



Positive effect of AgNPs and AuNPs in in vitro cultures of *Lavandula angustifolia* Mill.

Paula Jadczyk¹ · Danuta Kulpa¹ · Magdalena Bihun² · Włodzimierz Przewodowski³

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Abstract

The aim of this study was determined how an addition of gold nanoparticle (AuNPs) and silver NPs (AgNPs) into culture media affects plant development and formation of oil glands in narrow-leaved lavender (*Lavandula angustifolia*) cv. 'Munstead'. Plant shoots were propagated on media supplemented with 1, 2, 5, 10, 20 and 50 mg dm⁻³ AuNPs or AgNPs (diameter of 24.2 ± 2.4 nm and 27.5 ± 4.8 nm). Both of NPs positively influenced the growth and development of lavender propagated in vitro. The culture media with NPs stimulated formation of shoots and increased plant weight. Roots of plants propagated on the media supplemented with NPs were usually longer than those in the control. Only high concentrations of NPs (20 and 50 mg dm⁻³) in the culture media were toxic to plants, as demonstrated by restricted shoot length and gradual decrease in the value of other morphological features. Increases in AgNPs concentration caused the number of secretory trichomes to decrease. The diameter of the trichomes on both sides of the leaf blade was larger when the plants were propagated on the media supplemented with 1 and 2 mg dm⁻³ NPs. The diameter of trichomes formed on the adaxial surface of the leaf blade was greatest in the media enriched with 2 mg dm⁻³ AgNPs and 5 mg dm⁻³ AuNPs, and smallest in the media enriched with 5 mg dm⁻³ AgNPs. The diameter of trichomes formed on the abaxial surface was largest in plants exposed to 1, 2, 5 and 10 mg dm⁻³ AuNPs, 1 mg dm⁻³ AgNPs, and smallest in plants exposed to 5 mg dm⁻³ AgNPs.

Key message

Silver and gold nanoparticles had a significantly improved the growth and development of narrow-leaved lavender propagated in vitro. The number and size of secretory trichomes formed on the leaves of narrow-leaved lavender grown in in vitro cultures depends on the concentration of silver or gold nanoparticles in the media.

Keywords Micropropagation · Metal nanoparticles · Oil glands · Nanosilver · Nanogold

Abbreviations

NPs Nanoparticles
AgNPs Silver nanoparticles

AuNPs Gold nanoparticles
MS Murashige and Skoog medium
KIN Kinetin
IAA 3-Indoleacetic acid

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✉ Paula Jadczyk
paula.jadczyk@zut.edu.pl; paulajadczyk@gmail.com

¹ Department of Plant Genetics, Breeding and Biotechnology, Faculty of Environmental Management and Agriculture, West Pomeranian University of Technology in Szczecin, Słowackiego 17, 71-434 Szczecin, Poland

² Molecular Biology and Biotechnology Center, Laboratory of Environmental Research, University of Szczecin, Małkocin 37, 73-110 Stargard, Poland

³ Plant Breeding, Acclimation Institute in Bonin National Research Institute, Poland, Bonin 3, 73-009 Bonin, Poland

Narrow-leaved lavender (*Lavandula angustifolia* Mill.) belonging to *Lamiaceae* family is an evergreen perennial plant with a high number of beneficial properties. The herb is native to the Mediterranean region, but is also grown in many other countries in the world (Wesołowska et al. 2019). Lavender infusions show carminative, diuretic, anti-rheumatic and anti-epileptic properties, and are effective painkillers, especially for nervous headaches and migraines (Gilani et al. 2000). Essential oils and extracts of the plant demonstrate antibacterial and antifungal properties (D'Auria et al. 2005). Apart from medicinal applications, lavender is

highly popular in the cosmetics, perfume and aromatherapy or food industries (Gonçalves and Romano 2013). The most important substance isolated from lavender is the essential oil produced by the oil glands located on the surface of the calyx, in furrows between fine hairs (Wornouk et al. 2011). The composition of lavender essential oils depends on many factors such as: genotype, growing location, climatic conditions, propagation and morphological characteristics (Wornouk et al. 2011; Andrys and Kulpa 2018).

Plant tissue cultures, being an alternative to conventional cultivation, ensure rapid and large scale production of valuable biologically active compounds. This technique yields contamination-free material of the highest quality. The elicitation, i.e. using natural plant defense mechanisms against various types of threats (e.g. pathogens), is a common treatment used in in vitro cultures for obtaining secondary metabolites (Andrys et al. 2018). The stress factors, known as elicitors, stimulate secondary metabolite formation in plant cell cultures, thus reducing processing time and providing a high concentration of the product (Mulabagal and Tsay 2004). Nanoparticles (NPs) of heavy metals may be successfully used as elicitors in in vitro cultures. The studies by Hatami et al. (2016) and Ghanti and Somayeh (2013) showed that treating plants growing under natural conditions with metal NPs changed both the content and composition of their essential oils. Recent studies confirmed the suitability of silver NPs (AgNPs) as elicitors in plant tissue cultures (Ghanti and Somayeh 2013, 2014). The micropropagation of lavender on media supplemented with AgNPs and gold nanoparticles (AuNPs) resulted in significant changes in the composition of essential oils isolated from its tissues (Wesołowska et al. 2019). This may indicate the possibility of using these molecules as elicitors. Research performed in different plants demonstrated the positive effects of NPs (El-Temsah and Joner 2012; Mirzajani et al. 2013; Priyadarshini et al. 2014; Salama 2012). However, Yang and Watts (2005) reported root elongation inhibition by alumina particles in *Zea mays*, *Cucumis sativus*, *Glycine max*, *Brassica oleracea* and *Daucus carota*. In order to use metal NPs as elicitors in in vitro cultures, the concentration and type of NPs need to be precisely selected to ensure that the irritant effect is not too toxic for plants.

The aim of this study was to find out how the addition of AuNP and AgNPs to the culture media affects the development of *L. angustifolia* Mill. and its specific structures (secretory glands) when propagated in vitro. The experiment provided useful information on the possibilities of using AuNP and AgNPs as elicitors in the propagation of narrow-leaved lavender in plant tissue cultures.

Materials and methods

In this study, we examined material from plants of narrow-leaved lavender (*L. angustifolia* Mill.), cv. Munstead. Single-node shoot fragments 1–1.5 cm long were placed in 300 ml glass jars filled with 30 ml of a Murashige and Skoog (MS) medium (Murashige and Skoog 1962), supplemented with 2 mg dm⁻³ KIN and 0.2 mg dm⁻³ IAA and AuNPs or AgNPs in concentrations of 1, 2, 5, 10, 20 and 50 mg dm⁻³. We used aqueous suspensions of AuNP and AgNPs (with diameters of 24.2 ± 2.4 nm and 27.5 ± 4.8 nm), which were synthesized using Turkevich et al. (1951) and Liu et al. (2003) methods with modified synthesis conditions and two-stage microwave-convection heating. Media also contained 30 g dm⁻³ of sucrose, 100 mg dm⁻³ of inositol, and were solidified with agar at 7 g dm⁻³. The pH was set at 5.7 with 0.1 M HCl and NaOH. The jars were sterilized at 121 °C for 20 min. Once they contained cultures, the jars were kept in a phytotron (humidity 70–80%, temperature 24 °C, illumination for 16 h a day at 35 µE M⁻² s⁻¹ PAR). Biometric features such as shoot length (cm), shoot number, aboveground mass (g), underground mass (g), root length (cm) and percentage of rooted plants were measured after 4 weeks of cultivation. Micromorphology of the leaves was analyzed with a scanning electron microscope (SEM). Central sections of the leaves were dried in a Critical Point dryer (Quorum Technologies, Germany) and sprayed with gold in the Sputter Coater (Quorum Technologies, Germany). Observations were conducted using the Carl Zeiss EVO LS 10 microscope with accelerating voltage 1 or 15 kV. Trichome diameter and number on both the adaxial and abaxial surfaces of the blades were determined in field-grown and in vitro propagated plants.

The experiment was set in a one-factor completely randomized design. The significance of differences was determined by analysis of variance and the Tukey's *t* test at *p* = 0.05. Homogeneous groups in the examined combinations were labeled with successive letters of the alphabet.

Result

Irrespective of AgNPs concentration, we observed no visible toxic effects such as plant organ necrosis. However, AuNPs at 50 g dm⁻³ caused slight yellowing of leaf blades and changes in their structure (Fig. 1). Enriching the media with AgNPs and AuNPs significantly affected plant development. The plants growing in the presence of the lowest NP concentration (1–5 mg dm⁻³ AgNPs and 1–2 mg dm⁻³ AuNPs) developed shoots of similar length

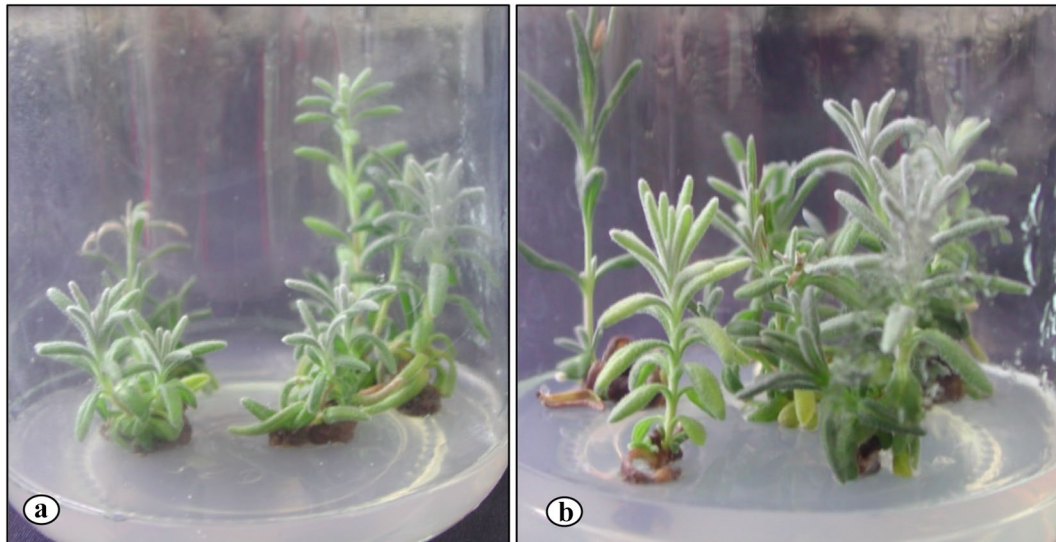


Fig. 1 Lavender propagated on control media (a) and enriched with 50 mg dm^{-3} AgNPs (b)

Table 1 Morphology of lavender plants cultivated in the media enriched with AgNPs and AuNPs

	NPs concentration (mg dm^{-3})	Plant height (cm)	Shoots (pcs)	Plant mass (g)	Root length (cm)	Rooted plants (%)
0	MS (control)	3.17bc	1.59i	0.15g	1.70g	11.8
1	AgNPs	3.36b	2.70gh	0.60ef	4.01b	39.1
2	AgNPs	3.21bc	2.77f-h	0.62ef	2.62f	39.2
5	AgNPs	2.97bcd	3.22e-g	0.80bc	2.38fg	88.9
10	AgNPs	2.68d	3.95bc	0.82bc	4.77a	85.0
20	AgNPs	2.84cd	5.31a	0.81cd	3.62b-d	82.0
50	AgNPs	2.24e	4.05b	0.66de	2.89d-f	84.6
1	AuNPs	3.23bc	2.93e-g	0.75cd	3.40b-e	36.8
2	AuNPs	3.23bc	2.29h	1.24a	3.70bc	37.9
5	AuNPs	3.95a	3.86b-d	0.91b	2.94d-f	38.1
10	AuNPs	2.97bcd	3.33d-f	0.62ef	2.73ef	47.6
20	AuNPs	2.11e	3.25e-g	0.50f	2.50fg	14.3
50	AuNPs	2.19e	3.40c-e	0.54f	3.05c-f	12.5
	LSD _{0.05}	0.39	0.60	0.12	0.73	

a–c Values followed by the same letter are not significantly different at $p \leq 0.05$ according to the LSD (least significant differences) Tukey test

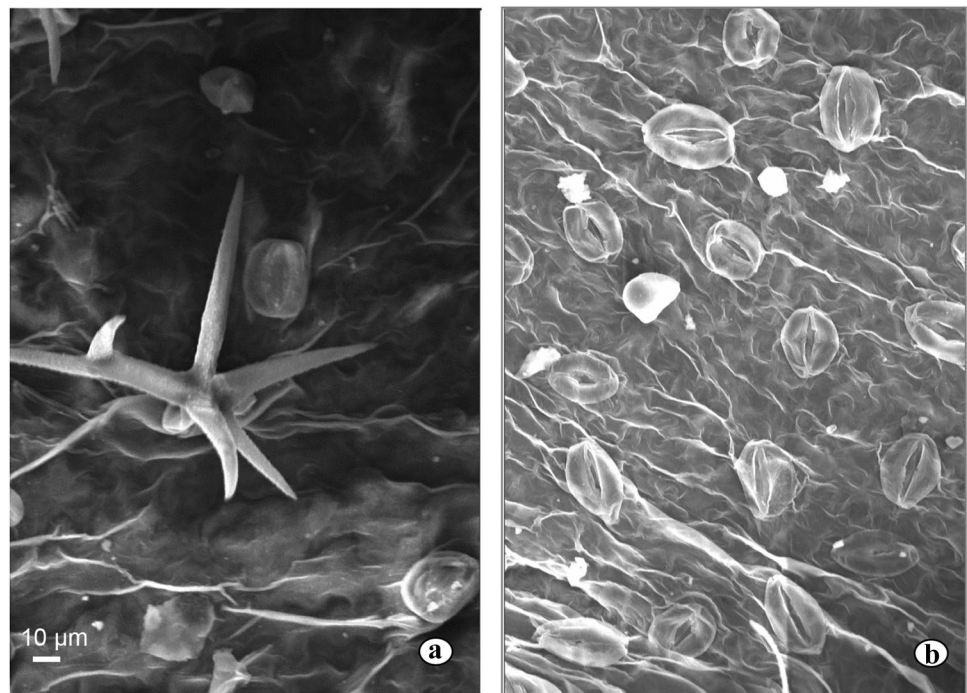
to those in the control (Table 1). Higher concentrations of the NPs reduced plant height. The regenerants cultivated in the presence of 50 mg dm^{-3} AgNPs and 20 and 50 mg dm^{-3} AuNPs developed the shortest shoots, while those cultivated in media supplemented with 5 mg dm^{-3} AuNPs developed the highest. Regardless of their concentration, NPs added to the media increased the formation of lateral shoots. Lavender plants grown in the control media developed the lowest number of lateral shoots (1.59). Among variants cultivated in the media enriched with NPs, plants exposed to the lowest content of AgNPs and AuNPs ($1\text{--}2 \text{ mg dm}^{-3}$) developed the lowest number

of lateral shoots. Those cultured in media with the highest concentration of AgNPs ($10, 20$ and 50 mg dm^{-3}) formed the highest number of lateral shoots. Enriching media with NPs resulted in increased lavender plant weight. Plants propagated in media enriched with $5\text{--}50 \text{ mg dm}^{-3}$ AgNPs or AuNPs were heavier than those growing in the control medium. The plants cultivated in the presence of the lowest concentration of NPs ($1\text{--}2 \text{ mg dm}^{-3}$ AgNPs and AuNPs) reached weight similar to the control. The addition of NPs considerably affected lavender root system development. Only 11.8% of shoots rooted in the control medium developed roots, while the rooting rate in plants treated with

Table 2 Effects of nanometals on the oil glands of *Lavandula angustifolia* Mill. cultivated in the media enriched with AgNPs and AuNPs

	NPs concentration (mg dm ³)	Leaf blade (adaxial surface)		Leaf blade (abaxial surface)	
		Number of oil glands (per mm ²)	Diameter of oil glands (μm)	Number of oil glands (per mm ²)	Diameter of oil glands (μm)
0	MS (control)	6.5c	68.2fe	7.1bc	67.3cd
1	AgNPs	6.4c	72.9cb	7.0bc	69.4ab
2	AgNPs	6.6c	76.3a	6.8cd	70.2ab
5	AgNPs	6.0d	64.2g	5.5e	64.2e
10	AgNPs	5.3f	66.9f	5.2e	66.2de
20	AgNPs	5.4f	68.1fe	4.0f	67.8cd
50	AgNPs	3.5g	66.6f	3.2g	64.5e
1	AuNPs	6.6c	73.1bc	7.1bc	70.4a
2	AuNPs	6.4c	70.2de	7.3b	71.2a
5	AuNPs	7.3a	74.8ab	8.0a	71.4a
10	AuNPs	6.9b	71.4cd	7.1bc	70.9a
20	AuNPs	5.9e	66.8f	6.8cd	67.2cd
50	AuNPs	5.2f	67.3f	6.6d	66.4de
	LSD _{0.05}	0.25	2.33	0.38	2.41

a–c Values followed by the same letter are not significantly different at $p \leq 0.05$ according to the LSD (least significant differences) Tukey test

Fig. 2 Lavender propagated on control media (a) and with 50 mg dm⁻³ AgNPs, secretory structures under magnification $\times 1000$ (b)

AgNPs ranged from 39.1 to 88.9% depending on the metal concentration. In plants exposed to AuNPs the rooting rate was lower and reached from 47.6% (10 mg dm⁻³ AuNPs) to 12.5% (50 mg dm⁻³ AuNPs). Roots developed in the media supplemented with AgNPs and AuNPs were usually longer than in the control (1.70 cm), and their length differed from 2.62 cm (2 mg dm⁻³ AgNPs) to 4.77 cm (10 mg dm⁻³ AgNPs).

The plants exposed to 5 mg dm⁻³ AgNPs developed roots of similar length to the control ones. The Results presented in Table 2 indicate the significant effects of AuNPs and AgNPs on the number and diameter of secretory trichomes on the abaxial and adaxial surface of lavender leaves (Fig. 2). Low concentrations of NPs significantly increased the number of trichomes. They were the most abundant in the media containing 5 mg dm⁻³ AuNPs (7.3 and 8 on

adaxial and abaxial surface, respectively). Lavender plants treated with 50 mg dm^{-3} AgNPs developed the lowest number of trichomes (3.5 and 3.2 per adaxial and abaxial surface, respectively). The diameter of trichomes formed on the adaxial surface was greatest in the media enriched with 2 mg dm^{-3} AgNPs and 5 mg dm^{-3} AuNPs (76.3 and $74.8 \mu\text{m}$, respectively), and smallest ($64.2 \mu\text{m}$) in the media enriched with 5 mg dm^{-3} AgNPs. These differences were significant. Higher concentrations of NPs did not increase the diameter of the secretory trichomes on the adaxial surface of lavender leaf blades compared to the control medium. The diameter of trichomes formed on the abaxial surface of the leaf blade was also the greatest in plants exposed to 5 mg dm^{-3} AuNPs ($71.4 \mu\text{m}$), 2 mg dm^{-3} AuNPs ($71.2 \mu\text{m}$), 10 mg dm^{-3} AuNPs ($70.9 \mu\text{m}$), and 1 mg dm^{-3} AuNPs and AgNPs (70.4 and $69.4 \mu\text{m}$). Similarly to the trichomes on the adaxial surface, those on the abaxial surface had the smallest diameter in the presence of 5 mg dm^{-3} AgNPs ($64.2 \mu\text{m}$), which differed significantly from those in plants exposed to 50 mg dm^{-3} AgNPs and AuNPs and 10 mg dm^{-3} AgNPs.

Discussion

Nanometal particles show a strong affinity to plant tissues and they activate enzymatic pathways responsible for the production of secondary metabolites (Shakeran et al. 2015). They also contribute to the peroxidation of cellular membranes in plant cells and affect the expression of genes responsible for the production of biologically active compounds (Raei et al. 2014). Oxidative stress induced by heavy metal molecules may stimulate secretion of secondary metabolites by plant cells. However, high concentrations of NPs may cause damage to the cell wall and plasma membrane, and disturb different plant processes (Mirzajani et al. 2013). Our study demonstrated that the addition of AuNP and AgNPs to the culture media significantly affected growth and development of narrow-leaved lavender cultivated in vitro. The effects depended on metal concentration and NP type. The lowest concentration of nanogold (1 mg dm^{-3}) in the nutrient medium had a growth-stimulating effect. In our study, lavender plants growing in the presence of the lowest concentrations of NPs ($1\text{--}5 \text{ mg dm}^{-3}$ AgNPs and $1\text{--}2 \text{ mg dm}^{-3}$ AuNPs) developed shoots of similar length to the control plants. Also, Timoteo et al. (2019) did not prove the toxic effect of low concentrations of AgNPs on the development of *Campanesia rufa* plants in in vitro cultures. Also, AuNPs at 50 mg dm^{-3} caused visible changes in plant appearance, which were not observed for AgNPs. Feichtmeier et al. (2015) described visible toxic effects, such as leaf yellowing, root darkening and decreasing biomass, which they attributed to increasing concentrations of AuNPs. Kumar

et al. (2013) reported that an exposure to 80 mg dm^{-3} AuNPs significantly improved seed germination rate, vegetative growth and free radical scavenging activity in *Arabidopsis thaliana*. Mirzajani et al. (2013) demonstrated the toxicity of AgNPs in *Oryza sativa*. AgNPs of 25 nm in diameter and a concentration of 60 mg dm^{-3} damaged the cell wall and root cell vacuoles. However, they were incapable of penetrating the root cells when present at low concentrations (up to 30 mg dm^{-3}). The authors proved that AgNPs at 30 mg dm^{-3} accelerated root development, while at 60 mg dm^{-3} it significantly restricted root elongation. Dimpka et al. (2013) investigated phytotoxic effects of AgNPs in a hydroponic culture of *Triticum aestivum* L., and Spinoso-Castillo et al. (2017) investigated the development of *Vanilla planifolia* in temporary immersion systems. As in our study, they confirmed toxicity of nanosilver in plants but only at high concentrations. Our experiments revealed that root system development was inhibited at concentrations as low as 20 mg dm^{-3} AuNPs. Qian et al. (2013) demonstrated that AgNPs at 0.2, 0.5 and 3 mg dm^{-3} inhibited root growth, reduced the content of chlorophyll *a*, chlorophyll *b*, and total chlorophyll, and altered transcription of antioxidant and aquaporin related genes in *A. thaliana*. In our study, low concentrations of both NPs stimulated lavender root elongation. This disparity may be due to the different diameters of experimental NPs.

The elongation of the root system could be caused by the stress reaction of plants and in response to the production of phytohormones. Recently, it was recognised that a broad range of nano-optimal environmental conditions can induce generic, “stress induced morphogenic responses” (SIMRs) such as alerted root elongation. Key components of the SIMR control mechanism are reactive oxygen species (ROS) and the phytohormone: auxin (Potters et al. 2007). ROS molecules serve as signals to coordinate a wide range of plant cellular events, including hormone perception and transduction (Geche et al. 2006). Furthermore, ROS plays a positive role in ABA signaling, which plays a key role in lateral root development when plants are exposed to environmental stress (De Smet et al. 2003, 2006). Rezvani et al. (2012) proved that in under flooding conditions, AgNP may promote root growth by blocking ethylene (ET) signaling in *Crocus sativus*. Syu et al. (2014) proved that different morphologies of AgNPs exhibited different levels of phyto-stimulatory effects in *A. thaliana*. Moreover, it was demonstrated that AgNPs interacted with genes that are involved cell proliferation, photosynthesis, and hormone signaling, including auxin, ABA and ET.

Our experiment demonstrated the significant effects of AgNP and AuNPs on the number and size of oil glands formed on the leaves of narrow-leaved lavender cultured in vitro.

The increase in the number of trichomes may be associated with a change in the level of endogenous phytohormones under the influence of NPs.

NPs of metals present in the environment affect the level of phytohormones in plants. For instant, in the research of Vinković et al. (2017), was demonstrated significant increase in levels of cytokinins in pepper plants (*Capsicum annuum* L.) exposed to AgNPs. Phytohormones modulates epidermal differentiation programs and interfere with trichome maturation and their size (Maes and Gossens 2010; Maes et al. 2008). Phytohormones that have a positive effect on the formation of trichomes are primarily cytokinins (Ishida et al. 2008), gibberellins (Perazza et al. 1998) and jasmonic acid (Traw and Bergelson 2003). Gazdovska-Simic et al. (2013) also proved that elicitation with polysaccharides such as chitin, pectin and dextran changed the morphology of secretory structures in *Hypericum perforatum* L.

In conclusion, it can be stated that AgNP and AuNPs had a significantly improved the growth and development of narrow-leaved lavender propagated in vitro. Enriching the culture media with AuNP or AgNPs stimulates formation of shoots and increases plant weight. Only high concentrations of these NPs (20 and 50 mg dm⁻³) in the culture media are toxic to lavender plants as manifested by restricted shoot length and decreased pacheters of other morphological features. AuNPs at concentrations up to 5 mg dm⁻³ stimulate shoot elongation. The addition of NPs has a positive effect on lavender root system development. The number and size of secretory trichomes formed on the leaves of narrow-leaved lavender grown in in vitro cultures depends on the concentration of AgNP or AuNPs in the media. Low concentrations of AgNPs and AuNPs stimulate the formation of secretory trichomes and enlarge their diameter on both sides of the leaf blade, but this effect is greater when AuNPs are used. In view of the observed lack of a highly toxic effect on the development of narrow-leaved lavender plants on media supplemented with low concentrations of AgNPs and AuNPs, this method can be used to produce a large amount of biomass, which is necessary to isolate essential oils. However, in order to be used commercially, depending on the proposed use, it would be necessary to determine the content of NPs in essential oils and to examine their potential toxicity.

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