# Essential Oil Compositions and Photosynthetic Pigments Content of *Pelargonium graveolens* in Response to Nanosilver Application

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## Abstract

**Background: Nanosilver particles are among the potentially candidates for modulating the redox status and changing the growth, performance and quality of plants because of their physicochemical characteristics.**

**Objective: This study was carried out to elucidate the potential effects of nanosilver particles application on variations of photosynthetic pigments (chlorophyll and carotenoids) and essential oil content and composition of *Pelargonium graveolens*.**

**Methods: Four nanosilver particles (5 - 20 nm) concentration (0, 20, 40, and 80 mg.L$^{-1}$) were used as foliar application. The essential oils were isolated from aerial flowering parts of the plants by hydro-distillation method and then subjected to GC and GC-MS apparatus to determine the oil constituents.**

**Results: Chlorophyll and carotenoids content increased in leaves of nanosilver treated plants in compare with the control. Nanosilver application at 40 mg.L$^{-1}$ was the most effective treatments in pigments and essential oil content. Also, results showed that 26 components were identified in essential oil and citronellol was the major component under all employed treatments. Maximum citronellol/geraniol ratio was obtained at 80 mg.L$^{-1}$ nanosilver treatment, indicating the positive influence of nanosilver particles on essential oil quality of *Pelargonium* plants.**

**Conclusion: The nanosilver particles application could maintain and enhanced the photosynthetic pigments and essential oil content of *P. graveolens* plants. Our findings suggested that nanosilver application improved quality and quantity of essential oil.**

**Keywords: *Pelargonium graveolens*, Carotenoids, Citronellol, Essential oil Chlorophyll, Nanosilver**

# Introduction

*Pelargonium graveolens* (Geraniaceae), with common name geranium, is one of the most important sources of essential monoterpene oil (s) called the oil of geranium. The aerial flowering parts contained essential oil with several medicinal values (as antidepressant, antiinflammatory effects [1]. Its oils are also used in fragrance industry due to its strong rose-like odor, especially in soap industry. In addition, geranium leaves are known to have antifungal activity and repel insects [1]. Essential oil of geranium mainly consisted of monoterpenol, geraniol, citronellol and linalool and their esters and aldehydes [2]. The production of essential oils not only depends on the metabolic state and present developmental differentiation program of the synthesizing tissue, but also is highly integrated with the physiology of the whole plant. Besides, production the oil of is due to ecophysiological, environmental and other factors [3].

The development of nanotechnology in physiology and biochemistry fields has expanded the application area of nanomaterials in different fields due to their unique characteristics. Among the latest line of technological innovations, nanotechnology offers a prominent function in improving the existing plant management techniques [4]. However in the field of medicinal plants, the use of nanomaterials is relatively new and needs more researches. In recent years, many researchers have studied the effects of nanomaterials on seed germination and plant growth with the aim of promotion of its use for agricultural applications. Most of these studies are focused on the potential toxicity of nanoparticles on higher plants and both positive and negative or inconsequential effects presented. As reported by Lu et al. [5] treatment of soybean (*Glycine max*) plants with a mixture of nano $Sio_2$ and $Tio_2$ increased nitrate reductase activity, stimulate its antioxidant system, and accelerate its germination and growth. While, it is reported that nano ZnO could be one of the most toxic nanoparticles which stop root growth of examined plants [6]. Recently, one of the studies has reported that silver nanoparticle treatment of *Brassica juncea* seedlings induced the activities of specific antioxidant enzymes [7]. Also, a study has reported that nanoparticles ($TiO_2$) improved seed germination and plant growth [8]. Furthermore, the presence of these nanoparticles has induced the increasing in the dry weight, chlorophyll synthesis, and metabolisms in photosynthetic organisms [9]. Therefore, there is potential for expanding the range of nanoparticles use for improvement of physiological and morphological characteristics of plants [10, 11]. Previous studies also have showed that nanoparticles ameliorate accumulation of malondialdehyde content by induction of plant antioxidant systems [12].

Recently, Aghajani et al., reported that moderate levels of nanosilver concentrations (up to 60 ppm) had the highest effect on *Thymus kotschyanus* Boiss. & Hohen essential oil yield and percentage; while nanosilver dose at 100 ppm had the minimal positive impact on them [13].

However, the mechanism of action of these nanoparticles has not been completely established yet. No previous literature reviews exist about the impact of nanoparticles on physiology and metabolism of *p. graveolens*, and their possible positive or negative effects on secondary metabolites production.

Therefore, the present study was carried out to elucidate the potential effects of nanosilver particles application on variations of photosynthetic pigments (chlorophyll and carotenoids), essential oil content and composition of *Pelargonium graveolens*.

# Materials and Methods

## Plant materials

*Pelargonium gravolens* stock was obtained from the research farm nursery of the department of medicinal plants in Arak University, Arak, Iran. Thereafter, the uniform plant cuttings (5 - 7 cm in height with 2 - 3 leaves) were taken from the stems of source plants and were initially planted in pots (10 × 25 cm) filled with soil, cocopeat and peat (0.5:1:1) at the rate of one cutting per pot. Subsequently, cuttings were grown in the greenhouse at the University of Guilan, Rasht, Iran, under normal environmental conditions: 25 ºC day/17 ºC night temperatures, natural light (16 h light: 8 h dark), 75% relative humidity (RH). Then, three month-old uniforms plants (before blooming) were sprayed with nanosilver particles treatments.

## Nanosilver particles characterization and treatments

Nanosilver particles were purchased from Iranian Nanomaterials Pioneers Company, NANOSANY (Mashhad, Iran). The size of NS particles was estimated to be 5 - 20 nm in diameter, metal basis and spherical. Transmission Electron Microscope (TEM) image and size distribution graph of nanosilver particles were showed in Figure 1. Deionised water was used to prepare 20, 40, and 80 $mg.L^{-1}$ nanosilver solutions. Control plants were only treated with deionised water. Whole plants foliage (both sides of the leaves, and stems) were sprayed with equal amounts of 50 ml aqueous solution of nanosilver by hand atomizer. All measurements were made in the end of blooming stage.

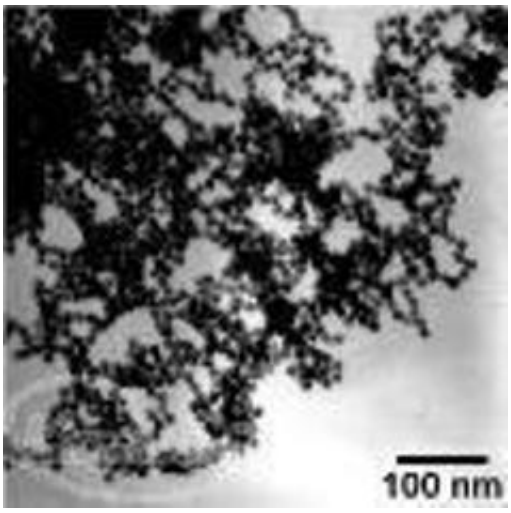


**Figure 1- Transmission Electron Microscope (TEM) image of spherical nanosilver particles from Iranian Nanomaterials Pioneers Company, NANOSANY**

**Pigments measurement**

To extract chlorophyll and carotenoids, 0.5 g of fresh leaf (which is taken from 3rd leaf of growing point) was homogenized in 0.5 mL of acetone (80% V/V). The extract was read at 645 nm (Chlorophyll *α*), 663 nm (Chlorophyll *b*) and 470 nm (carotenoids) in an UV-160 spectrophotometer. Measurements were performed on twenty leaves per treatment (n = 20). Pigments content were calculated using the equations suggested by Lichtenthaler [14] as below:

Chl *α* ($mg.g^{-1}$ fw) = $11.75 \times A_{663} - 2.35 \times A_{645}$

Chl *b* ($mg.g^{-1}$ fw) = $18.61 \times A_{645} - 3.96 \times A_{663}$

Carotenoids ($mg.g^{-1}$ fw) = $4.69 \times A_{470} - 0.268 \times (20.2 \times A_{645} + 8.02 \times A_{663})$

**Essential oil extraction**

In order to extraction of essential oil, aerial parts of *P. graveolens* were collected at the full flowering stage. The oils were extracted by hydrodistillation of air-dried samples using a Clevenger-type apparatus in 3 hours. The oil samples were collected and their volumes measured before drying over anhydrous sodium sulphate. Oil yield was computed by multiplying the fresh shoot yield by the oil content [15].

**Essential oil analysis procedure**

GC-FID analysis was performed using a Thermoquest gas chromatograph, TRACE GC, with a flame ionization detector (FID). The analysis was carried out using fused silica capillary DB-5 column (60 m × 0.25 mm i.d.; film thickness 0.25 μm). The operating conditions were as follows: injector and detector temperatures, 250°C and 300°C, respectively; oven temperature, 60 - 250°C at the rate of 5°C/min and finally held isothermally for 10 min; carrier gas, $N_2$ at a flow rate of 1.0 ml/min; split ratio, 150. GC-MS analysis was performed using a ThermoQuest-Finnigan gas chromatograph equipped with above mentioned column and coupled with a TRACE mass quadrupole analyzer (Manchester, UK). Helium was used as carrier gas with ionization voltage of 70 eV. Ion source and interface temperatures were 200°C and 250°C, respectively. Mass range was from m/z 43- 456. Gas chromatographic conditions were as given for GC-FID. The constituents of the essential oil were identified by calculating the retention indices under temperature-programmed conditions for n-alkanes (C6-C24) and the oil on a DB-5 column under the same chromatographic conditions. The identification of individual compounds was made by comparison of their mass spectra with those of the internal reference mass spectra library or with authentic compounds and confirmed by comparing their retention indices with authentic compounds or with those reported in the literature. For quantification purpose, relative area percentages obtained by GC-FID were used without the use of correction factors [16].

**Statistical Analysis**

Data analyses were performed based on a completely randomized design (CRD) with three replications (n=3). Data were subjected to analysis of variance (ANOVA) with the general linear model (Proc GLM) using computer SAS software, version 9.1 (CoHort Software). Multiple comparisons among treatment means was done by Tukey's Honest Significant Difference (HSD) test at P = 0.05.

# Results

**Photosynthetic pigments**

Significant differences ($p < 0.05$) in photosynthetic pigments were found under

employed nanosilver treatments (table 1). Chlorophyll *α*, *b* and total (*α*+*b*) content had an upward trend with increasing nanosilver concentration up to 40 mg.L$^{-1}$ and then followed a rapid decrease with increase of nanosilver treatment. Furthermore, it was also found that the maximum (1.91) and minimum (1.37) ratio between chlorophyll *α* and *b* (*α*/*b*) was calculated at control and 40 mg.L$^{-1}$ nanosilver concentration, respectively. The carotenoids content of the plants depended on nanosilver concentration and increased significantly (35.29% of the control) at 40 mg.L$^{-1}$ nanosilver. But the decrease of carotenoids content under the highest nanosilver treatment was not significantly lower than that of control (table 1).

**Essential oil content, yield and composition**

Foliar application of nanosilver significantly augmented the essential oil content (Figure 2) and yield (table 2) of *P. graveolens* plants compared to the control. The highest essential oil percentage (2.08%) and yield (0.46 g/plant) were obtained at 40 mg.L$^{-1}$ nanosilver treatment. However, there was no significant difference between 40 and 80 mg.L$^{-1}$ nanosilver concentrations on essential oil content of plants (Figure 2). Moreover, the lowest essential oil yield (0.25 g/plant) was recorded at the highest nanosilver concentration (80 mg.L$^{-1}$).

Essential oil composition of *P. graveolens* under different nanosilver concentrations is given in table 2. Generally, a total of 26 compounds, representing 92.4%-98.5% (at 40 and 20 mg.L$^{-1}$ nanosilver, respectively) of the total oil composition were identified in the employed treatments. However, no statistically difference was observed between control untreated plants and 20 mg.L$^{-1}$ nanosilver concentrations on maximum total oil identified. The data on quality of essential oils under different treatments are summarized in table 2. As it can be seen, the oil of *P. graveolens* plants was rich of monoterpens. Interestingly, citronellol was the major component of oils under all employed nanosilver concentrations. The most abundant components of the oil and their variations with treatments were citronellol (48.1-49.3%),

**Table 1- Variations of chlorophyll (Chl *a*, Chl *b*, Chl *a* + *b* and Chl *a/b*) and cartonoids content in *Pelargonium graveolens* under different nanosilver particles concentrations (0, 20, 40 and 80 mg.L$^{-1}$). Values represent mean ± SD (n=3)**

| Photosynthetic pigments (mg.g$^{-1}$ fw$^{-1}$) | Nanosilver (mg.L$^{-1}$) | | | |
|---|---|---|---|---|
| | 0 | 20 | 40 | 80 |
| *Chl a* | 0.044 ± 0.004 | 0.065 ± 0.06 | 1.048 ± 0.053 | 0.021 ± 0.005 |
| *Chl b* | 0.023 ± 0.006 | 0.035 ± 0.004 | 0. 76 ± 0.032 | 0.011 ± 0.007 |
| *Chla+b* | 0.067 ± 0.008 | 0.010 ± 0.005 | 1.124 ± 0.046 | 0.032 ± 0.009 |
| *Chl a/b* | 1.91 ± 1.10 | 1.85 ± 0.82 | 1.37 ± 0.23 | 1.9 ± 1.10 |
| *Caretonoids* | 0.022 ± 0.005 | 0.028 ± 0.007 | 0.034 ± 0.009 | 0.021 ± 0.011 |

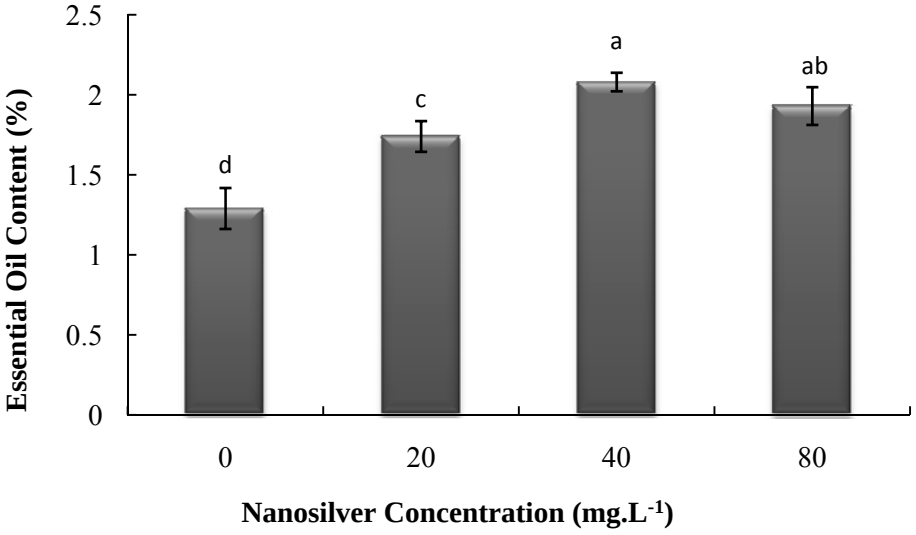


**Figure 2- Effect of different nanosilver concentrations on *Pelargonium graveolens* essential oil content. The error bars represent the standard deviation (±SD) for three replications (n=3). Different letters indicate significant differences in each treatment as determined by Tukey's Honest Significant Difference (HSD) test at P = 0.05**

**Table 2- Essential oil composition of *Pelargonium graveolens* under different nanosilver (NS) particles concentrations (0, 20, 40 and 80 mg.L$^{-1}$). The data presented as mean ± standard deviation (SD) for three replications (n=3)**

| No. | RI | Compound | $NS_0$ | $NS_{20}$ | $NS_{40}$ | $NS_{80}$ | Formula |
|---|---|---|---|---|---|---|---|
| 1 | 940 | α-Pinene | tr | 0.3±0.1 | 0.6±0.1 | tr | $C_{10}H_{16}$ |
| 2 | 1028 | *p*-Cymene | tr | tr | tr | tr | $C_{10}H_{14}$ |
| 3 | 1075 | Z-Linalool oxide | tr | 1.5±0.6 | tr | tr | $C_{10}H_{18}O_2$ |
| 4 | 1099 | Linalool | 2.0±0.3 | 1.7±0.4 | 0.8±0.2 | 2.0±0.6 | $C_{10}H_{18}O$ |
| 5 | 1112 | E-Rose oxide | tr | 0.6±0.1 | tr | tr | $C_{10}H_{18}O$ |
| 6 | 1130 | Z-Rose oxide | tr | tr | tr | tr | $C_{10}H_{18}O$ |
| 7 | 1152 | Citronellal | tr | 0.1±0.03 | tr | tr | $C_{10}H_{18}O$ |
| 8 | 1171 | *iso*-Menthone | 6.4±0.5 | 8.7±0.9 | 9.3±1.2 | 7.4±0.8 | $C_{10}H_{18}O$ |
| 9 | 1230 | Citronellol | 48.4±1.2 | 49.3±1.1 | 49.0±0.8 | 48.1±1.3 | $C_{10}H_{18}O$ |
| 10 | 1255 | Geraniol | 6.5±0.4 | 8.7±0.4 | 4.4±1.1 | 4.5±0.2 | $C_{10}H_{18}O$ |
| 11 | 1351 | Cirtonellyl formate | 12.9±1.1 | 15.8±0.9 | 17.9±1.8 | 16.1±2.1 | $C_{11}H_{20}O_2$ |
| 12 | 1390 | Geraniol formate | 1.0±0.4 | 2.2±0.2 | tr | 1.0±0.3 | $C_{11}H_{18}O_2$ |
| 13 | 1401 | Citronellyl acetate | tr | 0.5±0.1 | tr | tr | $C_{12}H_{22}O_2$ |
| 14 | 1465 | E-Caryophyllene | 2.9±0.2 | 2.9±0.1 | 3.0±0.4 | 2.5±0.4 | $C_{15}H_{24}$ |
| 15 | 1472 | 6,3-Guaiadiene | 1.2±0.1 | tr | tr | 1.1±0.1 | $C_{15}H_{24}$ |
| 16 | 1480 | gumma-Muurolene | 0.7±0.1 | 0.6±0.1 | 0.2±0.1 | 0.9±0.1 | $C_{15}H_{24}$ |
| 17 | 1499 | Geranyl propionate | 0.8±0.2 | tr | tr | 0.5±0.1 | $C_{13}H_{22}O_2$ |

**Table 2- Continued**

| No. | RI | Compound | $NS_0$ | $NS_{20}$ | $NS_{40}$ | $NS_{80}$ | Formula |
|---|---|---|---|---|---|---|---|
| 18 | 1513 | Aromadendorene | 2.4±0.4 | 2.3±0.2 | 1.1±0.3 | 2.1±0.2 | $C_{14}H_{26}O_2$ |
| 19 | 1527 | Germacrene-D | 1.5±0.3 | 1.4±0.3 | tr | 1.2±0.1 | $C_{15}H_{24}$ |
| 20 | 1536 | Geranyl propanoate | tr | tr | tr | tr | $C_{14}H_{24}O_2$ |
| 21 | 1559 | Citronellyl butyrate | 5.1±0.7 | tr | 1.2±0.8 | 5.4±0.3 | $C_{14}H_{26}O_2$ |
| 22 | 1572 | delta-Cadinene | 2.7±0.5 | 1.0±0.2 | tr | 2.2±0.6 | $C_{15}H_{24}$ |
| 23 | 1579 | Geranyl butyrate | 0.5±0.1 | tr | tr | 0.2±0.1 | $C_{14}H_{24}O_2$ |
| 24 | 1605 | Phenyl ethyl tiglate | tr | 0.8±0.1 | 0.8±0.1 | tr | $C_{13}H_{16}O_2$ |
| 25 | 1685 | E-Citronellyl tiglate | 2.3±0.9 | 0.1±0.04 | 0.7±0.1 | 2.1±0.8 | $C_{15}H_{26}O_2$ |
| 26 | 1701 | Geranyl tiglate | 0.7±0.1 | tr | tr | 0.1±0.1 | $C_{15}H_{24}O_2$ |
| | | **Oil Yield (gr.plant$^{-1}$)** | 0.31±0.08 | 0.38±0.03 | 0.46±0.04 | 0.25±0.01 | |
| | | Monoterpene hydrocarbons | tr | 0.3±0.1 | 0.4±0.1 | tr | |
| | | Oxygenated monoterpenes | 77.2±3.14 | 89.1±2.16 | 80.6±2.51 | 79.1±2.72 | |
| | | Sesquiterpene hydrocarbons | 17.8±1.2 | 8.2±1.4 | 10.5±1.6 | 16.1±1.3 | |
| | | Oxygenated sesquiterpenes | 3.0±0.9 | 0.1±0.1 | 0.8±0.1 | 2.3±0.4 | |
| | | Other compounds | tr | 0.8±0.2 | 0.1±0.1 | tr | |
| | | Total identified (%) | 98.0±1.26 | 98.5±1.15 | 92.4±2.28 | 97.5±2.1 | |

RI: Retention Index. tr: trace value (< 0.05%)

citronellyl formate (12.9-17.9%), *iso*-menthone (6.4-9.3%), geraniol (4.4-8.7%), E-caryophyllene (2.5-3%), linalool (0.8-2.0%) and E-citronellyl tiglate (0.1-2.3%). It was observed from the data that the essential oil composition of *P. graveolens* was significantly affected by nanosilver application. The maximum and minimum citronellol and geraniol content were found at 20 and 40 mg.L$^{-1}$ nanosilver treatments, respectively. Also, linalool and citronellyl butyrate content decreased steadily with increasing nanosilver levels until 40 mg.L$^{-1}$ and then followed a rapid increase.

# Discussion

Application of nanotechnology is widely distributed overall the life now, especially in agricultural systems. Physicochemical characteristics of nanoparticles including large surface area to volume ratio, ability to engineer electron exchange and high catalytic capabilities can imprive favorable interactions with various biomolecules in a cell. Nanosilver particles are among the most potential candidates for modulating the redox status of plants, because of their ability to support electron exchange with two elements, $Fe^{2+}$ and $Co^{3+}$, which participate in several biological redox reactions [17].

Our current experiment confirmed that nanosilver application could improve pigments status of *P. graveolens* plants. Also, the minimum ratio between chlorophyll *α* and *b* under 40 mg.L$^{-1}$ nanosilver concentrations indicate that chlorophyll *b* was the better improved pigment than chlorophyll *α* under

such conditions. In our study, only plant exposure to the highest nanosilver concentration resulted in reduction in the content of pigments when compared with the other nanosilver treatments (table 1). It has been recently reported that nanosilver treatments increased the chlorophyll contents (40% in chlorophyll *α* and 25% in total chlorophyll) of *Brassica juncea* seedlings at 100 $mg.L^{-1}$ nanosilver concentration [18]. They have also stated that improved quantum efficiency in the leaves of nanosilver treated seedlings positively correlates with higher chlorophyll content. Research on the influence of magnetic nanoparticles in maize crops revealed that the chlorophyll *α* increased at low ferro fluid concentrations, while a reverse effect was observed with increasing concentration of solution [19]. It is reported that higher chlorophyll levels coupled with an increase in photosynthesis might have contributed to increased levels of carbohydrates, and higher levels of carbohydrate and their possible diversion to secondary metabolism might contribute to elevated levels of the essential oil in the geranium plants [20].

The essential oil of geranium is one the most important items in the perfumery, cosmetic, food and pharmaceutical industries [21]. The geranium oils are characterized by presence of citronellol, geraniol, *iso*-menthone, linalool and wide range of esters such as geranyl formate, citronellyl formate, geranyl acetate and geranyl propionated [22]. The first two main oil compound (citronellol and geraniol) produced from same precursor (geranyl pyrophosphate) but it is presumed that different enzymes produced these two alcohols. This could be the reason of variation in the content of citronellol and geraniol with respect to the employed treatments. Citronellol/geraniol ratio (C/G) in *Pelargonium* species oils has been previously reported [23]. It is imperative to mention that the geranium oil possesses C/G ratio equivalent to one which is considered as the oil with best odor quality and hence, preferred by industry [24]. In our current research, maximum C/G ratio (10.68) was obtained at 80 $mg.L^{-1}$ nanosilver treatment, indicating the potential positive influence of nanosilver particles on essential oil quality of *Pelargonium* plants.

It has been demonstrated that signal molecules are very potential elicitors for induction of plant secondary metabolites [25]. Recent years, the applications of signal components as elicitors have evolved an effective strategy for the production of target secondary metabolites in plant cell cultures. However, it is still uncommon for commercial application [25]. It therefore, suggested that application of elicitors *in vivo* is an easy and direct channel to promote the yield of plant secondary metabolites at the whole plant scale. Nanomaterials could as signal molecule to generate metabolic and physiological responses. However, the mechanism of these nanoparticles has not been completely established yet. No previous literature reviews exist about the impact of nanoparticles on physiology and metabolism of medicinal plants, and their possible effects on secondary metabolites production.

# Conclusion

According to these results, it can be concluded that using appropriate nanosilver particles concentration to maintain and enhance the photosynthetic pigments and improve the essential oil quality and quantity, could be a new strategy in the field of secondary metabolites production.

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