia officinalis*.

Application of 1.5 mg/L of cycocel and salicylic acid led to a respective increase of 20.4% and 21.5% in camphene content compared to the control. In the case of β-pinene, treatment with 1 mg/L cycocel significantly enhanced its content by 33.4%, whereas application of 1.5 mg/L salicylic acid resulted in a

34.2% reduction. Notably, the highest β -pinene concentration was recorded in plants grown in the Shahrekord region (Table 2), suggesting a positive influence of cooler climates on the accumulation of this monoterpene.

For 1,8-cineole, 1 mg/L cycocel application in the Shahrekord region increased its concentration by 19.8%, indicating a synergistic effect between temperature and growth regulator in promoting this compound. In contrast, in the warmer ZobAhan region, the application of 1 mg/L cycocel and 1.5 mg/L salicylic acid increased α -thujone levels by 8% and 7.6%, respectively. However, application of 1.5 mg/L cycocel in this region led to a 19% decrease in α -thujone, while the same concentration of salicylic acid increased it by 29% (Table 2).

In total, 34 chemical constituents were identified in the leaf essential oil samples, comprising 97.6% of the total oil content. The major constituents included germacrene-D, manool, borneol, and β -ocimene. The combined application of 0.5 mg/L cycocel and 0.5 mg/L salicylic acid resulted in the highest concentration of these key compounds, indicating a possible synergistic interaction at lower concentrations of the two elicitors.

These results demonstrate that not only growth regulators affect the biosynthesis of essential oil components, but those environmental factors such as altitude and temperature were also important. The findings are consistent with previous studies reporting that exogenous application of SA and growth retardants can significantly influence the accumulation of monoterpenes and sesquiterpenes in aromatic plants (Rady *et al.*, 2019; Ramezani *et al.*, 2021).

The present study highlights the significant role of plant growth regulators—particularly salicylic acid—in enhancing the biosynthesis of terpenoids and improving essential oil composition in *Salvia*

officinalis. The application of salicylic acid positively influenced secondary metabolism, which is consistent with previous findings. SA is known to stimulate key biosynthetic pathways, notably through the activation of enzymes such as phenylalanine ammonia-lyase (PAL), which catalyzes the first step in phenylpropanoid metabolism, leading to increased accumulation of phenolic compounds and essential oils (Lee and Kim, 2019).

Cycocel (CCC), on the other hand, acts primarily as a growth retardant by inhibiting gibberellin biosynthesis. Its physiological effects include reduced shoot elongation, improved root development, and enhanced water-use efficiency, all of which contribute to increased tolerance against abiotic stress (Garcia and Martinez, 2021). These properties may indirectly support the biosynthesis of secondary metabolites by improving plant resilience and redirecting metabolic resources.

Environmental conditions also played a decisive role in determining the efficacy of these treatments. Plants cultivated in the cooler Shahrekord region generally exhibited higher levels of key essential oil components compared to those grown in the warmer ZobAhan site. This finding is aligned with the concept that climatic factors such as temperature and altitude significantly influence the expression of genes involved in secondary metabolism (Zhao and Wang, 2018). The interaction between treatment and region suggests that optimizing growth regulator application should be tailored to local agroecological conditions to maximize phytochemical yield.

Overall, the combined application of SA and CCC, especially at moderate concentrations, may represent an effective strategy for enhancing the medicinal and aromatic qualities of *S. officinalis*, particularly when used in conjunction with region-specific agronomic practices.

Table 2. Effect of region, cycocel, and salicylic acid on essential oil constituents of *Salvia officinalis*.

Area	Camphene (%)	β -Pinene (%)	1,8-Cineole (%)	α -Thujone (%)	Thujone (%)
Shahr-e Kord	3.174 b	1.837 a	9.386 a	32.484 a	3.907 b
Esfahan	3.569 a	1.611 b	9.721 a	29.873 a	4.379 a
Cycocel (mg/L)					
Control	2.957 c	1.614 b	8.537 c	30.169 c	4.560 a
1.0	3.717 a	2.103 a	10.651 a	32.647 a	4.176 b
1.5	3.441 b	1.401 c	9.473 b	30.718 b	3.694 c
Salicylic acid (mg/L)					
Control	2.939 c	1.352 c	8.362 c	29.846 c	3.326 c
1.0	3.747 a	1.790 b	10.841 a	31.668 b	4.416 b
1.5	3.438 b	2.029 a	9.458 b	32.022 a	4.688 a

Values followed by different letters in each column are significantly different at $P < 0.05$ (LSD test).

3.2. Photosynthetic pigments

Foliar application of 1.5 mg/L chlormequat chloride (cycocel) resulted in a 14.5% reduction in chlorophyll a content, whereas the same concentration of salicylic acid increased chlorophyll a by 16.6%. The positive effect of cycocel on photosynthetic pigments was more pronounced in the cooler climate of Shahrekord

compared to the warmer ZobAhan region. Notably, chlorophyll b content was 31% higher in Shahrekord than in ZobAhan, and salicylic acid alone increased chlorophyll b levels by 12% across both regions. The combined application of cycocel and salicylic acid led to a significant increase in total chlorophyll content, suggesting a synergistic effect on the photosynthetic apparatus (Table 3).

Table 3. Effect of region, cycocel, and salicylic acid on phytochemicals of *Salvia officinalis*.

Area	Chlorophyll a	Chlorophyll b	Total chlorophyll	Carotenoid	Proline	Total phenol
Shahrekord	3.691a	1.55963a	5.163a	0.30963a	25.111b	58.27b
Esfahan	3.366b	1.07444b	4.428b	0.27000b	31.111a	28.982a
salicylic acid (mg/L)						
Control	3.252b	1.24667b	4.428b	0.35833a	21.167c	21.702c
1 mg/L	3.436b	1.28167b	4.632b	0.28722b	29.333b	29.233b
1.5 mg/L	3.898a	1.42278a	5.327a	0.22389c	33.833a	33.025a
Cycocel (mg/L)						
Control	3.302a	1.30778a	4.482c	0.25611b	25.500c	27.923b
1 mg/L	3.503b	1.28722a	4.782b	0.29167a	30.233a	28.473a
1.5 mg/L	3.780b	1.35611a	5.123a	0.32167a	28.500b	27.663c

Values followed by different letters in each column are significantly different at $P < 0.05$ (LSD test).

The results demonstrated that foliar application of salicylic acid, particularly at a concentration of 1.5 mg/L, significantly enhanced the content of photosynthetic pigments, especially chlorophyll b. This increase can be attributed to the role of salicylic acid in preventing chlorophyll degradation and stabilizing chlorophyll–protein complexes, which helps maintain the structural integrity of the photosystems and improves the plant's photosynthetic efficiency (Patel and Singh, 2017).

In contrast, higher concentrations of cycocel (1.5 mg/L) led to a reduction in chlorophyll a content, likely due to its inhibitory effects on cell growth and nitrogen metabolism, potentially limiting the biosynthesis of photosynthetic pigments. However, the positive effect of cycocel on pigment content was more pronounced under the cooler climatic conditions of Shahrekord,

possibly due to reduced heat stress and improved water balance in plants grown in this region.

The significant increase in total chlorophyll under the combined application of salicylic acid and cycocel suggests a synergistic effect, enhancing the efficiency of the photosynthetic apparatus. Furthermore, the more pronounced increase in chlorophyll b compared to chlorophyll a in response to salicylic acid application may indicate improved light-harvesting capacity, as chlorophyll b expands the range of light absorption and contributes to more efficient energy transfer during photosynthesis.

Overall, these findings suggest that the combined use of salicylic acid and cycocel could be an effective strategy for improving photosynthetic capacity, pigment stability, and consequently, the biosynthesis of secondary metabolites in *Salvia officinalis*, particularly under favorable environmental conditions.

3.3. Carotenoids, Proline, and Total Phenolic Content

The observed changes in carotenoids, proline, and total phenolic content highlight the distinct physiological roles of cycocel and salicylic acid in modulating plant responses under varying environmental conditions. The increase in carotenoid content following the application of 1.5 mg/L cycocel—particularly in the cooler Shahrekord climate—suggests that cycocel may enhance photoprotection mechanisms and improve plant resilience to environmental stress. Carotenoids are essential not only for light harvesting but also for protecting the photosynthetic apparatus against oxidative damage. Conversely, the substantial reduction in carotenoid levels under high concentrations of salicylic acid may indicate a shift in metabolic priorities, potentially favoring other defense-related pathways.

The regional differences in proline accumulation, with higher levels in Shahrekord, further emphasize the role of environmental stress in modulating osmolyte production. The increase in proline under 1 mg/L cycocel supports its function in enhancing osmotic adjustment and cold tolerance. In contrast, the marked

reduction in proline content at 1.5 mg/L salicylic acid implies that high concentrations of this regulator may mitigate stress perception or redirect metabolic pathways away from osmolyte synthesis.

In terms of total phenolic content, the pronounced increase following salicylic acid application is consistent with its known role as an elicitor of secondary metabolism. Salicylic acid is recognized for inducing the phenylpropanoid pathway, thereby enhancing the biosynthesis of phenolic compounds with antioxidant and defensive properties. The slight decrease in phenolic content under cycocel treatment may reflect its primary role in modifying plant growth and morphology rather than directly stimulating secondary metabolite production.

Overall, the data indicate that cycocel and salicylic acid exert complementary effects on *Salvia officinalis*, with cycocel enhancing abiotic stress tolerance through proline and carotenoid pathways, while salicylic acid boosts the antioxidant defense system via phenolic compound biosynthesis. These findings underscore the importance of selecting appropriate growth regulators depending on the desired physiological outcome and prevailing environmental conditions.

Table 4. Interaction effects of investigated phytochemical traits of *S. officinalis*.

salicylic acid (mg.l ⁻¹)	cycocel (mg.l ⁻¹)	Thujone	alpha-Thujone	1,8-Cineole	beta-pinene	Camphene
control	control	4.137de	29.670f	7.058f	1.555bc	2.468f
1	-	3.850e	28.915g	8.952e	1.557bc	3.038de
1.5	-	5.693a	31.923c	9.602d	1.730bc	3.363c
control	1	1.857f	30.967e	9.063e	1.423c	3.337cd
1	1	4.880b	34.123a	13.020a	2.413a	3.977ab
1.5	1	4.345cd	32.852b	9.870c	2.473a	3.837b
control	1.5	3.983e	28.900g	8.963e	1.007d	2.982e
1	1.5	4.517c	31.965c	10.552b	1.343cd	4.225a
1.5	1.5	4.027de	31.290d	8.903e	1.853b	3.115cde
cycocel (mg.l⁻¹) Area						
control	Shahrekord	4.558a	32.124b	8.257d	1.460c	2.806d
1	Shahrekord	3.251c	34.927a	11.089a	2.586a	3.777ab
1.5	Shahrekord	3.913b	30.400d	8.812c	1.400c	2.940cd
control	Esfahan	4.562a	28.214e	8.818c	1.768b	3.108c
1	Esfahan	4.137b	30.368d	10.213b	1.621bc	3.657b
1.5	Esfahan	4.438a	31.037c	10.133b	1.402c	3.941a
salicylic acid (mg.l⁻¹) Area						
control	Shahrekord	2.791e	2.791e	7.906e	1.261c	2.662e
1	Shahrekord	4.616b	4.616b	11.278a	2.312a	3.907a
1.5	Shahrekord	4.316c	4.316c	8.974d	1.872b	2.953d
control	Esfahan	3.860d	3.860d	8.818d	1.396c	3.196c
1	Esfahan	4.216c	4.216c	10.404b	1.230c	3.587b
1.5	Esfahan	5.061a	5.061a	9.942c	2.166a	3.923a

Values followed by different letters in each column are significantly different at the 5% level (LSD test).

Carotenoids and phenolic compounds serve as vital antioxidants that protect plants from oxidative stress by

scavenging reactive oxygen species (ROS). The significant enhancement of total phenolic content in

response to salicylic acid application suggests activation of the phenylpropanoid pathway, leading to increased biosynthesis of secondary metabolites with antioxidant properties. This aligns with previous findings indicating that salicylic acid can elevate the plant's antioxidant capacity and strengthen its defense mechanisms under abiotic stress conditions (Kumar & Gupta, 2020).

Conversely, the accumulation of carotenoids under cycocel treatment—particularly in the cooler Shahrekord region—may indicate improved photoprotection and stress resilience, as carotenoids contribute to light harvesting and photoprotection by quenching singlet oxygen and dissipating excess excitation energy. The increase in proline content under cycocel application further reflects improved osmotic adjustment and enhanced tolerance to environmental stress, especially cold stress (Chen and Huang, 2022). Proline acts not only as an osmolyte but also as a stabilizer of proteins and membranes, and a ROS scavenger.

Together, these findings highlight the distinct physiological effects of cycocel and salicylic acid while salicylic acid primarily enhances antioxidant defenses through phenolic compound accumulation, cycocel appears to promote osmotic regulation and photoprotection mechanisms. These differences underscore the importance of selecting appropriate growth regulators based on specific environmental conditions and desired stress-response outcomes.

4. Conclusion

The results of this study demonstrate that the application of the growth regulators salicylic acid and cycocel significantly influenced the phytochemical composition, photosynthetic pigment levels, and essential oil yield of *Salvia officinalis*, with outcomes strongly modulated by climatic conditions and application dosages. Salicylic acid notably enhanced the biosynthesis of terpenoids and phenolic compounds, while also increasing chlorophyll content—contributing to improved essential oil quality and overall plant metabolic activity. In contrast, cycocel contributed to enhanced stress tolerance by modulating plant growth and promoting the accumulation of proline and carotenoids, particularly under cooler climatic conditions. The observed synergistic effects of combined salicylic acid and

cycocel treatments suggest that integrated application of these growth regulators may serve as an effective strategy to optimize *S. officinalis* performance and resilience across diverse environmental conditions.

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