Zachodniopomorski Uniwersytet Technologiczny
w Szczecinie
Wydział Kształtowania Środowiska i Rolnictwa
Studia doktoranckie

mgr inż. Paula Jadczak

ROZPRAWA DOKTORSKA

stanowiąca cykl publikacji pod wspólnym tytułem

Wpływ nanokoloidów złota i srebra na produkcję metabolitów wtórnych w kulturach *in vitro* lawendy wąskolistnej (*Lavandula angustifolia* Mill.)

Effect of gold and silver nanocolloids on secondary metabolite production in *in vitro* cultures of narrow-leaved lavender (*Lavandula angustifolia* Mill.)

Praca doktorska wykonana w Katedrze
Genetyki, Hodowli i Biotechnologii Roślin

Opiekun: dr hab. inż. Danuta Kulpa, prof. ZUT

Numer albumu: 17500

Spis treści

1. Wstęp

Nanocząstki, czyli cząstki materii o wielkości od 1 do 100 nm, dzięki swoim unikalnym właściwościom znajdują zastosowanie w wielu gałęziach przemysłu. Tworzą one stabilne roztwory koloidalne, nazywane nanokoloidami. Wykorzystywane są m.in. w medycynie i farmacji, kosmetologii, ochronie środowiska, przemyśle tekstylnym, czy opakowalnictwie. Coraz częściej stosuje się je także w ogrodnictwie i rolnictwie (Jamshidi i in., 2016; Zhao i in., 2001). Selektywne właściwości roślinnych ścian komórkowych umożliwiają transport tych cząstek, które przenikając do wnętrza komórek, przemieszczają się w organizmie roślinnym, a ostatecznie wpływają na szereg procesów biologicznych (Nair i in., 2010). Nanocząstki metali przyczyniają się do peroksydacji błon komórkowych i wpływają na ekspresję genów odpowiedzialnych za produkcję związków aktywnych biologicznie (Raei i in., 2014; Shakeran i in., 2015).

Nanokoloidy znalazły swoje zastosowanie również w roślinnych kulturach *in vitro*. Nanosrebro, ze względu na silne właściwości przeciwdrobnoustrojowe, wykorzystywane jest w mikrorozmnażaniu na etapie inicjacji kultur, w celu zapobiegania kontaminacjom (Sarmast i in., 2015). Prowadzone są, jak dotąd nieliczne, badania które mają na celu zbadanie przydatności nanocząstek jako elicytorów pobudzających produkcję metabolitów wtórnych w kulturach *in vitro* oraz określenie ich wpływu na organizm roślinny (Spinoso-Castillo i in., 2017). Jak dotąd udowodniono, że wywierają one istotny wpływ na produkcję metabolitów wtórnych w kulturach *in vitro* szałwii czerwonej (Zhang i in., 2004), bylicy rocznej (Zhang i in., 2013), burgmansji (Pitta-Alvarez i in., 2000), leszczyny pospolitej (Jamshidi i Ghanti, 2016), głowienki pospolitej (Fazal i in., 2014), czy aleosu zwyczajnego (Raei i in., 2014).

Aby można było odpowiednio pobudzić produkcję metabolitu należy precyzyjnie dobrać zarówno stężenie, rodzaj jak i wielkość nanocząstek. Stosowanie zbyt wysokich stężeń, zwłaszcza cząstek o najmniejszej średnicy, może wywierać zbyt toksyczny wpływ zarówno na wzrost i rozwój roślin, jak i ilość i skład produkowanych przez nie metabolitów. (Shakeran i in., 2015; Chung i in., 2018; Navarro i in., 2008).

Jak dotąd nie dowiedziono jednoznacznie, jaki jest mechanizm oddziaływania nanocząstek dodawanych do pożywek w kulturach *in vitro* na produkcję metabolitów wtórnych. Przypuszcza się, że nanocząstki metali wywołują stres oksydacyjny. Wzrost produkcji reaktywnych form tlenu (ROS) pod ich wpływem odnotowano u ryżu siewnego (Mirzajani i in., 2013), cebuli zwyczajnej (De i in., 2016), kapusty sitowej (Rao i Shekhawat, 2016)

i tytoniu szlachetnego (Dai i in., 2018). Jednak, zgodnie z przeciwstawną teorią, dodatek nanocząstek metali, zwłaszcza srebra, wpływa na produkcję metabolitów wtórnych poprzez łagodzenie stresu oksydacyjnego, przez obniżenie stężenia etylenu w naczyniu hodowlanym, produkowanego przez tkanki roślin (Sarmast i in., 2015; Sreelekshmi i in., 2021).

Jedną z roślin produkujących olejki eteryczne jest lawenda wąskolistna (*Lavandula angustifolia* Mill.), należąca do rodziny jasnotowatych (*Lamiaceae*). Jest ona wiecznie zieloną byliną, pochodzącą z regionu śródziemnomorskiego, uprawianą powszechnie w wielu częściach świata (Trejgell i Kęsy, 2019). Zioło to charakteryzuje się szeregiem właściwości prozdrowotnych i leczniczych. Napary lawendowe mają charakter karminatywny, moczopędny, przeciwreumatyczny oraz przeciwpadaczkowy. Są efektywnymi środkami przeciwbólowymi, szczególnie w przypadku nerwowych bóli głowy oraz migren (Gilani i in., 2000). Lawenda wykorzystywana jest powszechnie w przemyśle kosmetycznym i perfumeryjnym (Gonçalves i Romano, 2013). Obok atrakcyjnego zapachu, lawendowe ekstrakty i olejki eteryczne wykazują właściwości antybakteryjne, przeciwgrzybicze i antyoksydacyjne (D'Auria i in., 2005).

Olejki eteryczne to mieszanina kilkudziesięciu związków chemicznych, których skład jest zmienny i zależny od wielu czynników, takich jak: genotyp rośliny, miejsce i sposób uprawy, warunki klimatyczno-glebowe, czy nawet sposób pozyskiwania sadzonek (Wornouk i in., 2011; Andrys i Kulpa, 2018). Do pozyskiwania olejków eterycznych, jak również innych związków biologicznie czynnych z tkanek roślin leczniczych, coraz częściej wykorzystuje się roślinne kultury *in vitro*. Hodowla roślin w kulturach *in vitro* zapewnia produkcję materiału roślinnego wolnego od zanieczyszczeń i chorób. Dodatkowo, rozmnażanie roślin odbywa się w pełni kontrolowanych warunkach laboratoryjnych, niezależnie od warunków klimatyczno-glebowych, dając możliwość produkcji dużej ilości tkanek roślinnych, nieprzerwanie przez cały rok. Co więcej, metoda ta, dzięki zastosowaniu elicytorów, pozwala na zmianę ilości i kompozycji olejków produkowanych w tkankach roślinnych, co może wpływać na ich właściwości (Andrys i in., 2018 a). Jak wykazały Andrys i in. (2018 b) działanie jednym z elicytorów – kwasem jasmonowym, na tkanki lawendy w kulturach *in vitro* zwiększyło aktywność przeciwdrobnoustrojową i antyoksydacyjną wyizolowanych z nich olejków eterycznych.

Opracowanie wydajnej metody otrzymywania olejków eterycznych lub też fragmentów tkanek roślin o istotnie podwyższonej, w wyniku elicytacji aktywności antyoksydacyjnej i antymikrobiologicznej, pozwoliłoby na ich wykorzystanie jako substancji konserwujących produkty kosmetyczne, zwłaszcza te o krótkim terminie przydatności do użycia. Wpisywałoby

się to w obecne trendy produkcji kosmetyków, możliwie najbardziej naturalnych i niekonserwowanych chemicznie, bogatych w substancje pochodzenia roślinnego.

2. Cel badań

W ramach osiągnięcia naukowego, stanowiącego w rozumieniu ustawy Art. 15 Ust. 2 Ustawa z dnia 14 marca 2003 roku o stopniach naukowych i tytule naukowym oraz o stopniach i tytule w zakresie sztuki (Dz.U. 2016, poz. 882 ze zm.), przedstawiono jednotematyczny cykl publikacji.

Celem badań było:

1. Określenie wpływu dodatku do pożywki nanokoloidów zawierających nanocząstki złota i srebra na wzrost roślin, w tym rozwój trichomów wydzielniczych lawendy wąskolistnej (*Lavandula angustifolia* Mill.), odmiany Munstead w kulturach *in vitro*.
2. Ocena składu chemicznego olejków eterycznych oraz zawartości polifenoli ogółem, produkowanych przez lawendę wąskolistną namnażaną w kulturach *in vitro* na pożywkach wzbogaconych w nanokoloidy złota lub srebra.
3. Określenie wpływu nanocząstek złota i srebra na aktywność enzymów antyoksydacyjnych i zdolność do zmiatania wolnych rodników w tkankach roślin lawendy wąskolistnej namnażanych w kulturach *in vitro*.
4. Ocena możliwości wykorzystania tkanek lawendy wąskolistnej hodowanej w kulturach *in vitro* na pożywkach zawierających w swoim składzie nanocząstki złota lub srebra jako alternatywy dla syntetycznych konserwantów używanych w produktach kosmetycznych.

3. Materiał i metody badań

3.1. Charakterystyka materiału badawczego

Materiałem użytym do prowadzenia badań w ramach wykonanej pracy doktorskiej były rośliny lawendy wąskolistnej, odmiany Munstead pochodzące z kolekcji roślin Laboratorium Kultur *In Vitro*, Katedry Genetyki, Hodowli i Biotechnologii Roślin, mieszczącej się na Wydziale Kształtowania Środowiska i Rolnictwa, Zachodniopomorskiego Uniwersytetu Technologicznego w Szczecinie. Były to kultury namnażane na pożywce o składzie mineralnym według Murashige i Skoog (1962) z dodatkiem 2 $mg \cdot dm^{-3}$ KIN i 0,2 $mg \cdot dm^{-3}$ IAA (Andrys i in., 2018a). Pożywka ta stanowiła pożywkę podstawową w całym doświadczeniu, do której dodawano nanokoloidy srebra i złota.

Eksplantaty pobrane z tych roślin posłużyły do założenia doświadczenia, w którym określono: wpływ nanocząstek złota i srebra o wielkości cząsteczki 24,2 nm (AgNps) oraz 27,5 nm (AuNPs), dodawanych do pożywki podstawowej w stężeniach 1, 2, 5, 10, 20 i 50 $mg \cdot dm^{-3}$ na wzrost i rozwój roślin lawendy w kulturach *in vitro.* Prowadząc obserwacje przy pomocy SEM (skaningowego mikroskopu elektronowego) określono również morfologię wykształconych trychomów wydzielniczych (**publikacja 1**). W otrzymanych w ten sposób tkankach lawendy oznaczono aktywność enzymów antyoksydacyjnych (POX, APX, CAT, SOD), zdolność do zmiatania wolnych rodników metodą ABTS•+oraz zawartość polifenoli ogółem (**publikacja 3**).

Następnie porównano zawartość olejku eterycznego i jego skład (metodą GC-MS) w tkankach lawendy wąskolistnej namnażanej na pożywce podstawowej, z dodatkiem AgNPs lub AuNPs o wielkości cząsteczki odpowiednio 24,2±2,4 nm i 27,5±4,8 nm i stężeniu 10 lub 50 $mg \cdot dm^{-3}$ (**publikacja 2)**.

Na ostatnim etapie pracy doktorskiej (**publikacja 4**) porównano potencjał konserwujący emulsje kosmetyczne tkanek lawendy wąskolistnej hodowanych w kulturach *in vitro,* na pożywkach wzbogaconych w 1 lub 10 $mg \cdot dm^{-3}$ nanocząstki złota lub srebra o dwóch wielkościach cząstek 13 lub 30 nm.

3.2. Metody prowadzonych badań

3.2.1. Pochodzenie nanokoloidów

Nanokoloidy użyte w doświadczeniach pochodziły z Instytutu Hodowli i Aklimatyzacji Roślin Państwowego Instytutu Badawczego, Zakładu Nasiennictwa i Ochrony Ziemniaka

w Boninie **(publikacja 1, 2 i 3)** oraz z firmy NPIN (Polska) **(publikacja 4)**. W obu przypadkach do pożywek hodowlanych dodawano nanokoloidy zawierające nanocząstki złota lub srebra zawieszone w stężeniu 1 $mg \cdot ml^{-1}$ nanokoloidu.

3.2.2. Warunki prowadzenia kultur *in vitro*

Pożywką podstawową, w trakcie namnażania roślin w kulturach *in vitro*, była pożywka o składzie mineralnym według Murashige i Skoog (1962) z dodatkiem 2 $mg \cdot dm^{-3}$ KIN i 0,2 $mg \cdot dm^{-3}$ IAA. Uzupełniano ją w 30 $g \cdot dm^{-3}$ sacharozy, 100 $mg \cdot dm^{-3}$ inozytolu oraz 7 $g \cdot dm^{-3}$ agaru. Odczyn pożywek hodowlanych ustalano na poziomie 5,7 za pomocą 0,1 M HCL i NaOH. Szklane naczynia hodowlane o pojemności 300 ml wraz ze znajdującymi się w nich pożywkami hodowlanymi, sterylizowano w temperaturze 121°C przez 20 minut. W każdym naczyniu znajdowało się 30 ml medium hodowlanego. Naczynia hodowlane z materiałem roślinnym umieszczano w fitotronie, przy wilgotności powietrza 70-80 % i temperaturze 24°C. Kultury oświetlane były światłem o natężeniu 35 $\mu E\ M^{-2}\ s^{-1}$ PAR, przez 16 godzin na dobę.

3.2.3. Wpływ nanocząstek złota i srebra na wzrost roślin **(publikacja 1)**

Po czterotygodniowym wzroście roślin w fitotronie na pożywkach: podstawowej i z dodatkiem nanokoloidów złota lub srebra o wielkości cząsteczki 24,2±2,4 nm (AgNps) oraz 27,5±4,8 nm (AuNPs) oceniono rozwój roślin dokonując pomiarów biometrycznych, w których określono: liczbę i długość pędu (cm), masę roślin (g) oraz długość korzenia (cm). Określono także odsetek ukorzenionych roślin.

3.2.4. Wpływ nanocząstek złota i srebra na rozwój trichomów wydzielniczych **(publikacja 1)**

Analizę trichomów wydzielniczych liści za pomocą skaningowego mikroskopu elektronowego (SEM) przeprowadzono w Centrum Biologii Molekularnej i Biotechnologii Uniwersytetu Szczecińskiego w Małkocine. W celu wykonania pomiarów, środkowe odcinki liści suszono w suszarce Critical Point (Quorum Technologies, Niemcy) i spryskiwano złotem w Sputter Coater (Quorum Technologies, Niemcy). Obserwacje przeprowadzono przy użyciu mikroskopu Carl Zeiss EVO LS 10 o napięciu 1 lub 15 kV. Określono morfologię trichomów wydzielniczych, a także ich liczebność [$szt \cdot mm^{-2}$] i średnicę [mm] na dolnej i górnej stronie blaszek liściowych.

3.2.5. Określenie aktywności enzymów antyoksydacyjnych (**publikacja 3**)

Aktywność POX. Została określana zgodnie z protokołem Chance'a i Maehly'ego (1995), który zaadaptowano do czytnika mikropłytkowego, monitorując szybkość tworzenia się tetraguaiakolu z guaiakolu poprzez zwiększenie absorbancji przy 470 nm (ε=26,6 $mM^{-1}cm^{-1}$). Jedna jednostka POX była definiowana jako ilość enzymu tworzącego 1 μmol tetraguaiakolu na minutę. Wyniki wyrażono w jednostkach na mg wyekstrahowanego białka.

Aktywność APX. Została zmierzona zgodnie z protokołem Nakano i Asada (1981), poprzez monitorowanie spadku absorbancji przy 290 nm (ε=2,8 mM^{-1} cm^{-1}). Pomiary wykonano przy użyciu czytnika mikropłytek. Jedna jednostka APX była definiowana jako ilość enzymu utleniającego 1 μmol askorbinianu na minutę. Wyniki wyrażono w jednostkach na mg wyekstrahowanego białka.

Aktywność SOD. Została określona na podstawie zahamowania fotochemicznej redukcji tetrazolu nitroblue (NBT) zgodnie protokołem Beauchampa i Fridovicha (1971), dostosowanym do czytnika mikropłytek. Jedna jednostka SOD była definiowana jako ilość enzymu, który hamuje redukcję NBT o 50%. Wyniki wyrażono w jednostkach na mg wyekstrahowanego białka.

Aktywność CAT. Została oznaczona zgodnie z protokołem Li i Schellhorna (2007), na podstawie szybkości rozkładu nadtlenku wodoru poprzez pomiar spadku absorbancji przy 240 nm (ε=43,6 mM^{-1} cm^{-1}). Jedna jednostka CAT była definiowana jako ilość enzymu, który rozkłada 1 μmol nadtlenku wodoru na minutę. Wyniki wyrażono w jednostkach na mg wyekstrahowanego białka.

3.2.6. Określenie zawartości polifenoli ogółem (**publikacja 3**)

Całkowitą zawartość polifenoli w ekstraktach oznaczono za pomocą odczynnika Folin-Ciocalteu według metody Anastasiadi i in. (2010), którą zmodyfikowano do skali czytnika mikropłytkowego. Stężenie związków polifenolowych wyrażono w mg ekwiwalentu kwasu taninowego na 100 g suchej masy próbki (mg TAE/100 g DW).

3.2.7. Określenie zdolności unieczynniania kationorodników ABTS•+ (**publikacja 3**)

Aktywność zmiatania wolnych rodników oceniano w ekstraktach za pomocą testu przebarwień kationowych ABTS•+ dostosowanego do skali czytnika mikropłytkowego, zgodnie z metodą Shi i in. (2010). Jako wzorzec do kalibracji użyto kwasu askorbinowego. Wyniki wyrażono w μM ekwiwalentu kwasu askorbinowego na 100 g suchej masy próbki (μM AAE/100 g DW).

3.2.8. Analiza składu chemicznego olejków eterycznych **(publikacja 2)**

Badania mające na celu określenie stężenia i kompozycji olejków eterycznych wykonano na Wydziale Technologii i Inżynierii Chemicznej Zachodniopomorskiego Uniwersytetu Technologicznego w Szczecinie. W celu wyizolowania olejku eterycznego, 15 g powietrznie suchego ziela poddano dwugodzinnej hydrodestylacji przy użyciu aparatu Clevenger'a, zgodnie z zaleceniami Farmakopei Europejskiej 5.0. (Francja, 2005) Ekstrakty olejków eterycznych suszono nad bezwodnym siarczanem sodu, filtrowano, ważono i przechowywano w ciemnych, szczelnie zamkniętych fiolkach, w temperaturze 4°C, do momentu przeprowadzenia analizy metodą chromatografii gazowej/spektrometrii masowej (GC-MS). Zawartość procentową olejku eterycznego obliczono na podstawie suchej masy materiału roślinnego i wyrażono jako (% w/w). Składniki olejków eterycznych zidentyfikowano poprzez porównanie ich widm masowych z tymi przechowywanymi w bibliotekach masowych Wiley NBS75K.L i NIST/EPA/NIH (wersja 2002, National Institute of Standards and Technology, Gaithersburg, MD, USA). Tożsamość związków potwierdzono również poprzez porównanie ich obliczonych wskaźników retencyjnych z tymi, które podano w NIST Chemistry WebBook (http://webbook.nist.gov/chemistry/). Do obliczania wskaźników retencyjnych (RI) (Van Den Dool i Kratz, 1963, Babushok i in., 2011) zastosowano mieszaninę homologicznych serii n-alkanów C7-C40 (Supelco, Bellefonte, PA, USA), w tych samych warunkach chromatograficznych, które stosowano do analizy lawendowych olejków eterycznych.

3.2.9. Ocena stabilności i czystości mikrobiologicznej emulsji kosmetycznych z dodatkiem rozdrobnionych tkanek lawendy (**publikacja 4**)

Przygotowanie emulsji kosmetycznych. W oddzielnych zlewkach przygotowano składniki fazy wodnej i olejowej. Faza olejowa składała się z 18 g lanoliny, 12 g wosku pszczelego, 14 g masła shea oraz 88 ml oleju jojoba. W celu przygotowania fazy wodnej, do zlewki dodano 6 g 75% D-pantenolu, 6,4 g gliceryny i 68 ml sterylnej wody destylowanej. Obie zlewki z zawartością zostały szczelnie przykryte folią aluminiową i umieszczone w łaźni wodnej w celu rozpuszczenia wszystkich składników fazy olejowej. Całą fazę olejową dodawano do zlewki zawierającej fazę wodną, którą umieszczano w łaźni wodnej. Następnie zawartość zlewki mieszano przez około 1 minutę, po czym usuwano ją z łaźni wodnej. Mieszanie kontynuowano, aż do osiągnięcia przez emulsję temperatury 40°C. Emulsje kosmetyczne podzielono na porcje po 5 ml i uzupełniono o kwas dehydrooctowy oraz alkohol benzoesowy (DHA BA), jako chemiczne środki konserwujące lub o materiał w postaci tkanek lawendy namnażanych w kulturach *in vitro*, na pożywkach z dodatkiem nanocząstek srebra lub złota

o średnicy cząstki 13 lub 30 nm w stężeniu 1 lub 10 mg·dm^{-3}. W trakcie badań przygotowano 12 rodzajów emulsji kosmetycznych. Do każdego z wariantów dodano 0,1 g świeżej tkanki roślinnej, zmielonej w ciekłym azocie, co stanowiło 2% materiału roślinnego. Kontrola ujemna zawierała 12,5 µL DHA BA (Esent, Polska), który składał się z 7% kwasu dehydrooctowego (DHA) i 83% alkoholu benzylowego (BA) jako konserwantu, co stanowiło 0,25%.

Ocena czystości mikrobiologicznej. Emulsje kosmetyczne umieszczano w sterylnych szalkach Petriego i przechowywano w lodówce, w temperaturze +4°C. Próbki kontrolne stanowiły emulsje bez dodatku konserwantów i tkanek roślinnych. Jedna z próbek kontrolnych była przechowywana w lodówce, a druga w temperaturze pokojowej, która wynosiła 24°C. Po okresie 4 tygodni przechowywania, 0,1 g emulsji kosmetycznej rozpuszczono w 0,9 ml soli fizjologicznej przy użyciu Vortexu (IKA). Następnie 0,1 ml badanego materiału umieszczono na podłożu mikrobiologicznym i wykonano test kultury powierzchniowej. Do tego celu wykorzystano podłoże Sabourauda z chloramfenikolem oraz podłoże agarowe do infuzji mózgu (BHI) (Biomaxima SA, Polska).

4. Omówienie wyników badań przedstawionych w publikacjach stanowiących osiągnięcie naukowe

4.1. Publikacja nr 1

Jadczak P., Kulpa D., Bihun M., Przewodowski W. 2019. Possitive effect of AgNPs and AuNPs in *in vitro* cultures of *Lavandula angustifola* Mill. Plant Cell, Tissue and Organ Culture, 139, 191-197.

Najnowsze badania naukowe wskazują na możliwość wykorzystania nanocząstek złota i srebra jako elicytorów w kulturach *in vitro*. Jednocześnie substancje te niejednokrotnie opisywane są jako związki działające toksycznie lub też letalnie na rośliny i inne organizmy żywe. Wpływ na rośliny związany jest zwykle z rodzajem nanocząstki, jej wielkością oraz z zastosowanym stężeniem. Celem doświadczenia opisanego w pierwszej części pracy było zbadanie wpływu nanokoloidów zawierających nanocząstki złota lub srebra na rozwój roślin lawendy wąskolistnej hodowanych w kulturach *in vitro*, w tym także ich wpływ na trichomy wydzielnicze odpowiedzialne za sekrecję olejków eterycznych.

Niezależnie od stężenia AgNPs, nie zaobserwowano zmian w wyglądzie lawendy, takich jak przebarwienia czy nekrozy organów roślinnych. Jedynie AuNPs dodawane do pożywek w najwyższym stężeniu (50 $mg \cdot dm^{-3}$) powodowały nieznaczne zażółcenie blaszek liściowych i zmiany w ich budowie. Wzbogacenie pożywki w AgNPs i AuNPs istotnie wpłynęło natomiast na kształtowanie się cech morfologicznych badanych roślin. Rośliny rosnące na pożywkach o najniższym stężeniu NPs (1–5 $mg \cdot dm^{-3}$ AgNPs i 1–2 $mg \cdot dm^{-3}$ AuNPs) rozwijały pędy o podobnej długości jak rośliny kontrolne. Stężenia NPs wyższe niż 5 $mg \cdot dm^{-3}$ wpłynęły na zmniejszenie wysokości roślin. Najkrótszymi pędami charakteryzowały się rośliny lawendy namnażane na pożywkach z dodatkiem 50 $mg \cdot dm^{-3}$ AgNPs oraz 20 i 50 $mg \cdot dm^{-3}$ AuNPs. Niezależnie od ich stężenia, NPs dodawane do pożywki zwiększały liczbę pędów bocznych - najmniej pędów bocznych rozwijały rośliny lawendy uprawiane na pożywce kontrolnej (1,59 sztuk). W większości przypadków, wraz ze wzrostem stężenia nanoczastek w pożywce, rosła liczba pędów bocznych. Największą ich liczbę wykształciły rośliny lawendy namnażane na pożywkach z dodatkiem najwyższych stężeń AgNPs (10, 20 i 50 $mg \cdot dm^{-3}$). Wzbogacanie pożywek w NPs, niezależnie od ich rodzaju i stężenia, spowodowało wzrost świeżej masy roślin lawendy. Najwyższą masą charakteryzowała się lawenda rosnąca na pożywce z dodatkiem 2 $mg \cdot dm^{-3}$ AuNPs.

Dodanie NPs znacząco wpłynęło na rozwój systemu korzeniowego lawendy wąskolistnej (*Lavandula angustifolia* Mill.). Jedynie 11,8% pędów ukorzenionych w pożywce kontrolnej wykształciło korzenie, podczas gdy odsetek roślin ukorzenionych wśród tych, które namnażano na pożywce z dodatkiem AgNPs wahał się w zależności od stężenia NPs od 39,1 do 88,9%. W obrębie lawendy rosnącej na pożywkach z dodatkiem AuNPs procent roślin wykształcających system korzeniowy był niższy. Najniższy odsetek stwierdzono w przypadku roślin namnażanych na pożywkach uzupełnionych najwyższymi stężeniami AuNPs – 20 i 50 $mg \cdot dm^{-3}$ i było to odpowiednio 14,3% i 12,5%. Korzenie wykształcone przez rośliny rosnące na pożywce z dodatkiem AgNPs i AuNPs (za wyjątkiem rosnących na podłożu z dodatkiem 5 $mg \cdot dm^{-3}$ AgNPs) były dłuższe niż u roślin kontrolnych (1,70 cm), a ich długość wahała się od 2,62 (2 $mg \cdot dm^{-3}$ AgNPs) do 4,77 cm (10 $mg \cdot dm^{-3}$ AgNPs).

Wyniki powyższych badań wskazują na istotny wpływ AuNPs i AgNPs na liczbę i średnicę trichomów wydzielniczych na dolnej i górnej powierzchni blaszek liściowych lawendy wąskolistnej (*Lavandula angustifolia* Mill.). Niskie stężenia NPs zwiększały liczbę wykształconych trichomów wydzielniczych. Najliczniej struktury te występowały na blaszkach liściowych lawendy namnażanej na pożywkach zawierających 5 $mg \cdot dm^{-3}$ AuNPs. Lawenda namnażana na pożywkach z dodatkiem najwyższych stężeń AgNPs (50 $mg \cdot dm^{-3}$) wykształciła najmniejszą liczbę trichomów, na obu stronach blaszek liściowych. Średnica włosków utworzonych na powierzchni adaksjalnej była największa w pożywkach wzbogaconych o 2 $mg \cdot dm^{-3}$ AgNPs i 5 $mg \cdot dm^{-3}$ AuNPs (odpowiednio 76,3 i 74,8 µm). Wyższe stężenia NPs nie zwiększały średnicy włosków wydzielniczych na górnej powierzchni blaszek liści lawendy w porównaniu z pożywką kontrolną. Na zwiększenie się średnicy trichomów wydzielniczych w stosunku do kontroli na dolnej powierzchni blaszki liściowej wpłynęły od 1 do 10 $mg \cdot dm^{-3}$ AuNPs oraz 1 $mg \cdot dm^{-3}$ AgNPs dodawane do pożywek wzrostowych.

Przeprowadzone badania wskazują na możliwość otrzymania dużej ilości tkanek roślinnych, z prawidłowo wykształconymi trichomami wydzielniczymi poprzez namnażanie ich w kulturach *in vitro*, na pożywkach uzupełnionych AgNps oraz AuNPs. W związku z tym w kolejnym doświadczeniu określono zawartość i skład metabolitów wtórnych - olejków eterycznych **(publikacja 2)** i zawartość polifenoli ogółem **(publikacja 3)** w tkankach lawendy rosnącej na pożywkach z dodatkiem nanocząstek.

4.2. Publikacja nr 2

Wesołowska A., Jadczak P., Kulpa D., Przewodowski W. 2019 Gas Chromatography-Mass Spectrometry (GC-MS) Analysis of Essential Oils from AgNPs and AuNPs Elicited Lavandula angustifolia *In Vitro* Cultures. Molecules 24(3), 606.

Metabolity wtórne, w tym olejki eteryczne, produkowane są przez rośliny w niewielkich ilościach lub w ich szczególnych momentach rozwojowych, np. w okresie kwitnienia lub w odpowiedzi na niekorzystne warunki środowiskowe. Roślinne kultury *in vitro* dają możliwość produkowania cennego materiału roślinnego, wolnego od zanieczyszczeń i znacznie szybciej niż ma to miejsce przy uprawach polowych. Odpowiednio stosowane substancje elicytujące mogą wpływać korzystnie na wytwarzanie przez rośliny metabolitów wtórnych. Od niedawna do hodowli roślin leczniczych w kulturach *in vitro* wykorzystuje się nanocząstki metali w charakterze elicytorów, które znacząco wpływają na szereg procesów fizjologicznych roślin, w tym na produkcję i skład olejków eterycznych. Celem doświadczenia była analiza olejków eterycznych, pozyskanych z roślin lawendy wąskolistnej namnażanej w kultrach *in vitro* na pożywkach hodowlanych wzbogacanych w nanocząstki złota bądź srebra.

Dowiedziono, że hodowla lawendy wąskolistnej w kulturach *in vitro* na pożywkach zawierających w swoim składzie złoto i srebro istotnie wpływa na skład chemiczny i zawartość olejków eterycznych. Zawartość olejków eterycznych w tkankach lawendy wahała się od 0,81% (10 $mg \cdot dm^{-3}$ AuNPs) do 1,27% (10 $mg \cdot dm^{-3}$ AgNPs). Analizując skład olejków metodą GC-MS zidentyfikowano 97 różnych związków chemicznych reprezentujących, w zależności od stężenia nanocząstek od 99,29-99,95% zawartości olejków eterycznych. Spośród grup związków zawartych w badanych olejkach eterycznych największą stanowiły seskwiterpeny, (od 36,34 do 43,36%), następnie monoterpeny (27,77 do 38,23%), węglowodory seskwiterpenowe (od 10,27 do 14,35%) i monoterpenowe (5,57 do 10,40%). Związkami, których udział procentowy w tkankach roślin był najwyższy były: borneol (12,14-16,46%), τ-kadynol (12,96-16,63%), tlenek kariofilenu (8,79-12,23%), γ-kadynen (4,54-6,08%) oraz 1,8-kineol (2,80-4,58%). Innymi ważnymi zidentyfikowanymi składnikami były: cis-14-nor-murol-5-en-4-jeden (2,68-4,45%), β-pinen (1,93-3,14%), kamfora (2,05-2,79%), i α-santalen (1,42-2,64%).

Stwierdzono zróżnicowanie udziału procentowego związków chemicznych w analizowanych olejkach eterycznych, w zależności od stężenia i rodzaju nanocząstek w pożywce. Olejek eteryczny wyizolowany z lawend hodowanych na pożywkach kontrolnych,

bez dodatku nanocząstek był bogatszy w β-pinen (3,14%), α-pinen (1,46%), p-cymen (1,39%), kampen (1,08%) i δ-3-karen (0,93%). Nanocząstki złota i srebra dodawane do pożywek hodowlanych powodowały zmniejszenie udziału ilości i udziału procentowego związków o niższej masie cząsteczkowej, które zostały zastąpione przez te o wyższej masie cząsteczkowej. Olejki eteryczne pozyskane z tkanek lawendy hodowanej na pożywkach z dodatkiem 10 $mg \cdot dm^{-3}$ AgNPs najbardziej różniły się jakościowo w odniesieniu do kontroli. Najwyższe stężenia borneolu (16,46%) i 1,8-kineolu (4,58%) stwierdzono w olejku eterycznym pochodzącym z roślin namnażanych na pożywkach uzupełnionych w nanocząstki złota w stężeniu 50 $mg \cdot dm^{-3}$. Natomiast dodatek do pożywki nanocząstek srebra w stężeniu 50 $mg \cdot dm^{-3}$ zwiększył zawartość w olejku eterycznym γ-kadynenu (6,08%) oraz tlenku kariofilenu (12,23%). Zaobserwowano natomiast niższą, procentową zawartość kamfory w olejkach pozyskanych z roślin rosnących na pożywkach uzupełnionych w AgNPs w stężeniach 10 i 50 $mg \cdot dm^{-3}$.

Doświadczenie dowodzi, że nanocząstki złota i srebra mogą być z powodzeniem wykorzystywane w charakterze elicytorów w kulturach *in vitro* lawendy wąskolistnej, istotnie wpływając na zawartość i skład produkowanych przez nią olejków eterycznych. Nie jest jednak jasne, jaki jest mechanizm oddziaływania nanocząstek dodawanych do pożywek w kulturach *in vitro* na produkcję metabolitów wtórnych. Przypuszcza się, że nanocząstki metali wywołują stres oksydacyjny. Dlatego w kolejnym doświadczeniu określono wpływ nanocząstek na aktywność enzymów antyoksydacyjnych **(publikacja 3)**.

4.3. Publikacja nr 3

Jadczak P., Kulpa D., Drozd R., Przewodowski W., Przewodowska A. 2020. Effect of AuNPs and AgNPs on Antioxidant System and Antioxidant Activity of Lavender (*Lavandula angustifolia* Mill.) from *In Vitro* Cultures. Molecules, 25, (23), 5511.

Liczne badania naukowe opisują wysoką reaktywność nanocząstek metali w stosunku do roślin oraz ich wpływ na szereg procesów fizjologicznych, takich jak kiełkowanie nasion, wzrost, czy metabolizm. Badacze wskazują również na fitotoksyczność nanocząsteczek metali, która prowadzi do aktywacji układu antyoksydacyjnego w roślinach, co może wpływać na produkcję metabolitów wtórnych. Celem badania było określenie aktywności enzymów antyoksydacyjnych lawendy wąskolistnej (APX, SOD, POX, CAT) narażanej na stres wywołany nanocząstkami złota i srebra oraz jej zdolność do zmiatania wolnych rodników (ABTS•+) i zawartość polifenoli ogółem.

Stwierdzono, że nanokolidy złota i srebra zwiększają aktywność enzymów antyoksydacyjnych, takich jak peroksydaza askorbinianowa (APX) i dysmutaza ponadtlenkowa (SOD), jednak reakcja ta zależy od ich stężenia w pożywce. Najwyższą aktywność APX stwierdzono w tkankach roślin namnażanych na podłożach zawierających 2 i 5 mg·dm^{-3} AgNPs. Nanocząstki złota istotnie zwiększały aktywność APX w stosunku do kontroli, jedynie kiedy zastosowane były w stężeniu 10 mg·dm^{-3}. Najwyższą aktywność SOD zaobserwowano przy stężeniach 2 i 5 mg·dm^{-3} AgNP i AuNPs. Dodatek AgNPs do podłoży hodowlanych we wszystkich zastosowanych stężeniach oraz 1 mg·dm^{-3} AuNPs obniżył aktywność peroksydazy gwajakolowej (POX), w porównaniu do aktywności obserwowanej u roślin kontrolnych. Nie wykazano istotnego wpływu nanocząstek na wzrost aktywności katalazy (CAT) w stosunku do kontroli.

Przedstawione wyniki sugerują, że AgNPs i AuNPs dodane do pożywek w hodowlach *in vitro* wywierają wpływ na aktywność antyoksydacyjną określoną metodą ABTS•+. Wyjątek stanowiło najniższe stężenie AgNPs (1 mg·dm^{-3}) oraz najwyższe stężenie AuNPs (50 mg·dm^{-3}), w przypadku których nie stwierdzono różnic w aktywności antyoksydacyjnej,w porównaniu do kontroli. Najwyższe wartości zdolności do zmiatania wolnych rodników oznaczono dla roślin hodowanych na pożywkach z dodatkiem 10 mg·dm^{-3} AgNPs i 5 mg·dm^{-3} AuNPs, odpowiednio na poziomach 330,70 i 309,62 μM AAE/100 gDW.

Zawartość polifenoli ogółem zmieniała się w zależności od rodzaju i stężenia nanocząstek w podłożu hodowlanym. Wzrost zawartości polifenoli w stosunku do kontroli stwierdzono dla lawendy namnażanej na pożywkach z dodatkiem 2, 5 i 10 mg·dm^{-3} AuNPs oraz od 5 do 50 mg·dm^{-3} AgNPs (odpowiednio 0,214, 0,217 i 0,202 oraz od 0,212 do 0,318 mg TAE/100 gDW). Z kolei lawenda hodowana na podłożach z dodatkiem najwyższego stężenia

nanocząstek złota (50 $mg \cdot dm^{-3}$) oraz najniższych stężeń nanocząstek srebra (1 i 2 $mg \cdot dm^{-3}$) charakteryzowała się istotnie niższą od kontroli zawartością polifenoli.

W związku z tym, iż na podstawie wcześniejszych badań określono możliwość produkcji dużych ilości tkanki roślinnej **(publikacja 1)** o zmienionej zawartości metabolitów wtórnych **(publikacja 2 i 3)**, mając na uwadze aplikacyjny charakter pracy doktorskiej, w kolejnym etapie badań określono możliwość zastosowania wysuszonych i sproszkowanych fragmentów roślin lawendy, elicytowanych w kulturach *in vitro* nanocząstkami Ag i Au, jako substancji konserwujących emulsje kosmetyczne **(publikacja 4)**.

4.4. Publikacja nr 4

Jadczak P., Kulpa D. 2020. *Lavandula angustifolia* propagated in *in vitro* cultures on media containing AgNPs and AuNPs: an alternative to synthetic preservatives in cosmetics. Folia Pomeranae Universitatis Technologiae Stetinensis. 357(56)4, 5-8.

Produkcja kosmetyków jest jedną z najbardziej dynamicznie rozwijających się gałęzi przemysłu. Ograniczenie możliwości namnażania się mikroorganizmów w produktach kosmetycznych, przy jednoczesnym ograniczeniu stosowania konserwantów chemicznych jest jednym z najważniejszych wyzwań podczas produkcji kosmetyków. Zanieczyszczenie mikrobiologiczne produktu kosmetycznego skraca jego okres przydatności do użycia i może wywierać negatywny wpływ na zdrowie konsumenta. Liczne badania wykazały, że obecność konserwantów chemicznych w kosmetykach powoduje, między innymi alergie kontaktowe i choroby skóry. Dlatego też, coraz częściej przy produkcji kosmetyków sięga się po składniki pochodzenia naturalnego, które uznawane są za bardziej bezpieczne i sprzyjające zdrowiu konsumenta.

Lawenda wąskolistna jest z powodzeniem stosowana w przemyśle kosmetycznym. Rośliny lecznicze rozmnażane technikami *in vitro* charakteryzują się unikalną kompozycją olejków eterycznych. Dodanie do pożywek hodowlanych czynników stresogennych, jakimi są elicytory wpływa istotnie na zmianę składu metabolitów wtórnych, w tym olejków eterycznych. Zmieniony skład może wpływać na właściwości przeciwdrobnoustrojowe roślin. Celem ostatniego etapu badań było określenie możliwości wykorzystania rozdrobnionych tkanek lawendy wąskolistnej hodowanej na podłożach zawierających nanocząstki złota lub srebra o rozmiarach cząsteczek 13 i 30 nm do konserwacji emulsji kosmetycznych.

Wykazano, że tkanki lawendy hodowanej na podłożach zawierających AuNPs i AgNPs wykazały zadowalające zdolności konserwujące. W przypadku emulsji kosmetycznych kontrolnych, które nie zawierały dodatku tkanek roślinnych ani kwasu dehydrooctowego i benzoesowego (DHA BA), zaobserwowano pojawianie się kolonii bakteryjnych i grzybowych po drugim tygodniu trwania doświadczenia. Dodatek tkanek lawendy hodowanej na pożywkach nie zawierających nanocząstek chronił badane próbki przed zanieczyszczeniem mikrobiologicznym. W tym przypadku kolonie bakteryjne zostały wykryte po 4 tygodniach, a grzybowe po 6 tygodniach inkubacji. Dodatek tkanki lawendy rosnącej na podłożach uzupełnionych w AgNPs o wielkości cząstek 13 nm oraz w stężeniu 1 $mg \cdot dm^{-3}$ wydłużył czas pojawiania się kolonii bakteryjnych do 8 tygodni, a wynik ten był porównywalny z efektem konserwującym DHA BA. Wyższe stężenie AgNPs w podłożu

hodowlanym, a także większa średnica cząstek (30 nm), spowodowały zmniejszenie zdolności konserwujących tkanek roślinnych. Obecność AuNPs w podłożach hodowlanych także wykazała pozytywny wpływ na aktywność antymikrobiologiczną lawendy, jednak w mniejszym stopniu niż w przypadku AgNPs.

Powyższe rezultaty badań wskazują, że rozdrobnione fragmenty tkanki lawendy, pozyskane z roślin hodowanych na pożywkach zawierających 1 $mg \cdot dm^{-3}$ AgNPs o wielkości cząstek 13 nm mogą być wykorzystane do konserwacji emulsji kosmetycznych o krótkim okresie przydatności do użycia.

5. Wnioski

1. Wyniki przeprowadzonych badań wykazały możliwość otrzymania dużej ilości tkanek roślinnych lawendy, z prawidłowo wykształconymi trichomami wydzielniczymi poprzez namnażanie ich w kulturach *in vitro*, na pożywkach uzupełnionych nanokoloidami zawierającymi AgNps oraz AuNPs.
2. Rośliny lawendy rozmnażane na pożywkach z dodatkiem nanokoloidów zawierających AgNPs i AuNPs, niezależnie od ich stężenia w pożywce, charakteryzowały się wyższą świeżą masą i liczbą wykształconych pędów oraz dłuższym korzeniem.
3. Dodatek do pożywek niskich stężeń AgNPs (1 i 2 $mg \cdot dm^{-3}$) nie wywierał wpływu na wysokość namnożonych roślin lawendy. Zwiększenie jego stężenia w pożywce powyżej 5 $mg \cdot dm^{-3}$ wpłynęło na ograniczenie wzrostu roślin i zmniejszenie liczby wykształconych trichomów wydzielniczych, a jednocześnie stymulowało rozwój systemu korzeniowego.
4. Namnażanie lawendy w kulturach *in vitro* na pożywkach z dodatkiem od 1 do 10 $mg \cdot dm^{-3}$ AuNPs wpłynęło na zwiększenie liczby wykształconych trichomów wydzielniczych.
5. Najważniejszymi związkami występującymi w olejkach eterycznych lawendy wąskolistnej były: borneol, τ-kadinol, tlenek kariofilenu, γ-kadinen i 1,8-cineol.
6. Nanocząstki złota i srebra dodawane do pożywek miały wpływ na skład chemiczny olejków eterycznych. Powodując zmniejszenie udziału procentowego związków o niższej masie cząsteczkowej, takich jak α- i β-pinen, kamfen, δ-3-karen, p-cymen, eucaliptol, czy borneol) oraz wzrost udziału procentowego związków o wyższej masie cząsteczkowej (τ- i α-kadinol, 9-cedranon, kadalen, α-bisabolol, cis-14-nor-muurol-5-en-4-one, (E,E)-farnesol).
7. Olejek eteryczny pozyskany z tkanek lawendy hodowanej na pożywkach z dodatkiem 10 $mg \cdot dm^{-3}$ AgNPs najbardziej różnił się jakościowo w odniesieniu do kontroli. W jego składzie zidentyfikowano 13 związków chemicznych nieobecnych w olejku izolowanym z roślin kontrolnych, takich jak fenchol, heptadekan, oktadekan, fytan, disobutylphathothate, m-kamforen, eicosane, octadekanol, 1-ocadecanal czy 1-trikoscen.
8. Namnażanie lawendy wąskolistnej na pożywkach z dodatkiem nanocząstek złota i srebra skutkowało wzmożoną aktywnością enzymów antyoksydacyjnych takich jak:

peroksydaza askorbinianowa (APX) i dysmutaza ponadtlenkowa (SOD), wpływało natomiast na obniżenie się aktywności peroksydazy gwajakolowej POX. Poziom aktywności enzymów zależał od stężenia i rodzaju nanocząstki.

9. Dodatek do pożywek namnażających nanocząstek w stężeniu od 1 do 20 $mg \cdot dm^{-3}$ AuNPs oraz od 2 do 50 $mg \cdot dm^{-3}$ AgNPs zwiększał aktywność antyoksydacyjną określoną przez zdolność do zmiatania wolnych rodników ABTS•+. Wyjątek stanowiło tu najniższe stężenie AgNPs (1 $mg \cdot dm^{-3}$) oraz najwyższe stężenie AuNPs (50 $mg \cdot dm^{-3}$), których zdolność pozostawała na poziomie kontroli.
10. Rodzaj nanocząstek dodawanych do pożywki oraz ich stężenie w podłożu hodowlanym wpłynęło na zawartość w lawendzie polifenoli ogółem. Rośliny namnażane na pożywkach z dodatkiem od 2 do 10 $mg \cdot dm^{-3}$ AuNPs oraz od 5 do 50 $mg \cdot dm^{-3}$ AgNPs charakteryzowały się wyższą zawartością polifenoli ogółem w porównaniu z kontrolą.
11. Tkanki lawendy wąskolistnej namnażanej na pożywkach wzbogaconych w 1 $mg \cdot dm^{-3}$ nanocząstek srebra o wielkości cząsteczki 13 nm wykazywały właściwości konserwujące, zabezpieczając emulsje kosmetyczne przed zanieczyszczeniem mikrobiologicznym przez okres do 6 tygodni. Po okresie 8 tygodni obserwowano jedynie niewielki wzrost mikroorganizmów.
12. Fragmenty tkanki lawendy pochodzące z podłoży wzbogacanych w 1 $mg \cdot dm^{-3}$ AgNPs o wielkości cząstek 13 nm mogą być z powodzeniem wykorzystywane do konserwacji emulsji kosmetycznych o krótkim terminie przydatności.

6. Literatura

1. Anastasiadi M., Pratsinis H., Kletsas D., Skaltsounis, A.L., Haroutounian S.A. 2010. Bioactive noncoloured polyphenols content of grapes, wines and vinification by-products: Evaluation of the antioxidant activities of their extracts. Food Research International, 43(3): 805–813.
2. Andrys D., Adaszyńska-Skwirzyńska M., Kulpa D. 2018a. Jasmonic acid changes the composition of essential oil isolated from narrow-leaved lavender propagated in *in vitro* cultures Natural Product Research, 32(7): 834-839.
3. Andrys D., Kulpa D. 2018. *In vitro* propagation affects the composition of narrow-leaved lavender essential oils. Acta Chromatographica, 30(4): 225–230.
4. Andrys D., Kulpa D., Grzeszczuk M., Białecka B. 2018b. Influence of jasmonic acid on the growth and antimicrobial and antioxidant activities of *Lavandula angustifolia* Mill. propagated *in vitro* Folia Horticulturae, 30(1): 3-13.
5. Babushok V.I., Linstrom P.J., Zenkevich I.G. 2011. Retention indices for frequently reported compounds of plantessential oils. Journal of Physicial and Chemical Reference Data,40(4): 043101.
6. Beauchamp C., Fridovich I. 1971. Superoxide dismutase: Improved assay and an assay applicable to acrylamide gels. Analytical Biochemisty, 44(1): 276–287.
7. Chance B., Maehly, A.C. 1995. Assay of catalase and peroxidases. Methods in Enzymology 2, 764–775.
8. Chung I., Rajakumar G., Thiruvengadam M. 2018. Effect of silver nanoparticles on phenolic compounds production and biological activities in hairy root cultures of *Cucumis Anguria*. Acta Biologica Hungarica, 69, 97–109.
9. D'Auria F.D., Tecca M., Strippoli V.S., Battinelli G.M. 2005. Antifungal activity of *Lavandula angustifolia* essential oil against *Candida albicans* yeast and mycelia form. Journal of Medical Mycology, 43(5): 391–396.
10. Dai Y., Wang Z., Zhao J., Xu L., Yu X., Wei Y., Xing B. 2018. Interaction of CuO nanoparticles with plant cells: Internalization, oxidative stress, electron transport chain disruption, and toxicogenomic responses. Environmental Sciience: Nano, 5(10): 2269–2281.
11. De A., Chakrabarti M., Ghosh I. 2016. Evaluation of genotoxicity and oxidative stress of aluminium oxide nanoparticles and its bulk form in *Allium cepa*. Nucleus. 59, 219–225.
12. European Pharmacopoeia 5.0; EDQM: Strasbourg, France, 2005; p. 1894.
13. Fazal H., Abbasi B.H., Ahmad N. 2014. Optimization of adventitious root culture for production of biomass and secondary metabolites in *Prunella vulgaris* L. Applied Biochemistry and Biotechnology, 174(6): 2086–2096.
14. Gilani A.H., Aziz N., Khan M.A., Shaheen F., Jabeen Q., Siddiqui B.S. 2000. Ethnopharmacological evaluation of the anticonvulsant, sedative and antispasmodic activities of *Lavandula* stoechas L. Journal of Ethnopharmacology, 71(1-2):161–167.
15. Gonçalves S., Romano A. 2013. *In vitro* culture of lavenders (*Lavandula* spp.) and the production of secondary metabolites. Biotechnology Advances, 31(1):166–174.
16. Jamshidi M. Ghanti F. 2016. Taxanes content and cytotoxity of hazel cells extract after elicitation with silver nanoparticles. Plant Physiology and Biochemistry, 110, 178–184.
17. Jamshidi M., Ghanati F., Rezaei A., Bemani E. 2016. Change of antioxidant enzymes activity of hazel (*Corylus avellana* L.) cells by AgNPs. Cytotechnology. 68(3): 525–530.
18. Li Y., Schellhorn H.E. 2007. Rapid kinetic microassay for catalase activity. Journal of Biomolecular Techniques, 18(4): 185–187.
19. Mirzajani F., Askari H., Hamzelou S., Farzaneh M., Ghassempour A. 2013. Effect of silver nanoparticles on *Oryza sativa L*. and its rhizosphere bacteria. Ecotoxicology and Environmental Safety, 88(1): 48–54.
20. Murashige T., Skoog F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiologia Plantarum, 15, 473–497.
21. Nair R., Varghese S.H., Nair B.G., Maekaa T., Yoshida Y., Kumar D.S. 2010. Nanoparticulate material delivery to plants. Plant Science, 179(3): 154–163
22. Nakano Y., Asada K. 1981. Hydrogen peroxide is scavenged by ascorbate specific peroxidase in spinach chloroplasts. Plant & Cell Physiology, 22(5): 867–880.

23. Navarro E., Baun A., Behra R., Hartmann N.B., Filser, J., Miao A., Quigg A., Santschi, P.H. Sigg I. 2008. Environmental behavior and ecotoxicity of engineered nanoparticles to algae, plants and fungi. Ecotoxicology. 17, 372–386.
24. Pitta-Alvarez S.I., Spollansky T.C., Giulietti A.M. 2000. The influence of different biotic and abiotic elicitors on the production and profile of tropane alkaloids in hairy root cultures of *Brugmansia candida*. Enzyme and Microbial Technology, 26(2-4): 252–258.
25. Raei M., Angaji A.A., Omidi M., Khodayari M. 2014.Effect of abiotic elicitors on tissue culture of *Aloe vera*. International Journal of Biosciences, 5(1): 74-81.
26. Rao S., Shekhawat G.S. 2016. Phytotoxicity and oxidative stress perspective of two selected nanoparticles in *Brassica juncea*. 3 Biotech, 6(2): 244.
27. Sarmast MK, Niazi A, Salehi H, Abolimoghadam A. 2015. Silver nanoparticles affect ACS expression in *Tecomella undulata in vitro* culture. Plant Cell, Tissue and Organ Culture, 121, 227–236.
28. Shakeran Z., Keyhanfari M., Asghari G., Ghanadian M. 2015.Improvement of atropine production by different biotic and abiotic elicitors in hairy root cultures of *Datura metel*. Turkish Journal of Biology, 39, 111-118.
29. Shi F., Jia X., Zhao C., Chen Y. 2010. Antioxidant activities of various extracts from *Artemisisa selengensis* turcz (LuHao). Molecules. 15(7): 4934–4946.
30. Spinoso-Castillo J.L., Chavez-Santoscoy R.A., Bogdanchikova N., Pérez-Sato J.A., Morales-Ramos V., Bello-Bello J.J. 2017. Antimicrobial and hormetic effects of silver nanoparticles on *in vitro* regeneration of vanilla (*Vanilla planifolia* Jacks. ex Andrews) using a temporary immersion system. Plant Cell, Tissue and Organ Culture, 129, 195–207.
31. Sreelekshmi R., Siril E. A., Muthukrishna S. 2021. Role of Biogenic Silver Nanoparticles on Hyperhydricity Reversion in *Dianthus chinensis* L. an *In Vitro* Model Culture. Journal of Plant Growth Regulation,
32. Trejgell A, Kęsy J. 2019. Evaluation of shoot development and terpenes production in *Lavandula angustifolia* plantlets *in vitro* cultured under red and blue light. Acta Biologica Cracoviensia, 61(1): 35.
33. Van Den Dool H., Kratz P.D. 1963. A generalization of the retention index system including linear temperature programmed gas-liquid partition chromatography. Journal of Chromatography. A, 11, 463–471.
34. Wornouk G., Demisse Z., Rheut M., Mahmoud S. 2011. Biosynthesis and Therapeutic properties of *Lavandula* essential oil constituent. Planta Medica, 77(1): 7–15.
35. Zhang B., Zheng L.P., Wan Wen W.J. 2013. Stimulation of Artemisinin Production in *Artemisia annua* hairy roots by Ag-SiO2 core-shell nanoparticles. Current Nanoscience, 9(3): 363–370.
36. Zhang C., Yan Q., Cheuk W., Wu J. 2004. Enhancement of Tanshinone Production in *Salvia miltiorrhiza* hairy root culture by Ag elicitation and nutrient feeding. Planta Medica, 70(20): 147–151.
37. Zhao J., Hu Q., Guo Y.Q., Zhu W.H. 2001. Elicitor-induced indole alkaloid biosynthesis in *Catharanthus roseus* cell cultures is related to Ca? Influx and the oxidative burst. Plant Science, 161, 423–431.

7. Streszczenie

Lawenda wąskolistna to ceniona roślina lecznicza i ozdobna, uprawiana powszechnie na całym świecie. Produkowane przez nią olejki eteryczne, które są mieszaniną szeregu związków lotnych, szeroko stosowane są w przemysłach kosmetycznym, perfumeryjnym, farmaceutycznym, a także spożywczym, czy tekstylnym, w których w ostatnim czasie szczególną uwagę przywiązuje się związkom pochodzenia naturalnego, uznawanym za bezpieczne dla środowiska i konsumentów. Światowe trendy technologiczno-przemysłowe poszukują alternatyw dla sztucznych, pozyskiwanych chemicznie związków konserwujących, i zapachowych. Zanieczyszczenie środowiska oraz zmniejszające się nieprzerwanie zasoby stanowisk pod uprawy polowe są niejednokrotnie ograniczeniem przy produkcji związków biologicznie czynnych. Z tego właśnie powodu opracowuje się nowe technologie, które będą jak najbardziej wydajne, niezależne od warunków klimatyczno-glebowych i pozwalające na szybką i nieprzerwaną produkcje związków pochodzenia roślinnego. Olejki eteryczne roślin produkowane są przez nie często w niewielkich ilościach lub w szczególnych momentach rozwojowych (m.in. w okresie kwitnienia lub w odpowiedzi na czynniki stresowe). Z powodzeniem do produkcji ich wykorzystywane mogą być roślinne kultury *in vitro*. Za ich sprawą produkowany materiał roślinny jest wolny od zanieczyszczeń oraz chorób i pozbawiony pozostałości środków ochrony roślin. Co więcej, kontrolowane warunki laboratoryjne pozwalają na nieprzerwaną oraz niezależną od czynników środowiskowych produkcję roślin leczniczych bogatych w związki biologicznie czynne. Technika elicytacji może wpływać na wzrost sekrecji olejków eterycznych. Może także prowadzić do uzyskiwania związków o zamierzonym składzie oraz o pożądanych właściwościach i przeznaczeniu. Coraz częściej w procesie elicytacji próbuje się wykorzystywać nanocząstki metali, co do których dowiedziono, że ze względu na swoje właściwości są w stanie wpływać na procesy biologiczne zachodzące w roślinach, w tym na proces produkcji przez nie metabolitów wtórnych (m. in. olejków eterycznych). Celem niniejszej pracy doktorskiej było określenie wpływu nanocząstek złota i srebra na produkcję metabolitów wtórnych w kulturach *in vitro* lawendy wąskolistnej.

W pierwszym etapie badań zdefiniowano, w jaki sposób dodatek nanocząstek złota (AuNPs) i srebra (AgNPs) do pożywek hodowlanych wpływa na rozwój roślin i trichomy wydzielnicze lawendy wąskolistnej. Pędy roślin namnażano na pożywkach z dodatkiem 1, 2, 5, 10, 20 i 50 $mg \cdot dm^{-3}$ AuNPs lub AgNPs (o średnicy cząstki 24,2 ± 2,4 nm i 27,5 ± 4,8 nm). Oba NPs pozytywnie wpłynęły na wzrost i rozwój roślin w kulturach *in vitro*. Pożywki z NPs stymulowały tworzenie się pędów i zwiększały masę roślin. Korzenie roślin

namnażanych na pożywkach z dodatkiem NPs były zwykle dłuższe niż w kontroli. Jedynie wysokie stężenia NPs (20 i 50 mg·dm^{-3}) w pożywkach były toksyczne dla roślin, o czym świadczyło ograniczenie długości pędów i stopniowe obniżanie wartości innych cech morfologicznych. Wzrost stężenia AgNPs powodował zmniejszenie liczby trichomów wydzielniczych. Średnica trichomów wydzielniczych po obu stronach blaszki liściowej była większa, gdy rośliny były namnażane na pożywkach z dodatkiem 1 i 2 mg·dm^{-3} NPs. Średnica trichomów wydzielniczych znajdujących się na adaksjalnej powierzchni blaszki liściowej była największa u roślin pochodzących z podłoży wzbogaconych w 2 mg·dm^{-3} AgNPs i 5 mg·dm^{-3} AuNPs, a najmniejsza w przypadku podłoży wzbogaconych w 5 mg·dm^{-3} AgNPs. Średnica trichomów wydzielniczych tworzących się na powierzchni abaksjalnej była największa u roślin eksponowanych na 1, 2, 5 i 10 mg·dm^{-3} AuNPs, 1 mg·dm^{-3} AgNPs, a najmniejsza u roślin eksponowanych na 5 mg·dm^{-3} AgNPs.

Następnie określono, w jaki sposób dodatek nanocząstek do podłoży hodowlanych wpływa na skład olejków eterycznych lawendy. Rośliny namnażano na podłożach MS z dodatkiem 10 i 50 mg·dm^{-3} nanokoloidów złota (24,2 ± 2,4 nm) i srebra (27,5 ± 4,8 nm). Olejek pozyskany z tkanek lawendy namnażanej na podłożu z dodatkiem 10 mg·dm^{-3} AgNPs różnił się najbardziej w stosunku do kontroli; nie wykryto w nim w ogóle 10 związków, a wykryto 13 innych, które nie występowały w olejku kontrolnym. Dodatek AuNPs i AgNPs do pożywek spowodował zmniejszenie ilości związków o mniejszej masie cząsteczkowej, takich jak: α- i β-pinen, kamfen, δ-3-carene, p-cymen, 1,8-cyneol, trans-pinokarveol, kamforiborneol, które zostały zastąpione przez te o wyższej masie cząsteczkowej (τ- i α-kadynol, 9-cedranon, kadalen, α-bisabolol, cis-14-nor-muurol-5-en-4-on, (E,E)-farnesol).

Zbadano także wpływ nanocząstek złota i srebra na aktywność enzymów antyoksydacyjnych (peroksydazy askorbinianowej (APX), dysmutazy ponadtlenkowej (SOD), peroksydazy gwajakolowej (POX) i katalazy (CAT)), zdolność do zmiatania wolnych rodników oraz zawartość polifenoli ogółem w lawendzie wąskolistnej. W niniejszym doświadczeniu rośliny lawendy hodowano *in vitro* na podłożu z dodatkiem 1, 2, 5, 10, 20 i 50 mg·dm^{-3} AgNPs lub AuNPs, o rozmiarach cząstek odpowiednio 24,2 ± 2,4 i 27,5 ± 4,8 nm. Stwierdzono, że nanocząstki zwiększają aktywność enzymów antyoksydacyjnych APX i SOD, przy czym reakcja ta zależy od stężenia NPs. Najwyższą aktywność APX stwierdzono u roślin namnażanych na pożywkach wzbogaconych w 2 i 5 mg·dm^{-3} AgNPs. AuNPs istotnie zwiększyło aktywność APX po dodaniu do pożywki w stężeniu 10 mg·dm^{-3}. Najwyższą aktywność SOD odnotowano przy stężeniach 2 i 5 mg·dm^{-3} AgNP i AuNP. Dodanie większych stężeń nanocząstek do pożywek hodowlanych powodowało spadek aktywności APX i SOD.

Dodatek AuNPs do pożywek hodowlanych w stężeniach od 2 do 50 mg·dm^{-3} powodowało wzrost aktywności POX, w porównaniu z jej aktywnością po dodaniu AgNPs do pożywek hodowlanych. Nie wykazano istotnego wpływu NPs na wzrost aktywności CAT. AgNPs i AuNPs zwiększały zdolność zmiatania wolnych rodników (ABTS•+). Dodatek NPs w stężeniach 2 i 5 mg·dm^{-3} zwiększał produkcję polifenoli, natomiast w niższych stężeniach obniżał ich zawartość w tkankach lawendy.

Wcześniej uzyskane wyniki były powodem podjęcia próby zweryfikowania właściwości konserwujących lawendy wąskolistnej namnażanej na podłożach z nanocząstkami złota lub srebra o rozmiarach cząstek 13 i 30 nm. Emulsje kosmetyczne przygotowane z wykorzystaniem tkanek lawendy pochodzących z hodowli na podłożach zawierających AuNPs i AgNPs wykazywały zwiększone zdolności konserwujące, w porównaniu z emulsjami kontrolnymi. W przypadku kontrolnych emulsji kosmetycznych, które nie zawierały dodatku tkanek roślinnych oraz DHA BA, kolonie bakterii i grzybów pojawiły się już po drugim tygodniu eksperymentu. Dodatek tkanki lawendy namnożonej na podłożach bez AuNPs lub AgNPs chronił badane próbki przed skażeniem mikrobiologicznym; w tym przypadku skażenie bakteryjne wykryto po 4 tygodniach, a grzybowe po 6 tygodniach. Dodatek tkanki lawendowej pochodzącej z hodowli na pożywkach zawierających AgNPs o wielkości cząstek 13 nm w stężeniu 1 mg·dm^{-3} wydłużył czas pojawiania się kolonii bakteryjnych do 8 tygodni (0,9) i był to wynik zbliżony i porównywalny z działaniem DHA BA. Wyższe stężenie AgNPs w podłożu hodowlanym, jak również większa średnica cząstek (30 nm), powodowały obniżenie zdolności konserwujących tkanek roślinnych. Obecność AuNPs w podłożu hodowlanym wpływała pozytywnie na aktywność przeciwdrobnoustrojową lawendy, jednak w mniejszym stopniu niż w przypadku AgNPs. Doświadczenie dowiodło, że fragmenty tkanki lawendy pochodzące z podłoży wzbogacanych w 1 mg·dm^{-3} AgNPs, o wielkości cząstek 13 nm mogą być wykorzystywane do konserwacji emulsji kosmetycznych o krótkim terminie przydatności.

Przedstawiona praca doktorska ma charakter aplikacyjny oraz udowadnia zasadność dalszych badań w obszarze wykorzystania nanocząstek złota i srebra do produkcji wysokojakościowego materiału roślinnego, który mógłby być stosowany w przemyśle kosmetycznym, w charakterze naturalnego środka konserwującego.

8. Abstract

Narrow –Leaved Lavender is a valued medicinal and ornamental plant, widely cultivated all over the world. The essential oils it produces, which are a mixture of a number of volatile compounds, are widely used in cosmetic, perfumery, pharmaceutical, food and textile industries, where special attention has recently been paid to compounds of natural origin, considered safe for the environment and for consumers. Global technological and industrial trends are looking for alternatives to artificial, chemically produced preservative and fragrance compounds. Environmental pollution and the continually decreasing amount of land for field crops are often a limitation in the production of biologically active compounds. For this reason, new technologies are being developed to be as efficient as possible, independent of soil and climatic conditions and allowing rapid and uninterrupted production of compounds of plant origin. Essential oils are often produced by plants in small quantities or in specific moments of development (e.g. during flowering or in response to stress factors). Plant *in vitro* cultures can be successfully used for their production. Thanks to them, the produced plant material is free from contaminants and diseases, and free from plant protection agent residues. Moreover, controlled laboratory conditions allow the production of medicinal plants rich in biologically active compounds to be uninterrupted and independent from environmental factors. The elicitation technique can increase the secretion of essential oils. It can also lead to obtaining compounds with the intended composition and with the desired properties and purpose. More and more often metal nanoparticles are used in the elicitation process. It has been proven that due to their properties they are able to influence biological processes occurring in plants, including production of secondary metabolites (including essential oils). The aim of this dissertation was to determine the effect of gold and silver nanoparticles on the production of secondary metabolites in *in vitro* cultures of narrow-leaved lavender.

As a first step, this study defined how the addition of gold (AuNPs) and silver (AgNPs) nanoparticles to culture media affects plant development and secretory trichomes of narrow-leaf lavender. Plant shoots were propagated on culture media supplemented with 1, 2, 5, 10, 20 and 50 mg·dm^{-3} AuNPs or AgNPs (particle diameter 24.2 ± 2.4 nm and 27.5 ± 4.8 nm, respectively). Both NPs positively affected plant growth and development in *in vitro* cultures. The media with NPs stimulated shoot formation and increased plant weight. Roots of plants propagated on NPs-supplemented media tended to be longer than in the control. Only high concentrations of NPs (20 and 50 mg·dm^{-3}) in the media were toxic to

plants, as evidenced by a reduction in shoot length and a gradual decrease in the values of other morphological traits. An increase in AgNPs concentration resulted in a decrease in the number of secretory trichomes. The diameter of trichomes on both sides of the leaf blade was larger when plants were propagated on media supplemented with 1 and 2 mg·dm^{-3} NPs. The diameter of trichomes located on the adaxial surface of the leaf blade was the largest in plants derived from media enriched with 2 mg·dm^{-3} AgNPs and 5 mg·dm^{-3} AuNPs, and the smallest in media enriched with 5 mg· dm^{-3} AgNPs. The diameter of trichomes formed on the abaxial surface was the largest in plants exposed to 1, 2, 5, and 10 mg·dm^{-3} AuNPs, 1 mg·dm^{-3} AgNPs, and the smallest in plants exposed to 5 mg·dm-^{3} AgNPs.

Next, we determined how the addition of nanoparticles to culture media affects the composition of lavender essential oils. Plants were propagated on MS media supplemented with 10 and 50 mg·dm^{-3} nanocolloids of gold (24.2 ± 2.4 nm) and silver (27.5 ± 4.8 nm). The oil extracted from lavender tissues propagated on medium supplemented with 10 mg· dm^{-3} AgNPs differed the most from the control; 10 compounds were not detected in the oil at all, and 13 others were detected that were not present in the control oil. The addition of AuNPs and AgNPs to the media resulted in a reduction of lower molecular weight compounds (α- and β-pinene, camphene, δ-3-carene, p-cymene, 1,8-cyneol, trans-pinocarveol, camphoriborneol), which were replaced by those of higher molecular weight (τ- and α-cadinol 9-cedranone, cadalene, α-bisabolol, cis-14-nor-muurol-5-en-4-one, (E,E)-farnesol).

The effects of gold and silver nanoparticles on antioxidant enzyme activities (ascorbate peroxidase (APX), superoxide dismutase (SOD), guaiacol peroxidase (POX), and catalase (CAT)), free radical scavenging capacity, and total polyphenol content of narrow-leaf lavender were also investigated. In the present experiment, lavender plants were grown *in vitro* on medium supplemented with 1, 2, 5, 10, 20 and 50 mg·dm^{-3} AgNPs or AuNPs (with particle sizes of 24.2 ± 2.4 and 27.5 ± 4.8 nm, respectively). Nanoparticles were found to increase the activities of the antioxidant enzymes APX and SOD, with the response depending on the concentration of NPs. The highest APX activity was found in plants grown on media enriched with 2 and 5 mg·dm^{-3} AgNPs. AuNPs significantly increased APX activity when added to the medium at a concentration of 10 mg·dm^{-3}. The highest SOD activity was observed at concentrations of 2 and 5 mg·dm^{-3} AgNPs and AuNPs. Addition of higher concentrations of nanoparticles to culture media resulted in a decrease in APX and SOD activities. The addition of AuNPs to the culture media at concentrations

ranging from 2 to 50 mg·dm^{-3} resulted in an increase in POX activity compared to its activity when AgNPs were added to the culture media. There was no significant effect of NPs on the increase in CAT activity. AgNPs and AuNPs increased the free radical scavenging capacity (ABTS•+). The addition of NPs at concentrations of 2 and 5 mg·dm^{-3} increased the production of polyphenols, but at lower concentrations decreased their content in lavender tissues.

Previously obtained results were the reason for an attempt to verify the preservative properties of narrow-leafed lavender grown on media with gold or silver nanoparticles with particle sizes of 13 and 30 nm. Cosmetic emulsions prepared using lavender tissue derived from culture containing AuNPs and AgNPs showed enhanced preservative abilities compared to control emulsions. For the control cosmetic emulsions that did not contain the addition of plant tissue and DHA BA, bacterial and fungal colonies appeared as early as the second week of the experiment. The addition of lavender tissue grown on media without AuNPs or AgNPs protected the trial samples from microbial contamination; in this case, bacterial contamination was detected after 4 weeks and fungal contamination after 6 weeks. The addition of lavender tissue from cultures on media containing AgNPs with a particle size of 13 nm at a concentration of 1 mg·dm^{-3} increased the time for the appearance of bacterial colonies to 8 weeks (0.9) and this was similar and comparable to the effect of DHA BA. The higher concentration of AgNPs in the culture medium, as well as the larger particle diameter (30 nm), resulted in a decrease in the preservative capacity of plant tissues. The presence of AuNPs in the culture medium had a positive effect on the antimicrobial activity of lavender, but to a lesser extent than that of AgNPs. The experiment demonstrates that lavender tissue fragments from media enriched in 1 mg·dm^{-3} AgNPs with a particle size of 13 nm can be used for the preservation of cosmetic emulsions with a short shelf life.

The presented dissertation has an applied character and proves the validity of further research in the area of utilization of gold and silver nanoparticles in the production of high quality plant material, which could be used in the cosmetic industry as a natural preservative.

9. Jednotematyczny cykl publikacji

Wykaz publikacji stanowiący wskazanie osiągnięcia wynikającego z Art. 15 Ust. 2 Ustawy z dnia 14 marca 2003 roku o stopniach naukowych i tytule naukowym oraz o stopniach i tytule w zakresie sztuki (Dz.U. 2016, poz. 882) pod tytułem:

Wpływ nanokoloidów złota i srebra na produkcję metabolitów wtórnych w kulturach *in vitro* lawendy wąskolistnej (*Lavandula angustifolia* Mill.)

Lp.	Tytuł publikacji, autorzy, czasopismo naukowe, DOI, udział własny	Pkt.	IF**
P1	**Jadczak P.,** Kulpa D., Bihun M., Przewodowski W.2019. Possitive Effect Of AgNPs and AuNPs In *In Vitro* Cultures of *Lavandula angustifolia* Mill. Plant Cell, Tissue and Organ Culture. DOI: 10.1007/s11240-019-01656-w (udział własny: 51%; opracowanie koncepcji badań, prowadzenie doświadczeń w kulturach *in vitro* oraz współudział w przeprowadzeniu analizy statystycznej wyników i pisanie publikacji).	100*	2,329
P2	Wesołowska A., **Jadczak P**., Kulpa D., Przewodowski W. 2019. Gas chromatography mass spectrometry (GC-MS) analysis of essential oils from AgNPs and AuNPs elicited *Lavandula angustifolia in vitro* cultures. Molecules. DOI: 10.3390/molecules24030606 (udział własny: 40%; opracowanie koncepcji badań, prowadzenie doświadczeń w kulturach *in vitro* oraz współudział w przeprowadzeniu analizy statystycznej wyników i pisaniu publikacji)	140*	3,267
P3	**Jadczak P**., Kulpa D., Drozd R., Przewodowski W., Przewodowska A., 2020. Effect of AuNPs and AgNPs on antioxidant system and antioxidant activity of lavender (*Lavandula angustifolia* Mill.) from *in vitro* cultures. Molecules. DOI: 10.3390/molecules25235511. (udział własny: 50 %; opracowanie koncepcji badań, prowadzenie doświadczeń w kulturach *in vitro* oraz współudział w przeprowadzeniu analizy statystycznej wyników i pisanie publikacji)	140*	3,267
P4	**Jadczak P**., Kulpa D., *Lavandula angustifolia* propagated in *in vitro* cultures on media containing AgNPs and AuNPs – an alternative to synthetic preservatives in cosmetics. 2020. Folia Pomeranae Univrsitatis Technologiae Stetinensis seria Agricultura, Alimentaria, Piscaria et Zootechnica. DOI: 10.21005/AAPZ2020.56.4.01 (udział własny: 60%; współpraca przy opracowaniu koncepcji badań, współudział w przeprowadzeniu analizy statystycznej wyników i pisaniu publikacji)	40*	-
	Suma	420	8,863

* Liczba punktów według listy MNiSW z dnia 01.12.2021 r.
** Impact Factor (IF) wg bazy Journal Citation Reports (JCR) z roku wydania

Plant Cell, Tissue and Organ Culture (PCTOC)
https://doi.org/10.1007/s11240-019-01656-w

RESEARCH NOTE

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

Paula Jadczak[1] · Danuta Kulpa[1] · Magdalena Bihun[2] · Włodzimierz Przewodowski[3]

Received: 25 March 2019 / Accepted: 20 July 2019

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

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

Communicated by Nokwanda Pearl Makunga.

✉ Paula Jadczak
paula.jadczak@zut.edu.pl; paulajadczak@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

Published online: 31 July 2019

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

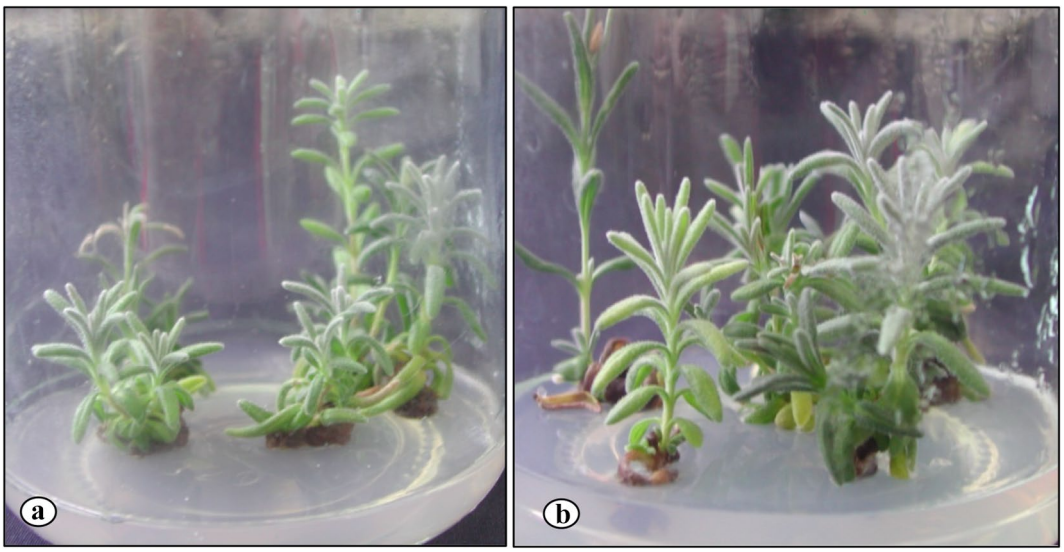

Fig. 1 Lavender propagated on control media (**a**) and enriched with 50 mg dm^{-3} AgNPs (**b**)

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 (1–2 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 5–50 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 (1–2 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

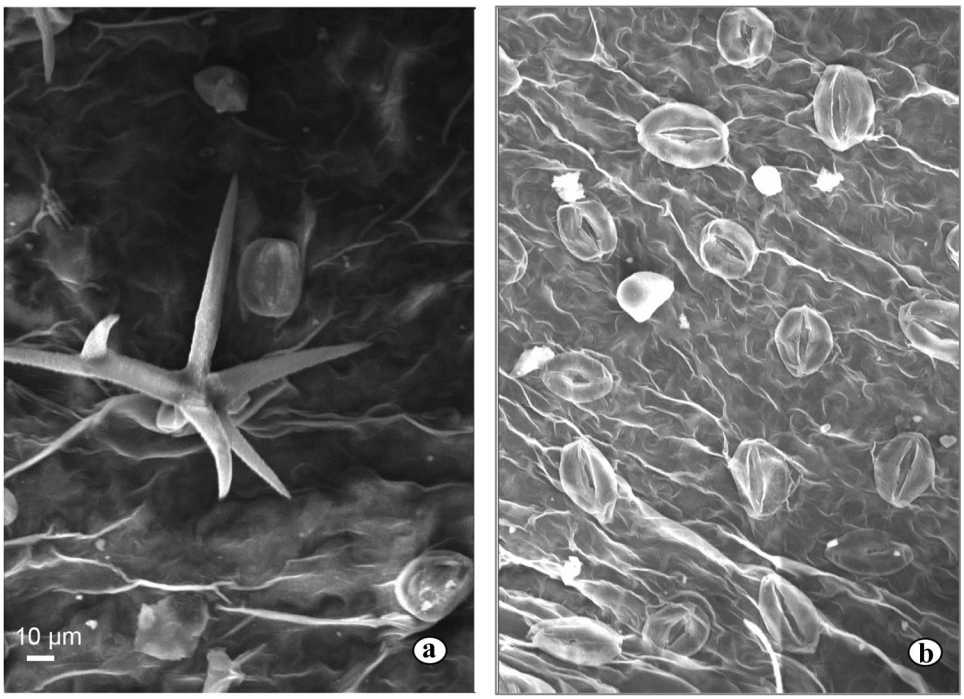

Fig. 2 Lavender propagated on control media (**a**) and with 50 mg dm^{-3} AgNPs, secretory structures under magnification × 1000 (**b**)

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 µm, respectively), and smallest (64.2 µm) 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 µm), 2 mg dm^{-3} AuNPs (71.2 µm), 10 mg dm^{-3} AuNPs (70.9 µm), and 1 mg dm^{-3} AuNPs and AgNPs (70.4 and 69.4 µm). 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 µm), 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 (1–5 mg dm^{-3} AgNPs and 1–2 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 *Campomanesia 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 phytostimulatory 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.

References

Andrys D, Kulpa D (2018) *In vitro* propagation affects the composition of narrow-leaved lavender essential oils. Acta Chromatogr 30:225–230

Andrys D, Adaszynska-Skwirzynska M, Kulpa D (2018) Jasmonic acid changes the composition of essential oil isolated from narrow-leaved lavender propagated in in vitro cultures. Nat Prod Res 32:834–839

D'Auria FD, Tecca M, Strippoli VS, Battinelli GM (2005) Antifungal activity of *Lavandula angustifolia* essential oil against *Candida albicans* yeast and mycelia form. Med Mycol 43:391–396

De Smet I, Signora L, Beeckman T, Inze D, Foyer CH, Zhang H (2003) An abscisic acid-sensitive checkpoint in lateral root development of *Arabidopsis*. Plant J 33:543–555

De Smet I, Zhang H, Inze D, Beeckman T (2006) A novel role for abscisic acid emerges from underground. Trends Plant Sci 11:434–439

Dimpka CO, Lean JE, Martineau N, Britt DW, Haverkamp R, Anderson AJ (2013) Silver nanoparticles disrupt wheat (*Triticum aestivum* L.) growth in a sand matrix. Environ Sci Technol 42(2):1082–1090

El-Temsah YS, Joner EJ (2012) Impact of Fe and Ag nanoparticles on seed germination and differences in bioavailability during exposure in aqueous suspension and soil. Environ Toxicol 27(1):42–49

Feichtmeier NS, Walther P, Leopold K (2015) Uptake, effects, and regeneration of barley plants exposed to gold nanoparticles. Environ Sci Pollut Res Int 22:8549–8558

Gazdovska-Simic S, Maury S, Delaunay A, Spasenoski M, Hagege D, Courtois D, Joseph C (2013) The influence of salicylic acid elicitation of shoots, callus and cell suspension cultures on production of naphtodianthrones and phenylpropanoids in *Hypericum perforatum* L. Plant Cell Tissue Organ Cult 113:25–39

Geche TS, Van Breusegem F, Stone JM, Denev I, Laloi C (2006) Reactive oxygen species as signals that modulate plant stress responses and programmed cell death. BioEssays 28:1091–1101

Ghanti F, Somayeh B (2013) Changes of natural compounds of *Artemisia annua* L. by methyl jasmonate and silver nanoparticles. Adv Environ Biol 7(9):2252–2258

Ghanti F, Somayeh B (2014) Effect of methyl jasmonate and silver nanoparticles on production of secondary metabolites by *Calendula officinalis* L. (*Asteraceae*). Trop J Pharm Res 13(11):1783–1789

Gilani AH, Aziz N, Khan MA, Shaheen F, Jabeen Q, Siddiqui BS (2000) Ethnopharmacological evaluation of the anticonvulsant, sedative and antispasmodic activities of *Lavandula stoechas* L. J Ethnopharmacol 71:161–167

Gonçalves S, Romano A (2013) *In vitro* culture of lavenders (*Lavandula* spp.) and the production of secondary metabolites. Biotechnol Adv 31:166–174

Hatami M, Hatamzadeh AB, Ghasemnezhad M, Sajidi RH (2016) Variations of the phytochemical compounds in rose scented geranium plant exposed to nanosilver particles. J Essent Oil Bear Plants 19(7):1747–1753

Ishida T, Kutata T, Okada K, Wada T (2008) A genetic regulatory network in the development of trichomes and root hairs. Annu Rev Plant Biol 59:365–386

Kumar V, Guleria P, Kumar V, Yadav SK (2013) Gold nanoparticles exposure indices growth and yield enhancement in *Arabidopsis thaliana*. Sci Total Environ 1:462–468

Liu F-K, Ker C-J, Chang Y-C, Ko F-H, Chu T-C, Dai B-T (2003) Microwave heating for the preparation of nanometer gold particles. Jpn J Appl Phys 42:4152–4158

Maes L, Gossens A (2010) Hormone-mediated promotion of trichome initiation in plants is conserved but utilizes species and trichome-specific regulatory mechanisms. Plant Signal Behav 5(2):205–207

Maes L, Inzé D, Goossens A (2008) Functional specialization of the TRANSPARENT TESTA GLABRA1 network allows differential hormonal control of laminal and marginal trichome initiation in *Arabidopsis* rosette leaves. Plant Physiol 148:1453–1464

Mirzajani F, Askari H, Hamzelou S, Farzaneh M, Ghassempour A (2013) Effect of silver nanoparticles on *Oryza sativa* L. and its rhizosphere bacteria. Exotoxicol Environ Saf 88:48–54

Mulabagal V, Tsay H-S (2004) Plant cell cultures—an alternative and efficient source for the production of biologically important secondary metabolites. Int J Appl Sci Eng 2(1):29–48

Murashige T, Skoog F (1962) A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiol Plant 15:473–497

Perazza D, Vachon G, Herzog M (1998) Gibberellins promote trichome formation by upregulating GLABROUS1 in *Arabidopsis*. Plant Physiol 117:375–383. https://doi.org/10.1104/pp.117.2.375

Potters G, Pasternak TP, Guisez Y, Jansen MAK (2007) Stress-induced morphogenic responses: growing out of trouble? Trends Plant Sci 12:98–105

Priyadarshini S, Sanajay G, Sandeep A (2014) Effect of silver nanoparticles on antioxidant status of *Brassica juncea* callus. Indian J Med Res 8:1–2

Qian H, Peng X, Han X, Ren J, Sun L, Fu Z (2013) Comparison of the toxicity of silver nanoparticles and silver ions on growth of terrestrial plant model *Arabidopsis thaliana*. J Environ Sci 25(9):1947–1956

Raei M, Abdolhamid Angaji S, Omidi M, Khodayari M (2014) Effect of abiotic elicitors on tissue culture of *Aloe vera*. Int J Biosci 5(1):74–81

Rezvani N, Sorooshzadeh A, Farhadi N (2012) Effect of nano-silver on growth of saffron in flooding stress. World Acad Sci Eng Technol 61:606–611

Salama HMH (2012) Effects of silver nanoparticles in some crop plants, common bean (*Phaseolus vulgaris* L.) and corn (*Zea mays* L.). IRJOB 3:190–197

Shakeran Z, Keyhanfari M, Asghari G, Ghanadian M (2015) Improvement of atropine production by different biotic and abiotic elicitors in hairy root cultures of *Datura metel*. Turk J Biol 39:111–118

Spinoso-Castillo JL, Chavez-Santoscoy RA, Bogdanchikova N, Pérez-Sato JA, Morales-Ramos V, Bello-Bello JJ (2017) Antimicrobial and hormetic effects of silver nanoparticles on in vitro regeneration of vanilla (*Vanilla planifolia* Jacks ex. Andrews) using a temporary immersion system. Plant Cell Tissue Organ Cult 129(1):195–207

Syu Y-Y, Hung J-H, Chen J-C, Chuang H-W (2014) Impact of size and shape of silver nanoparticles on *Arabidopsis* plant growth and gene expression. Plant Physiol Biochem 83:57–64

Timoteo C, Paiva R, dos Reis MV, Cunha Claro PI, Corrêa da Silva DP, Marconcini JM, de Oliveira JE (2019) Silver nanoparticles in the micropropagation of *Campomanesia rufa* (O. Berg) Nied. Plant Cell Tissue Organ Cult 137(2):359–368. https://doi.org/10.1007/s11240-019-01576-9

Traw MB, Bergelson J (2003) Interactive effects of jasmonic acid, salicylic acid and gibberellin on induction of trichomes in *Arabidopsis*. Plant Physiol 133:1367–1375. https://doi.org/10.1104/pp.103.027086

Turkevich J, Stevenson PC, Hillier J (1951) A study of the nucleation and growth processes in the synthesis of colloidal gold. Discuss Faraday Soc 11:55–75

Vinković T, Novák O, Strnad M, Goessler W, Domazet Jurašin DD, Parađiković N, Vrčeke IV (2017) Cytokinin response in pepper plants (*Capsicum annuum* L.) exposed to silver nanoparticles. Environ Res 156:10–18

Wesołowska A, Jadczak P, Kulpa D, Przewodowski W (2019) Gas chromatography–mass spectrometry (GC–MS) analysis of essential oils from AgNPs and AuNPs elicited *Lavandula angustifolia in vitro* cultures. Molecules 24(3):606

Wornouk G, Demisse Z, Rheut M, Mahmoud S (2011) Biosynthesis and Therapeutic properties of *Lavandula* essential oil constituent. Planta Med 77:7–15

Yang L, Watts DJ (2005) Particle surface characteristics may play an important role in phytotoxicity of alumina nanoparticles. Toxicol Lett 158(2):122–132

molecules

MDPI

Article

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis of Essential Oils from AgNPs and AuNPs Elicited *Lavandula angustifolia* In Vitro Cultures

Aneta Wesołowska [1], Paula Jadczak [2], Danuta Kulpa [2,*] and Włodzimierz Przewodowski [3]

[1] Faculty of Chemical Technology and Engineering, Department of Organic and Physical Chemistry, West Pomeranian University of Technology in Szczecin, Aleja Piastów 42, 71-065 Szczecin, Poland; aneta.wesolowska@zut.edu.pl

[2] Department of Plant Genetics, Breeding and Biotechnology, Faculty of Environmental Management and Agriculture, West Pomeranian University of Technology in Szczecin, ul. Słowackiego 17, 71-434 Szczecin, Poland; paula.jadczak@zut.edu.pl

[3] Plant Breeding and Acclimation Institute—National Research Institute, Bonin Reaserch Centre, Bonin 3, 73-009 Bonin, Poland; wlodzimierz.przewodowski@ihar.edu.pl

* Correspondence: danuta.kulpa@zut.edu.pl; Tel.: +91-48-449-6305

Academic Editor: Brendan M Duggan

Received: 21 January 2019; Accepted: 7 February 2019; Published: 9 February 2019

Abstract: The aim of this study was to determine how the addition of gold and silver nanoparticles to culture media affects the composition of essential oils extracted from *Lavandula angustifolia* propagated on MS media with the addition of 10 and 50 mg·dm^{-3} of gold (24.2 ± 2.4 nm) and silver (27.5 ± 4.8 nm) nanocolloids. The oil extracted from the lavender tissues propagated on the medium with 10 mg·dm^{-3} AgNPs (silver nanoparticles) differed the most with respect to the control; oil-10 compounds were not found at all, and 13 others were detected which were not present in the control oil. The addition of AuNPs (gold nanoparticles) and AgNPs to the media resulted in a decrease of lower molecular weight compounds (e.g., α- and β-pinene, camphene, δ-3-carene, p-cymene, 1,8-cineole, trans-pinocarveol, camphoriborneol), which were replaced by those of a higher molecular weight (τ- and α-cadinol 9-cedranone, cadalene, α-bisabolol, cis-14-nor-muurol-5-en-4-one, (*E*,*E*)-farnesol).

Keywords: nanoparticles; secondary metabolites; shoot cultures; micropropagation; elicitor

1. Introduction

Nanotechnology has become one of the fastest growing interdisciplinary fields of science today. Nanoparticles, i.e., compounds or elements reduced to the size of less than 100 nanometers, differ in terms of their atomic structure compared to the material they are derived from, and also differ in terms of their physical, chemical, and biological properties. The most important advantage of nanoparticles is a high surface-to-volume ratio, which tends to increase with the reduction in their diameter, whereby nanoparticles demonstrate very high chemical activity [1]. Their highly developed active surface area significantly affects their adsorption properties, material reactivity, and antimicrobial properties [2].

The most widely used and known nanoparticles are those of precious metals: Gold and silver. They undergo various processes which are not observed in macroscopic environments. Nanosilver has antimicrobial (antifungal and antibacterial) properties. Gold in its nanoform offers therapeutic effects due to its ease to penetrate body cells where it strongly stimulates their regeneration [3,4]. Nanoparticles of precious metals form stable colloidal solutions, which can be applied to plant in vitro cultures [5].

Nanoparticles are easily absorbed and accumulated by plants. The processes of nanopenetration into the cells of living organisms are still to be explored in detail. However, it has been confirmed they enter certain cells through endocytosis or through pass through surface pores in plant cell walls [6,7]. The selective properties of cell walls enable the transport of particles measuring from 5 to 20 nm, allowing nanoparticles to easily penetrate cells and spread throughout the entire organism, ultimately affecting biological processes occurring in the cells [8].

With their unique nanostructural properties, these materials are used in many key industries, such as pharmaceuticals, electronics, cosmetology, medicine, environmental protection, textiles, and packaging. They are also applied in biotechnology, and recently, in plant in vitro cultures [9]. Nanosilver is used in plant in vitro cultures at the culture initiation stage to prevent contaminations, offering a viable alternative to antibiotics used in plant micropropagation [10].

Ongoing studies are attempting to determine the suitability of nanoparticles as elicitors in the in vitro cultures. Currently used elicitors are either biotic agents derived from biological sources, such as components of fungal and bacterial cell wall structures (polysaccharides, glycoproteins, inactivated enzymes, curdlan, chitosan), or abiotic factors of chemical or physical origin (heavy metal salts, osmotic stress, mechanical damage, ultraviolet radiation) [11]. Nanometal particles have shown a high capacity for attaching to plant tissues and activate enzymatic pathways responsible for the production of secondary metabolites [12]. They also contribute to the peroxidation of cellular membranes in plant cells and influence the expression of genes responsible for the production of biologically active compounds [13].

So far, attempts to use gold and silver nanocolloids as elicitors in plant in vitro cultures have been limited. The addition of these substances increased the production of secondary metabolites in the cultures of *Salvia miltiorrhiza* [14], *Artemisia annua* [15], *Brugmensia candida* [16], *Corylus avellana* [17], *Prunella vulgaris* [18], and *Aloe vera* [13]. The influence of nanoparticles on plants depends on several factors, such as plant species, its age, growing conditions, culture medium, exposure time of the plant to nanomaterial, and administration method.

Essential oils constitute mixtures of volatile compounds, sesquiterpenes, and primarily monoterpenes [19]. The main components of the essential oils isolated from *L. angustifolia* tissue are, among others, linalool, borneol, geraniol, and linalool acetate [20,21]. The composition of an essential oil depends mainly on the plant genotype, yet its composition may differ under the influence of developmental and environmental factors, i.e., sun exposure, plant age, seedling collection method or essential oil isolation method [22,23].

There have been no literature reports so far regarding the influence of nanoparticles on the production of essential oils by plants propagated in in vitro cultures. The studies by Hatami et al. [24] and Ghanati and Bakhtarian [25] show that the application of metal nanoparticles to plants growing under natural conditions results in a change in the essential oil content extracted from their tissues. The aim of this study was to verify how gold and silver nanocolloids influence the composition of essential oils in narrow-leaved lavender (*Lavandula angustifolia*) propagated in in vitro cultures.

2. Results and Discussion

Hydrodistillation of the dried leafy shoots of *Lavandula angustifolia* generated pale yellow liquids with a yield from 0.81% (10 mg·dm^{-3} AuNPs) to 1.27% (10 Ag mg·dm^{-3} NPs) (Table 1). Interestingly, the results of this study are comparable with those obtained from lavender flowers. Kara and Baydar [26] studied four lavender cultivars and indicated that the oil content varied from 0.35 to 2.0%. Zheljazkov et al. [27] reported the content of volatile oil in dried flowers to range from 0.71 to 1.30%. However, the content of volatile oil in the leaves of lavender cultivated in Northwest Iran (0.64%) was lower when compared with the results discussed herein [28].

Table 1. Essential oil content in lavender plants as a function of medium used.

Medium [mg·dm^{-3}]	Essential Oil Content (%*w*/*w*)
0—control	1.15
50 Au	0.95
10 Au	0.81
50 Ag	0.82
10 Ag	1.27

The chemical composition of *L. angustifolia* essential oils is shown in Tables 2 and 3, where the percentage composition and retention indices of the constituents are given. A total of 97 different compounds representing 99.29–99.95% of the oils were identified. The main volatile constituents were: Borneol (12.14–16.46%), τ-cadinol (12.96–16.63%), caryophyllene oxide (8.79–12.23%), γ-cadinene (4.54–6.08%), and 1,8-cineole (2.80–4.58%). Other important constituents were: Cis-14-nor-muurol-5-en-4-one (2.68–4.45%), β-pinene (1.93–3.14%), camphor (2.05–2.79%), and α-santalene (1.42–2.64%). The extracted oils were the most abundant in oxygenated sesquiterpenes (36.34–43.36%), followed by oxygenated monoterpenes (27.77–38.23%), sesquiterpene hydrocarbons (10.27–14.35%), and monoterpene hydrocarbons (5.57–10.40%).

Table 2. Statistical analysis of main compounds.

	Compound	RI	Control	50 mg·dm^{-3} Au	10 mg·dm^{-3} Au	50 mg·dm^{-3} Ag	10 mg·dm^{-3} Ag
1	α-Pinene	933	1.46a	0.99b	1.03b	1.11b	0.64c
2	β-Pinene	977	3.14a	2.56b	2.25b	2.53b	1.93b
3	p-Cymene	1025	1.39a	1.18b	1.17b	0.91c	0.92c
4	1,8-Cineole	1031	4.49a	4.58a	2.80b	2.95b	2.95b
5	*trans*-Pinocarveol	1140	1.61a	1.65a	1.54b	1.42c	1.14d
6	Camphor	1145	2.75a	2.79a	2.41b	2.06c	2.05c
7	Pinocarvone	1164	1.32a	1.36a	1.32a	1.16b	0.90c
8	Borneol	1170	16.00a	16.46a	12.78b	12.14b	12.99b
9	Myrtenol	1198	2.25a	2.35a	1.94a	2.13a	1.85a
10	Geranylacetate	1385	1.20a	1.38a	1.14a	0.59b	1.41a
11	α-Santalene	1422	1.90bc	1.42d	2.16b	2.64a	1.74c
12	γ-Cadinene	1518	4.97c	4.54d	5.09c	6.08a	5.36b
13	Caryophylleneoxide	1589	9.12c	8.54c	11.06b	12.23a	8.79c
14	τ-Cadinol	1648	12.96c	14.35b	13.65bc	14.17b	16.63a
16	α-Cadinol	1662	1.33a	1.13b	1.35a	1.36a	1.08c
17	Cadalene	1675	1.87d	1.58e	2.34b	2.53a	2.03c
18	*cis*-14-nor-Muurol-5-en-4-one	1693	2.68c	3.72b	3.72b	3.37c	4.45a
19	(*E*,*E*)-Farnesol	1720	1.20d	1.43b	1.32c	1.45b	1.57a
20	Bisabolol oxide A	1750	1.97c	2.26b	2.23b	2.13b	2.60a

compounds with a significantly lower content as compared with the control oil; compounds with a significantly higher content as compared with the control oil; a, b, 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.

The growing medium applied affected the percentage composition of each essential oil constituent. The highest concentrations of borneol (16.46%) and 1,8-cineole (4.58%) were noticed in the volatile oil isolated from plants cultivated on the medium supplemented with gold nanoparticles (50 mg·dm^{-3} AuNPs). Addition of silver nanoparticles (50 mg·dm^{-3} AgNPs) to the growing medium increased the content of γ-cadinene (6.08%) and caryophyllene oxide (12.23%) in the oil (Figure 1).

Table 3. Relative percentage composition of lavender essential oils depending on the medium ±SD (n = 3).

No.		Compound	RI	Control		50 mg·dm^{-3} Au		10 mg·dm^{-3} Au		50 mg·dm^{-3} Ag		10 mg·dm^{-3} Ag	
1.	MH	α-Thujene	927	0.09	±0.01								
2.	MH	α-Pinene	933	1.46	±0.16	0.99	±0.10	1.03	±0.01	1.11	±0.18	0.64	±0.04
3.	MH	Camphene	948	1.08	±0.16	0.78	±0.07	0.85	±0.04	0.77	±0.11	0.48	±0.03
4.	MH	Thuja-2,4(10)-diene	954			0.09	±0.01						
5.	MH	β-Thujene	971	0.33	±0.02	0.24	±0.01	0.28	±0.01	0.20	±0.00	0.21	±0.01
6.	MH	Sabinene	974	0.31	±0.01	0.25	±0.03	0.23	±0.01	0.23	±0.03	0.21	±0.01
7.	MH	β-Pinene	977	3.14	±0.26	2.56	±0.26	2.25	±0.08	2.53	±0.46	1.93	±0.09
8.	MH	δ-3-Carene	1010	0.93	±0.08	0.72	±0.06	0.77	±0.03	0.78	±0.10	0.59	±0.03
9.	MH	m-Cymene	1022	0.52	±0.05	0.45	±0.04	0.49	±0.02	0.33	±0.01	0.32b	±0.01
10.	MH	p-Cymene	1025	1.39	±0.13	1.18	±0.10	1.17	±0.07	0.91	±0.06	0.92c	±0.04
11.	MH	D-Limonene	1029	0.81	±0.11	0.33	±0.47	0.64	±0.02	0.58	±0.04	0.27a	±0.38
12.	OM	1,8-Cineole	1031	4.49	±0.26	4.58	±0.01	2.80	±0.12	2.95	±0.43	2.95b	±0.33
13.	MH	γ-Terpinene	1060	0.10	±0.01	0.08	±0.01						
14.	OM	*cis*-Sabinenehydrate	1068	0.070	±0.00							0.09	±0.00
15.	MH	α-Terpinolene	1091	0.24	±0.01	0.23	±0.00	0.27	±0.02	0.15	±0.06		
16.	OM	Linalool	1101	0.65	±0.03	0.77	±0.05	0.55	±0.04	0.30	±0.06	0.51b	±0.01
17.	O	α-Pineneoxide	1110	0.210	±0.01								
18.	OM	Fenchol	1114			0.21	±0.01	0.26	±0.01	0.16	±0.01	0.19	±0.00
19.	O	3-Octanol acetate	1122	0.15	±0.00	0.15	±0.01	0.20	±0.01	0.13	±0.01		
20.	OM	α-Campholenal	1127	0.23	±0.00	0.22	±0.01	0.24	±0.01	0.21	±0.01	0.20	±0.01
21.	OM	1,2-Dihydrolinalool	1136	0.24	±0.01	0.21	±0.01	0.26	±0.01	0.20	±0.01	0.18	±0.00
22.	OM	*trans*-Pinocarveol	1140	1.61	±0.04	1.65	±0.10	1.54	±0.08	1.42	±0.05	1.14	±0.02
23.	OM	Camphor	1145	2.75	±0.08	2.79	±0.21	2.41	±0.13	2.06	±0.13	2.05	±0.03
24.	OM	Pinocarvone	1164	1.32	±0.01	1.36	±0.08	1.32	±0.08	1.16	±0.01	0.90	±0.00
25.	OM	Borneol	1170	16.00	±0.58	16.46	±1.51	12.78	±0.44	12.14	±1.20	12.99	±0.16
26.	OM	Terpinen-4-ol	1179	0.69	±0.04	0.64	±0.04	0.62	±0.01	0.60	±0.06	0.48	±0.00
27.	OM	p-Cymen-8-ol	1184	0.80	±0.04	0.84	±0.01	0.99	±0.06	0.57	±0.07	0.40	±0.02
28.	OM	Cryptone	1187	0.55	±0.25	0.71	±0.03	0.77	±0.04	0.52	±0.06	0.35	±0.01
29.	OM	α-Terpineol	1193	0.66	±0.01	0.65	±0.04	0.51	±0.00	0.52	±0.06	0.41	±0.01
30.	OM	Myrtenol	1198	2.25	±0.06	2.35	±0.11	1.94	±0.06	2.13	±0.22	1.85	±0.03
31.	OM	Verbenone	1210	0.78	±0.06	0.69	±0.08	0.61	±0.02	0.51	±0.07	0.45	±0.01
32.	OM	*cis*-Carveol	1221	0.18	±0.01	0.18	±0.00	0.24	±0.01	0.08	±0.11		
33.	OM	*trans*-Carveol	1224	0.20	±0.00	0.23	±0.01	0.23	±0.00	0.09	±0.12		
34.	OM	Bornylformate	1229	0.86	±0.01	0.85	±0.04	0.63	±0.00	0.62	±0.02	0.91	±0.01
35.	OM	D-Carvone	1247	0.24	±0.00	0.24	±0.01	0.32	±0.01	0.10	±0.14	0.20	±0.02

Table 3. *Cont.*

No.		Compound	RI	Control		50 mg·dm^{-3} Au		10 mg·dm^{-3} Au		50 mg·dm^{-3} Ag		10 mg·dm^{-3} Ag	
36.	OM	Geraniol	1254	0.46	±0.01	0.43	±0.01	0.40	±0.01	0.33	±0.02	0.31	±0.01
37.	OM	α-Citral	1272	0.12	±0.01	0.12	±0.00	0.11	±0.01				
38.	OM	Bornylacetate	1287	0.32	±0.01	0.29	±0.00	0.32	±0.04	0.27	±0.00	0.38	±0.03
39.	OM	Lavandulylacetate	1292	0.20	±0.00	0.16	±0.00	0.21	±0.01	0.19	±0.01	0.18	±0.01
40.	OM	Piperitenone	1341	0.11	±0.01	0.12	±0.01	0.14	±0.00	0.05	±0.06	0.12	±0.01
41.	OM	Nerylacetate	1367			0.10	±0.01						
42.	OM	Geranylacetate	1385	1.20	±0.06	1.38	±0.07	1.14	±0.06	0.59	±0.28	1.41	±0.27
43.	SH	α-Cedrene	1416	0.40	±0.06	0.39	±0.04	0.45	±0.04	0.47	±0.01	0.45	±0.04
44.	SH	α-Santalene	1422	1.90	±0.04	1.42	±0.02	2.16	±0.01	2.64	±0.00	1.74	±0.04
45.	SH	α-Bergamotene	1438	0.28	±0.01	0.25	±0.01	0.33	±0.01	0.38	±0.02	0.29	±0.00
46.	SH	Aromadendrene	1448	0.11	±0.01	0.08	±0.00	0.14	±0.00	0.14	±0.01	0.12	±0.01
47.	SH	β-Santalene	1450	0.11	±0.01			0.12	±0.01	0.15	±0.01	0.10	±0.00
48.	SH	*trans*-β-Bergamotene	1460	0.15	±0.01	0.09	±0.04			0.16	±0.06		
49.	SH	β-Chamigrene	1463	0.11	±0.01	0.09	±0.00	0.11	±0.00	0.14	±0.01	0.12	±0.00
50.	SH	Di-epi-α-Cedrene	1470	0.13	±0.00	0.14	±0.01	0.15	±0.00	0.15	±0.01	0.15	±0.01
51.	SH	*cis*-β-Farnesene	1488	0.10	±0.00	0.12	±0.06	0.19	±0.05	0.15	±0.04	0.16	±0.01
52.	SH	β-Bisabolene	1511							0.07	±0.09		
53.	SH	γ-Cadinene	1518	4.97	±0.06	4.54	±0.11	5.09	±0.03	6.08	±0.03	5.36	±0.05
54.	SH	β-Sesquiphellandrene	1522	0.39	±0.01	0.44	±0.04	0.56	±0.01	0.50	±0.01	0.52	±0.01
55.	SH	δ-Cadinene	1526	0.45	±0.01	0.42	±0.03	0.49	±0.01	0.54	±0.01	0.50	±0.02
56.	SH	*trans*-Calamenene	1533	0.24	±0.00	0.24	±0.03	0.33	±0.01	0.32	±0.03	0.35	±0.01
57.	SH	Cadina-1,4-diene	1536	0.53	±0.01	0.60	±0.06	0.59	±0.03	0.53	±0.02	0.60	±0.01
58.	SH	α-Cadinene	1543							0.19	±0.01	0.13	±0.18
59.	SH	α-Calacorene	1547	0.36	±0.01	0.52	±0.06	0.51	±0.03	0.45	±0.05	0.48	±0.01
60.	SH	Germacrene B	1557	0.89	±0.02	0.82	±0.07	1.14	±0.03	1.23	±0.05	0.86	±0.01
61.	SH	β-Calacorene	1563	0.10	±0.01	0.11	±0.03			0.06	±0.08	0.13	±0.01
62.	OS	Nerolidol	1569	0.51	±0.01	0.64	±0.07	0.62	±0.02	0.60	±0.02	0.66	±0.01
63.	O	(*Z*)-3-Hexenyl benzoate	1579	0.63	±0.01	0.76	±0.08	0.74	±0.04	0.73	±0.01	0.76	±0.01
64.	OS	Caryophylleneoxide	1589	9.12	±0.16	8.54	±0.31	11.06	±0.13	12.23	±0.21	8.79	±0.07
65.	O	Hexadecane	1600	0.26	±0.01	0.30	±0.05	0.32	±0.01	0.29	±0.01	0.36	±0.01
66.	OS	Humuleneepoxide	1605	0.21	±0.00	0.22	±0.04	0.30	±0.01	0.28	±0.01	0.26	±0.01
67.	OS	Humuleneepoxide II	1613	0.65	±0.02	0.69	±0.06	0.87	±0.01	0.89	±0.04	0.80	±0.01
68.	OS	epi-Cubenol	1619	1.55	±0.04	1.73	±0.12	1.70	±0.04	1.77	±0.08	2.01	±0.01
69.	OS	γ-Eudesmol	1628	0.25	±0.01	0.28	±0.04	0.32	±0.03	0.29	±0.01	0.20	±0.02
70.	OS	Isospathulenol	1638			0.17	±0.04	0.24	±0.02	0.25	±0.03	0.18	±0.01
71.	OS	Caryophylla-4(12),8(13)-dien-5β-ol	1642	0.30	±0.00	0.47	±0.04	0.26	±0.37	0.62	±0.12		

Table 3. *Cont.*

No.		Compound	RI	Control		50 mg·dm^{-3} Au		10 mg·dm^{-3} Au		50 mg·dm^{-3} Ag		10 mg·dm^{-3} Ag	
72.	OS	τ-Cadinol	1648	12.96	±0.69	14.35	±0.64	13.65	±0.14	14.17	±0.69	16.63	±0.06
73.	OS	α-Muurolol	1655	0.44	±0.02	0.47	±0.06	0.54	±0.01	0.53	±0.00	0.59	±0.00
74.	OS	α-Eudesmol	1659	0.46	±0.03	0.51	±0.06	0.55	±0.01	0.53	±0.07	0.69	±0.01
75.	OS	α-Cadinol	1662	1.33	±0.04	1.13	±0.06	1.35	±0.06	1.36	±0.02	1.08	±0.04
76.	OS	9-Cedranone	1667	1.13	±0.04	1.29	±0.16	1.33	±0.01	1.37	±0.06	1.41	±0.07
77.	O	Cadalene	1675	1.87	±0.05	1.58	±0.16	2.34	±0.04	2.53	±0.18	2.03	±0.01
78.	OS	α-Bisabolol	1681	0.77	±0.04	0.87	±0.09	0.96	±0.03	0.92	±0.04	0.91	±0.00
79.	OS	epi-α-Bisabolol	1691	0.69	±0.03					0.38	±0.53		
80.	OS	*cis*-14-nor-Muurol-5-en-4-one	1693	2.68	±0.08	3.72	±0.22	3.72	±0.05	3.37	±0.7	4.45	±0.03
81.	O	Heptadecane	1703			0.28	±0.05	0.30	±0.01	0.13	±0.18	0.31	±0.00
82.	O	5-Ethyl-5-methylpentadecane	1709	0.27	±0.01	0.31	±0.06	0.38	±0.01	0.33	±0.03	0.41	±0.01
83.	O	Pentadecanal	1714	0.46	±0.04	0.53	±0.07	0.57	±0.01	0.52	±0.01	0.68	±0.01
84.	OS	(*E*,*E*)-Farnesol	1720	1.20	±0.07	1.43	±0.14	1.32	±0.04	1.45	±0.04	1.57	±0.02
85.	O	5-Phenyldodecane	1733	0.50	±0.05	0.55	±0.13	0.68	±0.02	0.62	±0.03	0.81	±0.01
86.	OS	Bisabolol oxide A	1750	1.97	±0.12	2.26	±0.27	2.23	±0.03	2.13	±0.12	2.60	±0.03
87.	OS	(*E*)-α-Atlantone	1777	0.12	±0.02	0.19	±0.03	0.19	±0.00	0.22	±0.03	0.23	±0.05
88.	O	Octadecane	1805			0.13	±0.02	0.20	±0.03			0.27	±0.02
89.	DT	Phytane	1811									0.21	±0.00
90.	O	Diisobutylphthalate	1872					0.24	±0.01	0.25	±0.08	0.33	±0.01
91.	DT	m-Camphorene	1957					0.14	±0.01			0.26	±0.02
92.	O	Eicosane	2003									0.12	±0.08
93.	O	Octadecanal	2021									0.20	±0.03
94.	O	1-Octadecanol	2088					0.29	±0.03			0.66	±0.08
95.	O	1-Tricosene	2296									0.31	±0.16
96.	O	Tricosane	2300							0.25	±0.13	0.44	±0.09
97.	O	2-Heneicosanone	2307					0.66	±0.20	0.84	±0.21	2.22	±0.35
		Total identified [No.]		82		81		81		83		83	
		Total identified [%]		99.29		99.95		99.95		99.69		99.72	
		Monoterpene hydrocarbnons (MH)		10.40		7.90		7.98		7.59		5.57	
		Oxygenated monoterpenes (OM)		37.19		38.23		31.34		27.77		28.65	
		Sesquiterpene hydrocarbons (SH)		11.22		10.27		12.36		14.35		12.06	
		Oxygenated sesquiterpenes (OS)		36.34		38.96		41.21		43.36		43.06	
		Diterpenes (DT)		-		-		0.14		-		0.47	
		Other (O)		4.14		4.59		6.92		6.62		9.91	

RI: Retention indices relative to n-alkanes (C_7-C_{40}) on HP-5MS capillary column; -: Not detected.

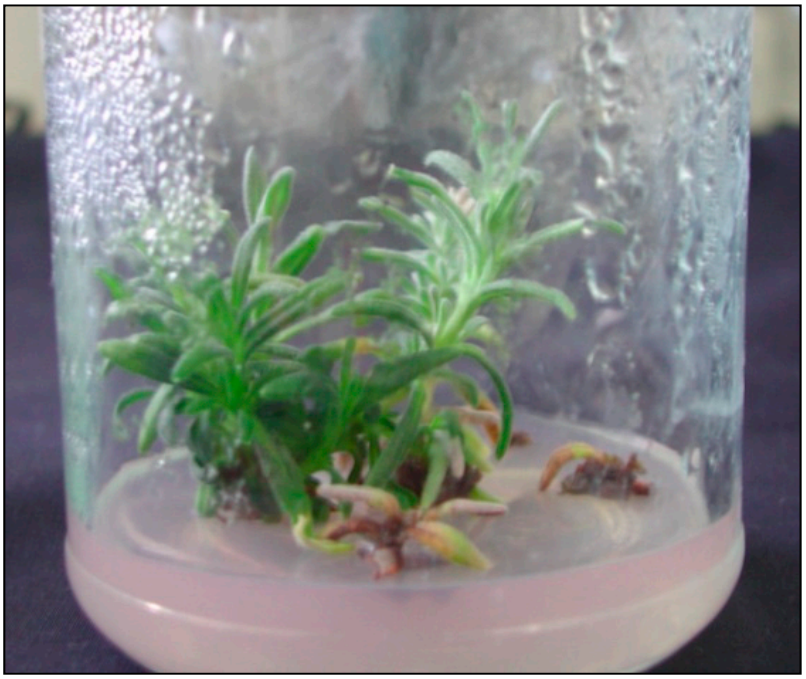

Figure 1. Plants of *Lavandula angustifolia* Mill. propagated on medium with 50 mg·dm^{-3} AuNPs.

However, the percentage content of camphor was lower in the plants cultivated on the medium supplemented with AgNPs (10 and 50 mg·dm^{-3}). Moreover, volatile oil derived from lavender cultivated on MS medium was richer in β-pinene (3.14%), α-pinene (1.46%), *p*-cymene (1.39%), camphene (1.08%), and δ-3-carene (0.93%).

Phytochemical studies revealed that linalool (9.3–68.8%) and linalyl acetates (1.2–59.4%) were the main components of the aerial parts and flowers of *Lavandula angustifolia* [29,30]. However, the essential oil obtained from plants cultivated in North Africa [31] had 1,8-cineole (29.4%) and camphor (24.6%) as the major constituents. 1,8-cineole (65.4%) and borneol (11.5%) dominated in the essential oils isolated from the leaves of *L. angustifolia* collected near Isfahan, Iran [32]. Borneol was the main compound in the essential oils isolated from leafy stems of three lavender cultivars propagated in in vitro cultures: 'Blue River' (25.75%), 'Elegance Purple' (32.17%), and Munstead (13.38%) [33].

The percentage contents of linalool (0.30–0.77%), 1,8-cineole (2.80–4.58%), and camphor (2.05–2.79%) found in volatile oils in this study were much lower than the results reported in the referenced literature. Essential oils isolated from plants grown on control medium and 50 mg·dm^{-3} AuNPs medium were the only ones with higher borneol content (16.00–16.46%) compared with the results obtained by Andrys and Kulpa [29]. Linalool, lavandulol, and their esters (linalyl acetate and lavandulyl acetate) are responsible for the fresh and floral smell of lavender oil. Moreover, the quality of oil depends on both a high content of linalool and linalyl acetate and their mutual proportions (preferably higher than 1) [34].

Contrary to the results obtained by other researches, lavandulol and linalyl acetate were not detected in the oils in this study, and the content of lavandulyl acetate did not exceed 0.21%. The data reported in the literature indicated that many terpenoids are biologically active and are used medicinally [35]. Camphor, with its specific camphoraceous odor, is used commercially as a moth repellent and as a preservative in pharmaceuticals and cosmetics [36]. Borneol, a widely-used food and cosmetic additive, possesses analgesic, anti-inflammatory, and antibacterial properties [37,38]. It is well known that 1,8-cineole and camphor are responsible for the insecticidal activity of the plants from *Lavandula* genus [39]. Based on these facts, it can be stated that the volatile oils extracted from leafy shoots of *L. angustifolia* may have commercial applications.

The oil extracted from the tissues of lavender propagated on the culture medium which was supplemented with 10 mg·dm^{-3} of AgNPs differed the most with respect to the control culture (plants propagated on the culture medium with no nanoparticles) in terms of the number of compounds: While 10 compounds were not found in it at all, 13 others were detected which were not observed in the control oil. The addition of AuNPs and AgNPs to the media resulted in a decrease in compounds with lower molecular weight (e.g., α- and β-pinene, camphene, δ-3-carene, p-cymene, 1,8-cineole (eucalyptol), trans-pinocarveol, camphor, and borneol), which were replaced by those of higher molecular weight (τ- and α-cadinol 9-cedranone, cadalene, α-bisabolol, cis-14-nor-Muurol-5-en-4-one, (*E*,*E*)-farnesol).

Heavy metal nanocolloids that have been recently used in plant in vitro cultures, as elicitors provoke the production of secondary metabolites. There are research reports confirming that these particles are capable of eliciting responses in plants consistent with those generated when typical elicitors are used [40,41]. It is commonly believed that the production of secondary metabolites in plants is significantly affected by environmental stress. Biotic and abiotic stresses delay cellular differentiation through the production of reactive oxygen species (ROS), which directly destroy cells by producing secondary metabolites [42,43]. The researchers suggest that oxidative stress induced by nanoparticles is correlated with the production of secondary metabolites in plants. Due to their small size, nanocolloids can easily attach to plant cell walls, destroy them, change their permeability, and thus significantly affect cellular metabolism [13]. Zhang et al. [14] confirmed the effectiveness of silver as an elicitor using silver ions in the production of diterpenoids in the cultures of root hairs of *Salvia miltiorrhiza* genus. The addition of silver to the culture media of root hairs resulted in an increase in the production of reactive oxygen species. Activation of ROS-based mechanisms following exposure of plants from *Calendula officinalis* L. genus to nanoparticles was also confirmed by Ghanati and Bakhtiarian [20] in the production of secondary metabolites. Fazal et al. [18] demonstrated that a callus of *Prunella vulgaris* genus treated with silver and gold nanocolloids produced significant quantities of antioxidant enzymes, such as POD and SOD, as well as phenolic and flavonoid compounds that are directly related to the protection of plants against environmental stress. Silver nanocolloids were used to produce capsaicin from *Capsicum* sp. and resulted in a significant increase in the production of this compound [44]. Hemm et al. [45] and Liu et al. [46] showed that growth regulators combined with elicitors resulted in a larger organogenic potential of plants and increased the production of primary and secondary metabolites.

The study showed that the addition of gold and silver nanocolloids to the culture media significantly affected the composition of essential oil derived from narrow-leaved lavender cultivated in in vitro cultures. In the oils extracted from plants propagated in vitro on culture media with the addition of nanoparticles, a variety of compounds were identified that were not present in the oil derived from plants grown on the control medium. The above suggests that gold and silver nanoparticles can be successfully used to obtain essential oils of different composition which may result in different properties: Fragrance and, above all, antioxidant and antimicrobial activity, but the latter requires further studies. It is also necessary to determine the toxicity of nanoparticles in relation to plant tissues.

3. Material and Methods

3.1. Nanoparticles

Aqueous suspensions of gold and silver nanoparticles were synthesized using Turkevich et al. [47] and Liu et al.'s [48] methods with modified synthesis conditions and a two-stage microwave-convection heating method. For this purpose, aqueous mixtures of 0.903 g·dm^{-3} of sodium citrate with 2.378 g·dm^{-3} of tetrachloroauric acid ($HAuCl_4$), and 1.189 g·dm^{-3} of silver nitrate ($AgNO_3$), respectively, were prepared. After their purification with a small (0.2 μm) pore antibacterial filter (Sartorius, Goettingen, Germany), they were placed in a microwave (MX 245), where they were stirred

and heated to 100 °C at 800 W, which allowed for reaching a heating rate of approx. 1.6 °C/s. Once the preset temperature was reached, the mixture was kept in the microwave for an additional 20 s, and then placed in HBR 4 digital IKAMAG heating bath (IKA, Staufen, Germany), where it was stirred with a magnetic stirrer and incubated at 95 °C for an additional 15 min. It was then gradually cooled to room temperature (0.8 °C/min). To obtain a similar distribution of nanoparticle diameters, the resulting mixtures were homogenized in a centrifugal force field in Beckmann JA-20 centrifuge to obtain similar nanoparticle concentrations in both mixtures. Once their spectra were plotted with UV-VIS EPOCH microplate spectrophotometer (BioTek, Bad Friedrichshall, Germany), the optical density of the fractions obtained was adjusted to a common DEV value, using the following spectra absorbance maxima λ_{max} = 520 nm and λ_{max} = 445 nm for gold and silver colloids, respectively (Figure 2). The similarities in morphology, shape, and size of the synthesized and prepared gold (24.2 ± 2.4 nm) and silver (27.5 ± 4.8 nm) nanoparticles were assessed after their application to the surface of a nylon membrane (Supelco, Park Bellefonte, PA, USA) and through an analysis of images from a scanning electron microscope (SEM, FEI Quanta 200 FEG model) (FEI Company, Tokyo, Japan, Figure 3).

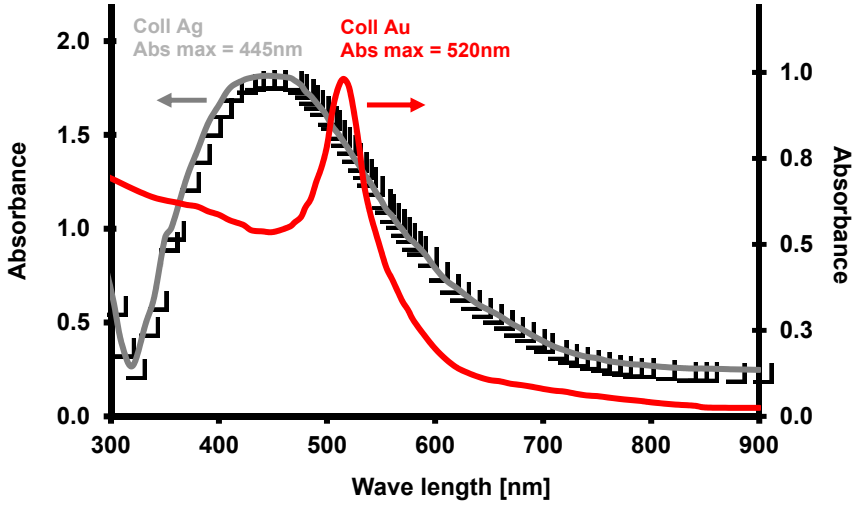

Figure 2. UV-VIS spectral spectra of the fraction of 4-5000 x g of gold and colloidal silver.

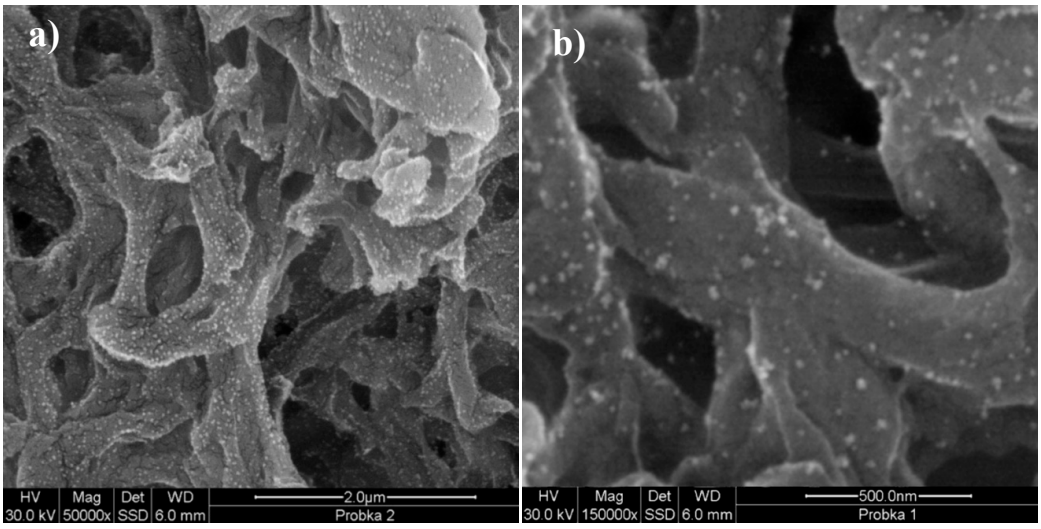

Figure 3. View of nanoparticles of gold colloids (**a**) and silver (**b**) after fractionation and placement on a nylon diaphragm (Supelco, Park Bellefonte, PA, USA) made with a help of FEI Quanta 200 FEG scanning electron microscope. The magnification applied to the observed colloidal gold and silver 50,000 (**a**) and 150,000 (**b**) times, respectively.

3.2. In Vitro Cultures

The materials examined in this study were plants of narrow-leaved lavender (*Lavendula angustifolia*), 'Munstead' cultivar. Single-node shoot fragments with a length of 1–1.5 cm were put in glass jars with a capacity of 300 mL, filled with 30 mL of the medium. The media, with a mineral composition developed by Murashige and Skoog [49] (MS media), were supplemented with 2 mg·dm^{-3} kinetin (KIN) and 0.2 mg·dm^{-3} indole-3-acetic acid (IAA) [50] with the addition of gold (AuNPs) with a diameter of 24.2 ± 2.4 nm and silver (AgNPs) with a diameter of 27.5 ± 4.8 nm nanocolloids with the concentrations of: 10 and 50 mg·dm^{-3}, respectively. Furthermore, the media were supplemented with: 30 g·dm^{-3} of sucrose, 100 mg·dm^{-3} of inositol, and solidified with agar at 7 g·dm^{-3}. Medium pH was set at 5.7 using 0.1 M solutions of HCl and NaOH. The jars were sterilized at 121 °C for 20 min. The jars with cultures were placed in a phytotron, with a humidity of 70–80% and temperature of 24 °C. The cultures were illuminated for 16 h a day, and the illuminance was kept at 35 μEM^{-2}s^{-1} PAR.

3.3. Extraction of Essential Oils

Fifteen grams of the entire dried aerial parts of lavender were placed in 1000 mL round-bottomed flasks along with 400 mL of distilled water and subjected to hydrodistillation (3 replicates) for two hours using a Clevenger apparatus as recommended by the European Pharmacopoeia 5.0 [51]. The essential oil extracts were dried over anhydrous sodium sulfate, filtered, weighed and stored in dark sealed vials at 4 °C until gas chromatography/mass spectrometry (GC-MS) analysis was performed. Essential oil percentage was calculated based on the dry weight of plant material and expressed as (% *w*/*w*) in Table 1.

3.4. Gas Chromatography/Mass Spectrometry (GC-MS) Analyses of Essential Oils

The qualitative GC-MS analysis of the extracted essential oils was carried out using HP 6890 gas chromatograph coupled with HP 5973 Mass Selective Detector (Agilent Technologies, Foster City, CA, USA) operating in 70 eV mode. Samples of 2 μL (40 mg of oil dissolved in 1.5 mL of dichloromethane) were injected in a split mode at a ratio of 5:1. The compounds were separated on a 30 m long capillary column (HP-5MS), 0.25 mm in diameter and with 0.25 μm thick stationary phase film ((5% phenyl)-methylpolysiloxane).

The flow rate of helium through the column was kept at 1.2 mL·min^{-1}. The initial temperature of the column was 45 °C, then it was increased to 200 °C at a rate of 5 °C·min^{-1} (kept constant for 10 min), and then heated up to a final temperature of 250 °C at a rate of 5 °C min^{-1}. The oven was kept at this temperature for 20 min. The injector temperature was 250 °C, the transfer line temperature was 280 °C, and the ion source temperature was 230 °C. The solvent delay was 4 min. The scan range of the MSD was set at 40 to 550 m/z. The total running time for a sample was about 71 min. The relative percentage of the essential oil constituents was evaluated from the total peak area (TIC) by apparatus software [52,53]. Essential oil constituents were identified by comparison of their mass spectra with those stored in the Wiley NBS75K.L and NIST/EPA/NIH (2002 version, National Institute of Standards and Technology, Gaithersburg, MD, USA) mass spectral libraries using various search engines (PBM, Nist02). The identity of compounds was also confirmed by comparison of their calculated retention indices with those reported in NIST Chemistry WebBook (http://webbook.nist.gov/chemistry/). For retention indices (RI) calculation [54,55], a mixture of homologus series of n-alkanes C_7-C_{40} (Supelco, Bellefonte, PA, USA) was used, under the same chromatographic conditions which were applied for the analysis of the lavender essential oils.

Author Contributions: A.W. isolated the essential oil from plant tissue and performed the analysis of GC-MS as well as analyzed the obtained data; P.J. performed the experiments in in vitro cultures, analyzed the data, and wrote the manuscript; D.K. contributed to the revisions of the manuscript; W.P. prepared the solution of nanoparticles. All authors were responsible for processing information and manuscript writing. All authors read and approved the final manuscript.

Funding: The study was supported by the Polish Ministry of Science and Higher Education (Project BMN 517-07-017-5799/17 ZUT).

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Song, J.Y.; Kim, B.S. Rapid biological synthesis of silver nanoparticles using plant leaf extracts. *Bioprocess Biosyst. Eng.* **2009**, *32*, 79–84. [CrossRef] [PubMed]
2. Penyala, N.R.; Pena-Mendez, E.M.; Havel, J. Silver or silver nanoparticles: A hazardous threat to the environment and human health? Review. *J. Appl. Biomed.* **2008**, *6*, 117–129.
3. Kim, Y.K.; Lee, Y.S.; Jeong, D.H.; Cho, M.H. Antimicrobial effect of silver nanoparticles. *Nanomedicine* **2007**, *3*, 95–101. [CrossRef]
4. Choi, O.; Deng, K.K.; Kim, N., Jr.; Ross, L.; Rao, Y.S.; Hu, Z. The inhibitory effects of silver nanoparticles, silver ionsand silver chloride colloids on microbial growth. *Water Res.* **2008**, *42*, 3066–3074. [CrossRef]
5. Hackenberg, S.; Scherzed, A.; Kessler, M.; Hummel, S.; Technau, A.; Froelich, K.; Ginzkey, C.; Koehler, C.; Hagen, R.; Kleinsasser, N. Silver nanoparticles: Evaluation of DNA damage, toxicity and functional impairment in human mesenchymal stem cells. *Toxicol. Lett.* **2011**, *25*, 27–33. [CrossRef] [PubMed]
6. Jamshidi, M.; Ghanti, F. Taxanes content and cytotoxity of hazel cells extract after elicitation with silver nanoparticles. *Plant Psychol. Chem.* **2016**, *110*, 178–184.
7. Zhao, J.; Hu, Q.; Guo, Y.Q.; Zhu, W.H. Elicitor-induced indole alkaloid biosynthesis in *Catharanthus roseus* cell cultures is related to Ca? Influx and the oxidative burst. *Plant Sci.* **2001**, *161*, 423–431. [CrossRef]
8. Nair, R.; Varghese, S.H.; Nair, B.G.; Maekaa, T.; Yoshida, Y.; Kumar, D.S. Nanoparticulate material delivery to plants. *Plant Sci.* **2010**, *179*, 154–163. [CrossRef]
9. Navarro, E.; Baun, A.; Behra, R.; Hartmann, N.B.; Filser, J.; Miao, A.; Quigg, A.; Santschi, P.H.; Sigg, I. Environmental behaviour and ecotoxicity of engineered nanoparticles to algae, plants and fungi. *Ecotoxicol.* **2008**, *17*, 372–386. [CrossRef]
10. Spinoso-Castillo, J.L.; Chavez-Santoscoy, R.A.; Bogdanchikova, N.; Pérez-Sato, J.A.; Morales-Ramos, V.; Bello-Bello, J.J. Antimicrobial and hormetic effects of silver nanoparticles on in vitro regeneration of vanilla (*Vanilla planifolia* Jacks. ex Andrews) using a temporary immersion system. *Plant Cell Tiss. Organ Cult.* **2017**, *129*, 195–207.
11. Ramachandra, R.S.; Ravishankar, G.A. Plant cell cultures: Chemical factories of secondary metabolites. *Biotechnol. Adv.* **2002**, *20*, 101–153. [CrossRef]
12. Shakeran, Z.; Keyhanfari, M.; Asghari, G.; Ghanadian, M. Improvement of atropine production by diferent biotic and abiotic elicitors in hairy root cultures of *Datura metel*. *Turk. J. Biol.* **2015**, *39*, 111–118. [CrossRef]
13. Raei, M.; Angaji, A.A.; Omidi, M.; Khodayari, M. Effect of abiotic elicitors on tissue culture of *Aloe vera*. *Inter. J. Biosc.* **2014**, *5*, 74–81.
14. Zhang, C.; Yan, Q.; Cheuk, W.; Wu, J. Enhancement of Tanshinone Production in *Salvia miltiorrhiza* hairy root culture by Ag elicitation and nutrient feeding. *Planta Med.* **2004**, *70*, 147–151. [PubMed]
15. Zhang, B.; Zheng, L.P.; Wan Wen, W.J. Stimulation of Artemisinin Production in *Artemisia annua* hairy roots by Ag-SiO_2 core-shell nanoparticles. *Curr. Nanosc.* **2013**, *9*, 363–370. [CrossRef]
16. Pitta-Alvarez, S.I.; Spollansky, T.C.; Giulietti, A.M. The influence of different biotic and abiotic elicitors on the production and profile of tropane alkaloids in hairy root cultures of *Brugmansia candida*. *Enz. Microb. Technol.* **2000**, *26*, 252–258. [CrossRef]
17. Jamshidi, M.; Ghanati, F.; Rezaei, A.; Bemani, E. Change of antioxidant enzymes activity of hazel (*Corylus avellana* L.) cells by AgNPs. *Cytotech.* **2016**, *68*, 525–530. [CrossRef] [PubMed]
18. Fazal, H.; Abbasi, B.H.; Ahmad, N. Optimization of adventitious root culture for production of biomass and secondary metabolites in *Prunella vulgaris* L. *Appl. Biochem Biotechnol.* **2014**, *174*, 2086–2096. [CrossRef] [PubMed]
19. De Falco, E.; Mancini, E.; Roscigno, G.; Mignola, E.; Taglialatela-Scafati, O.; Senatore, F. Chemical composition and biological activity of essential oils of *Origanum vulgare* subsp. *vulgare* L. under different growth conditions. *Molecules* **2013**, *18*, 14948–14960. [CrossRef]
20. Mancini, E.; Camele, I.; Elshafie, H.S.; De Martino, L.; Pellegrino, C.; Grulova, D. Chemical Composition and Biological Activity of the Essential Oil of *Origanum vulgare* ssp. *hirtum* from Different Areas in the Southern Apennines (Italy). *Chem. Biodiv.* **2014**, *11*, 639–651. [CrossRef] [PubMed]

21. Smigielski, K.; Prusinowska, R.; Stobiecka, A.; Kunicka-Styczyñska, A.; Gruska, R. Biological Properties and Chemical Composition of Essential Oils from Flowers and Aerial Parts of Lavender (*Lavandula angustifolia*). *J. Essent. Oil Bear. Pl.* **2018**, *21*, 1303–1314. [CrossRef]
22. Wesolowska, A.; Grzeszczuk, M.; Kulpa, D. GC-MS analysis of the essential oil from flowers of *Chrysanthemum coronarium* L. propagated conventionally and derived from in vitro cultures. *Acta Chromat.* **2015**, *27*, 525–539. [CrossRef]
23. Wesolowska, A.; Grzeszczuk, M.; Wilas, J.; Kulpa, D. Gas Chromatography-Mass Spectrometry (GC-MS) analysis of indole alkaloids isolated from *Catharanthus roseus* (L.) G. don cultivated conventionally and derived from in vitro cultures. *Not. Bot. Hort. Agrobot. Cluj-Napoca.* **2016**, *44*, 100–106. [CrossRef]
24. Hatami, M.; Hatamzadeh, A.; Ghasemnezhad, M.; Sajidi, R.H. Variations of the Phytochemical Compounds in Rosescented Geranium Plant Exposed to Nanosilver Particles. *J. Essent. Oil Bear. Pl. 2016. 19*, 1747–1753.
25. Ghanati, F.; Bakhtiarian, S. Changes of natural compounds of *Artemisia annua* L. by methyl jasmonate and silver nanoparticles. *Adv. Envir. Biol.* **2013**, *7*, 2251–2258.
26. Kara, N.; Baydar, H. Determination of lavender and lavandin cultivars (*Lavandula* sp.) containing high quality essential oil in Isparta, Turkey. *Turk. J. Field Crops.* **2013**, *18*, 58–65.
27. Zheljazkov, V.; Astatkie, T.; Hristov, A. Lavender and hyssop productivity, oil content and bioactivity as function of harvest time and drying. *Ind. Crops Prod.* **2012**, *36*, 222–228. [CrossRef]
28. Hassanpouraghdam, M.B.; Hassani, A.; Vojodi, L.; Asl, B.H.; Rostami, A. Essential oil constituents of *Lavandula officinalis* Chaix. from Northwest Iran. *Chemija.* **2011**, *22*, 167–171.
29. Verma, R.S.; Rahman, L.U.; Chanotiya, C.S.; Verma, R.K.; Chauhan, A.; Yadav, A.; Singh, A.; Yadav, A.K. Essential oil composition of *Lavandula angustifolia* Mill. cultivated in the mid hills of Uttarakhand, India. *J. Serb. Chem. Soc.* **2010**, *75*, 343–348. [CrossRef]
30. Śmigielski, K.; Prusinowska, R.; Raj, A.; Sikora, M.; Wolińska, K.; Gruska, R. Effect of drying on the composition of essential oil from *Lavandula angustifolia*. *J. Ess. Oil Bearing Plants.* **2011**, *14*, 532–542. [CrossRef]
31. Mostefa, M.B.; Kabouche, A.; Abaza, I.; Aburjai, T.; Touzani, R.; Kabouche, Z. Chemotypes investigation of *Lavandula* essential oils growing at different North African soils. *J. Mater. Environ. Sci.* **2014**, *5*, 1896–1901.
32. Hajhashemi, V.; Ghannadi, A.; Sharif, B. Anti-inflammatory and analgesic properties of the leaf extracts and essential oil of *Lavandula angustifolia* Mill. *J. Ethnopharmacol.* **2003**, *89*, 67–71. [CrossRef]
33. Andrys, D.; Adaszyńska-Skwirzyńska, M.; Kulpa, D. Jasmonic acid changes the composition of essential oil isolated from narrow-leaved lavender propagated in in vitro cultures. *Nat. Prod. Res.* **2018**, *32*, 834–839. [CrossRef] [PubMed]
34. Andrys, D.; Kulpa, D. In Vitro Propagation Affects the Composition of Narrow-Leaved Lavender Essential Oils. *Acta Chrom.* **2018**, *30*, 225–230. [CrossRef]
35. Prusinowska, R.; Śmigielski, K.B. Composition, biological properties and therapeutic effects of lavender (*Lavandula angustifolia* L.). A review. *Herba Pol.* **2014**, *60*, 56–66. [CrossRef]
36. Cherneva, E.; Pavlovic, V.; Smelcerovic, A.; Yancheva, D. The effect of camphor and borneol on rat thymocyte viability and oxidative stress. *Molecules* **2012**, *17*, 10258–10266. [CrossRef] [PubMed]
37. Elshafie, H.S.; Sakr, S.; Mang, S.M.; Belviso, S.; De Feo, V.; Camele, I. Antimicrobial activity and chemical composition of three essential oils extracted from Mediterranean aromatic plants. *J. Med. Food.* **2016**, *19*, 1096–1103. [CrossRef]
38. Elshafie, H.S.; Camele, I. An Overview of the Biological Effects of Some Mediterranean Essential Oils on Human Health. *BioMed Res. Int.* **2017**, *2017*, 9268468. [CrossRef]
39. Love, J.N.; Sammon, M.; Smereck, J. Are one or two dangerous? Camphor exposure in toddlers. *J. Emerg. Med.* **2004**, *27*, 49–54. [CrossRef]
40. Nakahashi, H.; Miyazawa, M. Biotransformation of (−)-camphor by *Salmonella typhimurium* OY1002/2A6 expressing human CYP2A6 and NADPH-P450 reductase. *J. Oleo Sci.* **2011**, *60*, 545–548. [CrossRef]
41. Dai, J.P.; Chen, J.; Bei, Y.F.; Han, B.X.; Wang, S. Influence of borneol on primary mice oral fibroblasts: A penetration enhancer may be used in oral submucous fibrosis. *J. Oral. Pathol. Med.* **2009**, *38*, 276–281. [CrossRef] [PubMed]
42. Srinivasan, K. Black pepper and its pungent principle-piperine:a review of diverse physiological effects. *Critical Rev. Food Sci. Nutr.* **2007**, *47*, 735–748. [CrossRef] [PubMed]
43. Abbasi, B.H.; Khan, M.; Guo, B.; Bokhari, S.A.; Khan, M.A. Efficient regeneration and antioxidative enzyme activities in *Brassica rapa* var. *turnip. Plant Cell Tiss. Organ Cult.* **2011**, *105*, 337–344. [CrossRef]

44. Bhat, P.; Bhat, A. Silver nanoparticles for enhancement of accumulation of capsaicin in suspension culture of *Capsicum* sp. *J. Exp. Scien.* **2016**, *7*, 1–6.
45. Hemm, M.R.; Rider, S.D.; Ogas, J.; Murry, D.J.; Chapple, C. Light induces phenylpropanoid metabolism in *Arabidopsis* roots. *Plant J.* **2004**, *38*, 765–778. [CrossRef] [PubMed]
46. Liu, C.Z.; Guo, C.; Wang, Y.; Ouyang, F. Effect of light irradiation on hairy root growth and artemisinin biosynthesis of *Artemisia annua*. *Proc. Biochem.* **2002**, *38*, 581–585. [CrossRef]
47. Turkevich, J.; Stevenson, P.C.; Hillier, J. A study of the nucleation and growth processes in the synthesis of colloidal gold. *Discuss. Faraday Soc.* **1951**, *11*, 55–75. [CrossRef]
48. Liu, F.K.; Ker, C.J.; Chang, Y.C.; Ko, F.H.; Chu, T.C.; Dai, B.T. Microwave heating for the preparation of nanometer gold particles. *Jpn. J Appl. Physic.* **2003**, *42*, 4152–4158. [CrossRef]
49. Murashige, T.; Skoog, F. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol Plant.* **1962**, *15*, 473–497. [CrossRef]
50. Andrys, D.; Kulpa, D.; Grzeszczuk, M.; Bihun, M.; Dobrowolska, A. Antioxidant and antimicrobial activities of *Lavandula angustifolia* Mill. field-grown and propagated in vitro. *Folia Hort.* **2017**, *29*, 161–180. [CrossRef]
51. *European Pharmacopoeia 5.0*; EDQM: Strasbourg, France, 2005; p. 1894.
52. Hassanpouraghdam, M.B.; Hassani, A.; Shalamzari, M.S. Menthone-and estragole-rich essential oil of cultivated Ocimum basilicum L. from Northwest Iran. *Chemija* **2010**, *21*, 59–62.
53. Rosas, J.F.; Zoghbi, M.G.B.; Andrade, E.H.A.; van den Berg, M.E. Chemical composition of a methyl-(E)-cinnamate *Ocimum micranthum* Willd. from the Amazon. *Flavour Fragr. J.* **2005**, *20*, 161–163. [CrossRef]
54. Van Den Dool, H.; Kratz, P.D. A generalization of the retention index system including linear temperature programmed gas-liquid partition chromatography. *J. Chromatogr. A.* **1963**, *11*, 463–471. [CrossRef]
55. Babushok, V.I.; Linstrom, P.J.; Zenkevich, I.G. Retention indices for frequently reported compounds of plant essential oils. *J. Phys. Chem. Ref. Data* **2011**, *40*, 043101. [CrossRef]

Sample Availability: Samples of the compounds are not available from the authors.

molecules

MDPI

Article

Effect of AuNPs and AgNPs on the Antioxidant System and Antioxidant Activity of Lavender (*Lavandula angustifolia* Mill.) from In Vitro Cultures

Paula Jadczak [1,*], Danuta Kulpa [1], Radosław Drozd [2], Włodzimierz Przewodowski [3] and Agnieszka Przewodowska [3]

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; danuta.kulpa@zut.edu.pl

2 Department of Microbiology and Biotechnology, Faculty of Biotechnology and Animal Husbandry, West Pomeranian University of Technology in Szczecin, Piastów 45, 70-311 Szczecin, Poland; radoslaw.drozd@zut.edu.pl

3 Plant Breeding and Acclimation Institute in Bonin National Research Institute, Bonin 3, 73-009 Bonin, Poland; w.przewodowski@ihar.edu.pl (W.P.); a.przewodowska@ihar.edu.pl (A.P.)

* Correspondence: paula.jadczak@zut.edu.pl

Academic Editors: Susana M. Cardoso and Olívia R. Pereira

Received: 27 August 2020; Accepted: 19 November 2020; Published: 25 November 2020

Abstract: The aim of this study was to determine the effect of gold and silver nanoparticles on the activity of antioxidant enzymes (ascorbate peroxidase (APX), superoxide dismutase (SOD), guaiacol peroxidase (POX), and catalase (CAT)), the free radical scavenging capacity, and the total polyphenol capacity of lavender (*Lavandula angustifolia* Mill.) cultivar "Munstead" propagated in vitro. In the experiment, fragments of lavender plants were cultivated in vitro on medium with the addition of 1, 2, 5, 10, 20, and 50 mg·dm^{-3} of AgNPs or AuNPs (particle sizes 24.2 ± 2.4 and 27.5 ± 4.8 nm, respectively). It was found that the nanoparticles increase the activity of the antioxidant enzymes APX and SOD; however, the reaction depends on the NP concentration. The highest APX activity is found in plants propagated on media with 2 and 5 mg·dm^{-3} of AgNPs. AuNPs significantly increase the APX activity when added to media with a concentration of 10 mg·dm^{-3}. The highest SOD activity is recorded at 2 and 5 mg·dm^{-3} AgNP and AuNP concentrations. The addition of higher concentrations of nanoparticles to culture media results in a decrease in the APX and SOD activity. The addition of AuNPs to culture media at concentrations from 2 to 50 mg·dm^{-3} increases the POX activity in comparison to its activity when AgNPs are added to the culture media. No significant influence of NPs on the increase in CAT activity was demonstrated. AgNPs and AuNPs increased the free radical scavenging capacity (ABTS$^{\bullet+}$). The addition of NPs at concentrations of 2 and 5 mg·dm^{-3} increased the production of polyphenols; however, in lower concentrations it decreased their content in lavender tissues.

Keywords: gold; silver; nanoparticles; antioxidant enzymes; free radical scavenging; shoot propagation; elicitation; micropropagation

1. Introduction

True lavender (*Lavandula angustifolia*), classified in the mint family (*Lamiaceae*), is an evergreen shrub native to southern Europe, primarily the Mediterranean region. It is commercially cultivated in, among other places, France, Portugal, Poland, Spain, Hungary, the UK, Bulgaria, Australia, China, and the USA [1–3]. Essential oil with the characteristic aroma is one of the most important commercial components of true lavender, as it is used in the food, perfume, and the cosmetic industry [4–6].

Lavender oil is a mixture of chemical substances comprising primarily mono- and sesquiterpene compounds, exhibiting a complex therapeutic effect [7,8]. It is commonly used individually or as an additive in aromatherapy and folk and conventional medicine [9–12]. Numerous studies have confirmed the bactericidal and fungicidal properties of lavender oil toward *Staphylococcus aureus*, *Enterococcus*, *Aspergillus nidulans* and even *Trichophyton metangrophytes*, which is considered to be resistant to certain synthetic antibiotics [1]. In addition, lavender oil is characterized by analgesic, sedative, anticancer, and anti-inflammatory properties. It is a perfect agent to control digestive disorders and helps to combat skin disorders of various origins [10]. A study has also proven its effectiveness in alleviating depression symptoms [13].

Nowadays, the production of high-quality propagation materials, free of viruses and identical to parent plants, is ensured by biotechnological methods, including in vitro plant culture. Plants propagated in vitro are genetically uniform, which is of special importance for the production of secondary metabolites [14,15]. The large-scale production of lavender requires efficient in vitro propagation techniques to avoid the overexploitation of natural populations and allow the application of biotechnology-based approaches for plant improvement and the production of valuable secondary metabolites [16].

A series of studies have confirmed that medicinal plants propagated in vitro are characterized by different contents and compositions of essential oils [16–19]. The composition of secondary metabolites can also be changed using biotic and abiotic elicitors. These elicitors induce a series of physiological reactions in the plant and stimulate the production of secondary metabolites [20,21].

Nanotechnology has been applied in many fields of science and industry as a matter of specific qualities that have been identified by nanoparticles [22]. Nanometals are increasingly used in commercial products, including in the production of drugs and cosmetics and as transporting molecules, biosensors, or drug delivery agents [23–25]. They are also used in optoelectronics, nano-devices, and nanoelectronics. Their antibacterial properties have found application in medicine, water treatment, and food processing [26]. Resent research describe the wide use of nanosilver and gold in medicine [27–30].

They are valued for their unique physical and chemical properties (e.g., a higher surface to volume ratio, atypical surface structure, increased reactivity), and for their specific impact on biological tissues [31–36].

In recent times, particular attention has been paid to the use of nanotechnology in in vitro cultures. Nanoparticles have been mainly used as antimicrobial agents for sterilization and prevention of contamination of plant cultures in vitro [37]. In recent years, their influence on seed germination and plant growth has been increasingly studied [38–40]. The influences of copper (Cu), zinc (Zn), iron (Fe), titanium (Ti), and aluminium (Al) nanoparticles on plants are widely described among other things in *Phaseolus radiatus* [41], *Stevia rebaudiana* [42], *Sinapis alba*, *Lepidium sativum*, [43], *Cicer arietinum* [44], and *Nicotiana tabacum* [45]. However, the influence of nanoparticles of two metals, silver and gold, is most often studied. Ag and Au nanoparticles have been selected because of the features related to their commercial applicability—their low price, easy availability, and high stability in aqueous solutions—and the numerous studies indicating their low toxicity [46]. The effect of nanoparticles on plant development has been determined, such as the influence of silver nanoparticles on *Solanum tuberosum* [47] and *Eichhornia crassipes* [48] and gold nanoparticles on *Brassica juncea* [49], *Artemisia absinthium*, and *Vigna radiata* [50].

These nanoparticles are among the latest currently studied elicitors [51–53]. It has been proven that metal nanoparticles are highly reactive toward plants. Due to their small size, they can easily penetrate the cell membrane and accumulate inside the cell [54]. The properties of nanoparticles are strongly dependent on many factors, such as their method of preparation, the temperature at which they are prepared, and the surfactants used for capping [55].

Certain metal nanoparticles have a considerable impact on the physiological processes of plants, such as seed germination, growth, and metabolism [56–59], and scientific research indicates that

their impact can be both positive and negative. The majority of studies describe that they exhibit a destructive influence that results in damage at the genomic level, chromosomal aberrations, cell growth inhibition, and programmed cell death-apoptosis [60–62]. Numerous studies have demonstrated the phytotoxicity of metal nanoparticles leading to the activation of the antioxidative system in plants [63,64]. Antioxidative enzymes, including guaiacol peroxidase (POX), superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), and alternative oxidase, and other low-molecular compounds in the form of polyphenols, vitamins, and minerals, are responsible for the inhibition of formation or the removal of excessive reactive oxygen species (ROS) from plant organisms [65,66].

The increase in the production levels of ROS under the influence of nanoparticles has been reported in many plants, such as *Oryza sativa* [67,68], *Alium cepa* [69], *Brassica juncea* [70,71], *Arabidopsis Tthaliana* [72], and *Nicotina tabacum* [73].

The oxidative stress induced by the metal nanoparticles may influence the production and qualitative and quantitative composition of secondary metabolites in plants [40,74]. The favorable influence of elicitation by metal nanoparticles on the production of biologically active compounds in plants has been confirmed by Zhang et al. [41,75] for *Artemisia anuua*, Sharafi et al. [42,76] for *Hypericum perforatum*, and Oloumi et al. [43,77] for *Glycyrrhiza glabra*. Silver and gold nanoparticles have been used as elicitors in cultures of *Prunella vulgaris* [78], *Stevia glycosides* [52], *Cucumis anguria* [79], and *Caralluma tuberculata* [80]. The latest scientific reports indicate significant changes in the metabolite profile of *Arabidopsis thaliana* under the influence of silver nanoparticles [81].

This paper is part of a cycle of research aimed at developing an efficient method of production of secondary metabolites, especially essential oils, by changing the profile of the secondary metabolites produced by *Lavandula angustifolia* plants from in vitro cultures. According to our previous studies, the addition to growth media of 1 to 50 mg·dm^{-3} of AgNPs and AuNPs has a significant impact on plant development [82]. Our research has also shown that the addition of 10 or 50 mg·dm^{-3} of AgNPs and AuNPs changes the composition of essential oils produced by lavender plants [83]. However, it is not clear to us whether this phenomenon is a result of stress or a change in the production of one of the endogenous plant growth regulators—e.g., ethylene, which modulates the endogenous production of other regulators. According to Akasaka-Kennedy [84], the addition of silver nanoparticles reduces the production of endogenous ethylene in *Brassica sp* tissues. Therefore, the main aim of the study is to answer the question of whether the addition of nanoparticles, which has been shown earlier, modifies their growth, affects the production of secondary metabolites, or activates ROS in plants grown on media in in vitro cultures. So far, there have been no scientific reports on the influence of AuNPs and AgNPs on the activation of the antioxidant system in *L. angustifolia* propagated in vitro.

The aim of the study was to analyze the influence of the addition to an in vitro culture of lavender of AgNPs and AuNPs on elements of the enzymatic antioxidant defences system, POX, APX, SOD, and CAT in plant tissue.

2. Results

2.1. Antioxidative Enzyme Activity

2.1.1. APX

Silver nanoparticles added to the propagation medium at the lowest concentration used (1 mg·dm^{-3}) did not affect the APX enzyme activity, which remained at the level of control plants (Figure 1). However, the addition of higher AgNP concentrations to the medium resulted in increasing the APX activity. The highest APX activity was determined in plants propagated on media with the addition of 2 and 5 mg·dm^{-3} of AgNPs, at 26.01 and 18.93 U·mg^{-1} DW, respectively. With the increased AgNP concentration in culture media in the range of 10 to 50 mg·dm^{-3}, the enzyme activity decreased, but it was at a significantly higher level ($p \leq 0.05$) compared to the activity of control plants. The addition of AuNPs to the culture media had a significant effect on the increase in APX activity in relation to the control, but only for AuNP concentrations equal to 10 mg·dm^{-3} (9.28 U·mg^{-1} DW).

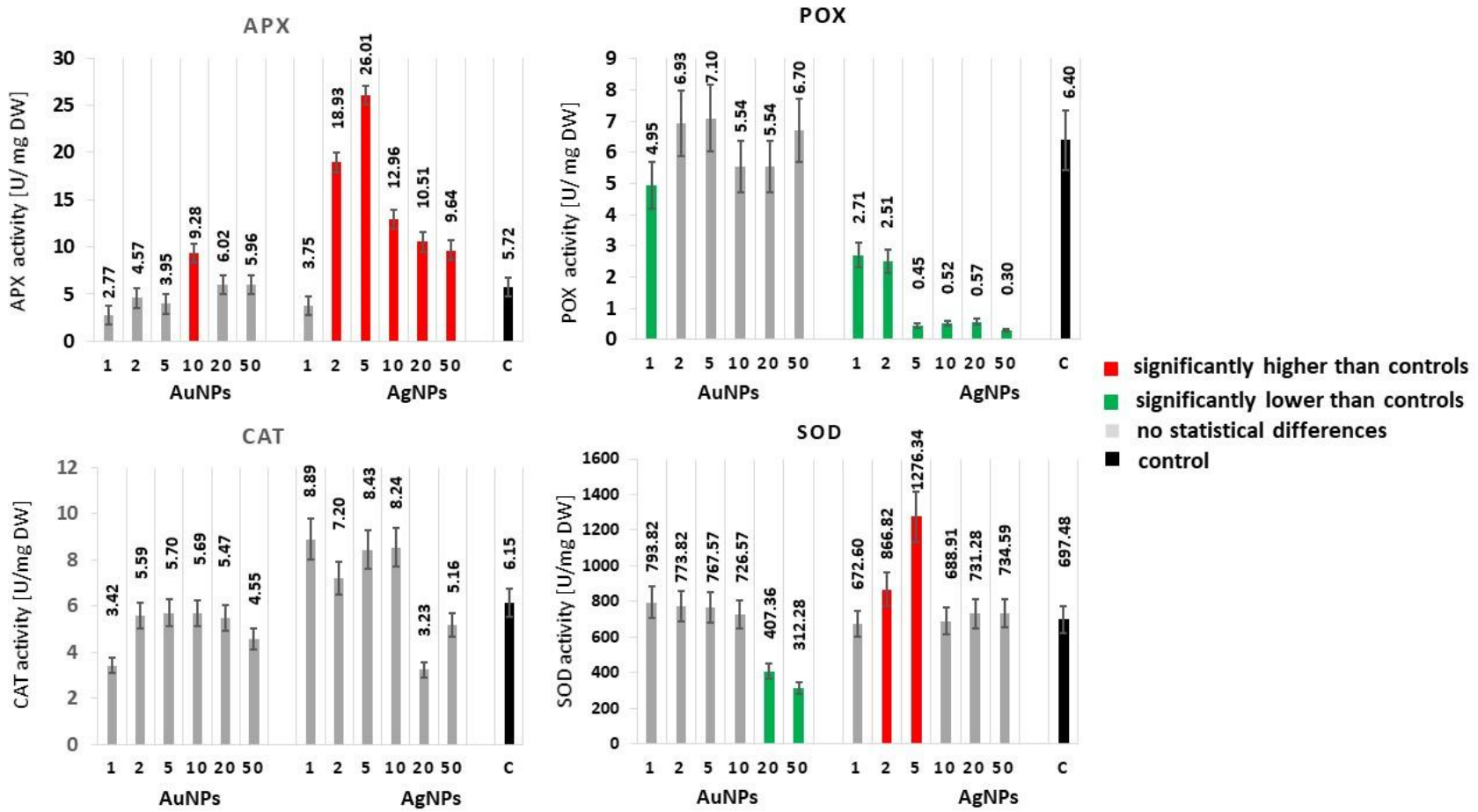

Figure 1. Effect of AgNPs and AuNPs (1–50 mg·dm^{-3}) on the antioxidant enzymes activity in *Lavandula angustifolia*. a-ascorbate peroxidase (APX), b-guaiacol peroxidase (POX), c-catalase (CAT), and d-superoxide dismutase (SOD). Values represent the means of three replications ± SE. Different colors of bars indicate significant statistical differences between the treatments and control samples according to Tukey's Test ($p < 0.05$).

2.1.2. POX

The POX activity varied depending on the nanoparticle type applied. The POX activity was close to that of the control when plants were propagated on media with an AuNP addition at concentrations between 2 and 50 mg·dm^{-3} (Figure 1). A reduction in activity in relation to the control was observed solely for the lowest of the concentrations used (1 mg·dm^{-3}). The propagation in media with the addition of AgNPs resulted in a dramatic reduction in the POX activity, relative to both the control as well as plants propagated on media with the addition of AuNPs. A particularly low POX activity was determined for plants propagated on media with an addition of 5 to 50 mg·dm^{-3} (0.30 to 0.57 U·mg^{-1} DW).

2.1.3. SOD

The activity of SOD above the control was recorded for the concentrations 2 and 5 mg·dm^{-3} of AgNPs (866.22 and 1276.34 U·mg^{-1} DW, respectively). The addition of higher AgNP concentrations to the culture media (10, 20, 50 mg·dm^{-3}) resulted in the decreased activity of the enzyme, thus it remained at the control level. Gold nanoparticles resulted in a decreased SOD activity ($p \leq 0.05$) only at the highest concentrations applied—20 and 50 mg·dm^{-3} (407.36 and 312.28 U·mg^{-1} DW). The activity of SOD for the lowest AuNPs concentrations remained at the control level (Figure 1).

2.1.4. CAT

The conducted study revealed that no significant impact of gold and silver nanoparticles could be found for the activity of catalase determined in the studied tissues (Figure 1).

2.2. Free Radical Scavenging Activity and Total Polyphenols Content

2.2.1. The Total Polyphenol Content

Polyphenols content varied depending on the nanoparticle type and its concentration in the medium (Figure 2). Increasing polyphenol contents relative to the control were determined for lavender cultivated on media with the addition of 2 and 5 mg·dm^{-3} of AuNPs and 5 to 50 mg·dm^{-3} of AgNPs (0.214 and 0.217, respectively, and from 0.212 to 0.318 mg of TAE/100 gDW). On the other hand, the lavender cultivated on media with the addition of the highest AuNP concentrations (20 and 50 mg·dm^{-3}) and lowest AgNPs concentrations (1 and 2 mg·dm^{-3}) was characterized by significantly lower polyphenol contents.

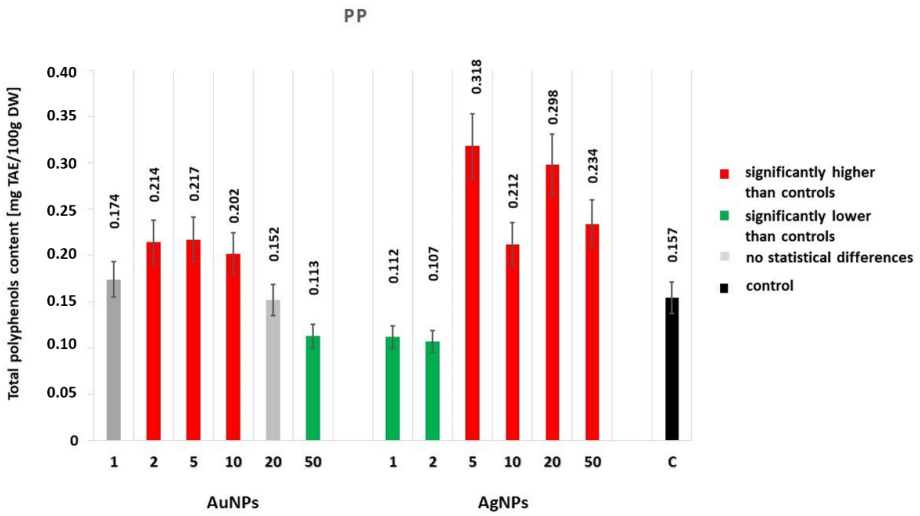

Figure 2. Effect of AgNPs and AuNPs (1–50 mg·dm^{-3}) on the total polyphenol content (PP) of *Lavandula angustifolia* (±SE). Different colors of bars indicate significant statistical difference between the treatments and control samples according to Tukey's Test ($p < 0.05$).

2.2.2. Free Radical Scavenging Activity ($ABTS^{\bullet+}$)

The presented outcomes suggest that gold and silver nanoparticles added to culture media in in vitro cultures have a positive impact on the free radical scavenging activity of true lavender (Figure 3). An exception here was the lowest AgNP concentration (1 mg·dm^{-3}–197.77 µM AAE/100 gDW) and highest AuNP concentration used (50 mg·dm^{-3}–158.86 µM AAE/100 gDW). The highest free radical scavenging capacity values were determined for plants cultivated on media with the addition of 5 mg·dm^{-3} of AgNPs and AuNPs, at 330.70 and 309.62 µM AAE/100 gDW, respectively. The addition of NPs in 1, 2, and 5 mg·dm^{-3} concentrations to the culture media resulted in a gradual increase in the antioxidant activity of lavender, whereas a reduced scavenging capacity was observed for higher concentrations (10, 20, and 50 mg·dm^{-3}).

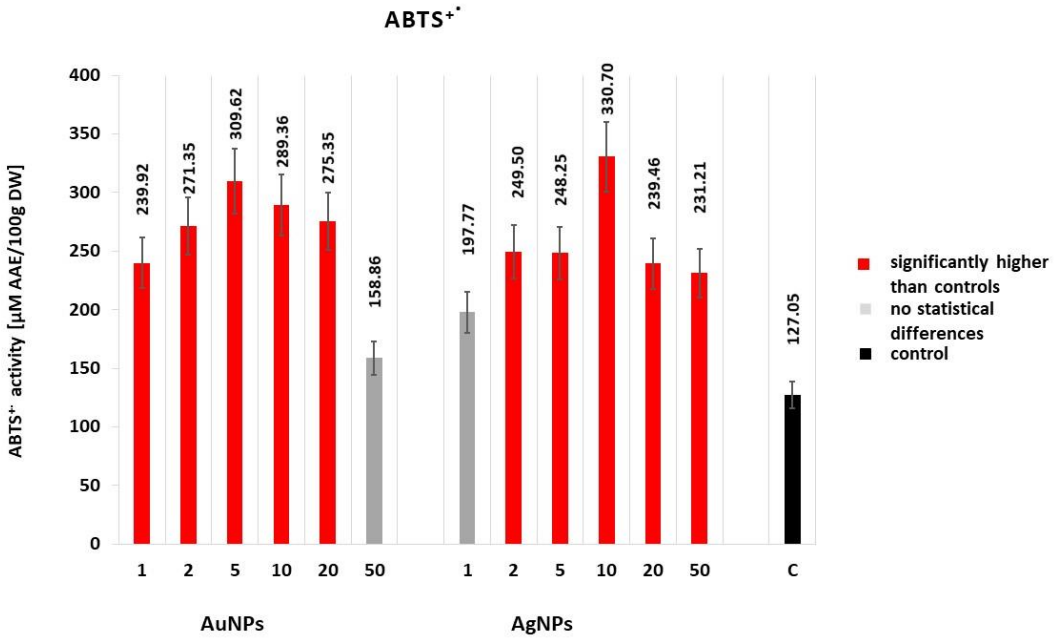

Figure 3. Effect of AgNPs and AuNPs (mg·dm^{-3}) on the antioxidant activity of *Lavandula angustifolia* in vitro cultures measured by the $ABTS^{\bullet+}$ radicals scavenging assay. Values represent the means of three replications ±SE. Different colors of bars indicate significant statistical differences between the treatments and control samples according to Tukey's test ($p < 0.05$).

3. Discussion

Gold and silver nanoparticles are used on a large scale in many industries. Due to their antibacterial, anti-angiogenic, and anti-inflammatory properties, silver nanoparticles are used in antibacterial coatings for coating medical devices, wound and burn dressings, numerous cosmetic products, and food packaging [85–87]. Gold nanoparticles are used in molecular imaging, targeted drug delivery, gene therapy, cancer treatment, and radiation [88]. Prociak et al. [89] presented the potential use of gold and silver nanocolloids in cosmetic production. Microbiological tests performed in this study, showed that emulsion with silver and gold nanocolloids possessed satisfactory fungicidal properties and had a positive effect on the characteristics of the creams such as their consistency and absorption. Ag and Au nanocolloids can also be used in medicine, e.g., for the treatment of wounds, including the most difficult to heal burn and post-surgery wounds [90–92] or in medical imaging when diagnosing cancer [93,94]. It has been also confirmed the use of nanocolloids in the food industry, mainly in food packaging or prevention of contamination [95,96].

In view of the wide range of possibilities for the use of nanoparticles, it is important to determine the toxic properties of nanoparticles for humans and the environment. The results of most published scientific studies exclude the toxicity of nanoparticles [85,97,98]. However, there are also studies showing

their adverse effects on living organisms and the environment, with this harmfulness depending on many factors, such as their physicochemical properties (shape, size, load) or coating agents [99,100]. Most of the studies show that nanoparticles are toxic to plants at higher concentrations. In addition, the type of target cells, tissues, or organisms or the type of test itself should be considered [100]. Therefore, it is vital to precisely determine the effect of nanoparticles on living organisms, including plants [100].

In response to oxidative damage, plants have developed a defense system which manifests itself, among other things, through the increased activity of antioxidant enzymes [101,102].

The activity of antioxidative enzymes may be a good marker of the toxic influence of the external environment on an organism. Mehrian et al. [103] studied the influence of AgNPs on the activity of antioxidant enzymes in *Lycopersicon esculentum*. It was found that, with the increase in the concentration of silver nanoparticles in the plant tissue medium, the activity of SOD, CAT, and POX in the shoots and roots increased. The increased activity of CAT, SOD, and POX was also observed in the callus tissue of sugar cane (*Saccharum spp.* cv CP-77,400) propagated on media with the addition of 20 to 60 AgNPs [104] (Barbasz et al.]. Iqbal et al. [105] carried out a study to investigate the effect of silver nanoparticles (AgNP) on the physiological, biochemical, and antioxidant parameters of wheat (*Triticum aestivum* L.) under heat stress conditions. It was found that the addition of AgNPs had a protective effect on plant tissues under stress conditions—wheat plants treated with AgNPs showed a significant increase in dry matter, with a simultaneous increase in SOD, POX, CAT, APX, and GPX activity under heat stress conditions. In the conducted study, we analysed the impact of the addition of gold or silver nanoparticles to culture media on the activity of CAT, APX, POX, and SOD antioxidative enzymes. The conducted study revealed a significant impact of the applied nanoparticles on the activity of all the tested antioxidative enzymes, with the exception of CAT. The observed effect was variable depending on the nanoparticle type and concentration. Lavender exposed to stress caused by AgNPs or AuNPs primarily activated the antioxidative system, consisting of APX and SOD. Increased activity of CAT, SOD, and POD due to Cu_2O and Zn nanoparticles has also been confirmed for cucumber *Cucumis sativus* L. in the study of Kim and Lee [106]. These authors, using low concentrations of both kinds of nanoparticles, observed a significant increase in the activity of these three antioxidative enzymes. However, with the increased concentration of metals nanoparticles in the culture media (above 100 mg dm^{-1}), the activity of these enzymes was markedly reduced. The concentration of the analyzed AgNPs which significantly reduced the activity of APX and SOD was 5 mg·dm^{-3}. The experiment carried out by Gunjan et al. [107] examined the impact of gold nanoparticles on the activity of antioxidative enzymes in *Brassica juncea* seedlings. It was established that the glutathione reductase (GR), ascorbate peroxidase (APX), and glutathione peroxidase (GPX) activity increases with high AuNP concentrations (200 mg·dm^{-3}), and that they are significantly higher than the CAT activity. These results suggest that the enzymatic complex in the form of APX, GPX, and GR forms part of the defensive mechanism against oxidative disturbance produced by nanometals in *Brassica juncea*, and that CAT does not have a substantial role in this case. In the study of Tripathi et al. [108], it was noted that silver nanoparticles (at concentrations from 100 to 300 µM) markedly stimulate superoxide dismutase and ascorbate peroxidase activity, while inhibiting the glutathione reductase and dehydroascorbate reductase activity in *Pisum sativum*.

Our study revealed that the true lavender antioxidative activity under in vitro cultures depends on the nanoparticle type and its concentration in the medium. *Lavandula angustifolia* is known for its health-promoting properties, resulting from the presence of antioxidative substances in its tissues (Miliasukas et al.) [109]. The chemical composition of plant metabolites and their antioxidative properties are influenced by various factors, including the i.a. cultivation method, growing location, climatic conditions, and plant genotype [2,110,111]. In our study, the free radical $ABTS^{\bullet+}$ scavenging analysis revealed a significant increase in the true lavender antioxidative activity after the addition of gold and silver nanoparticles to all culture media. This result is similar to the study of Chung et al. [79], where increased antioxidative activity was observed in transformed *Corylus avellana* roots under the

impact of AgNPs. Fazal et al. [78] tested AuNPs and AgNPs as elicitors of *Prunella vulgaris* callus cultures and observed a marked increase in antioxidative activity.

Increased antioxidant activity may be associated, for example, with an increase in the content of substances with antioxidant activity, including polyphenols, in plant tissues. In our research we also examined the influence of the addition of AgNPs and AuNPs to the culture media of in vitro cultures on the polyphenol content. Polyphenols are secondary plant metabolites with strong antioxidants properties and are produced by plant cells for protective purposes against pathogens and also contribute in adaptation process to the external environment. Polyphenol compounds have also been suggested to have the ability to support activity of the cellular enzymatic antioxidative system, as well as modulate low-molecular antioxidant concentrations in the form of ascorbic acid (vitamin C) and alpha-tocopherol (vitamin E) in plant organisms [112]. In conducted study for determination of total phenolic compounds content in *Lavandula angustifolia* has been used the Folin-Ciocalteu assay. The sensitivity of this method can be affected by other cellular compounds such as sugars, proteins and lipids. However, depend on the plant organs, the total phenolic content can differ. Leaves of many plants are especially rich in this compounds and eventual interference other co-extracted substances do not affect the quality of assay [113]. Despite its limitations, Folin-Ciocalteu method is widely used to determine the content of polyphenols in the tissues of plants grown in in vitro cultures [114]. It was used, inter alia, by Tian et al., (2018) [115] determining the content of polyphenols in *Atropa bellladonna* tissues treated with Mn_2O_3 NPs, callus tissues of *Caralluma tuberculata* treated with AgNPs [80] or *Stevia rebaudiana* shoots treated with ZnONPs [42]. Previous research has stated that the influence of metal nanoparticles on the content of polyphenols in vegetable plants is variable. An increased content of polyphenols under the impact of AgNPs has been determined for *Bacopa moonnieri* [116] and *Solanum tuberosum* [117] and a reduction for castor [118]. We obtained similar results in our research. The polyphenol content was higher than in control plants when the plants grew on media with the addition of 10–50 mg·dm^{-3} AgNPs and 2–5 AuNPs. The study of Jamshidi and Ghanati [119] provided evidence of the significant effect of silver nanoparticle elicitation on the increase in phenol and flavonoid contents in hazel plants. In their experiment, they utilized low AgNP concentrations (2, 5, 10 mg·dm^{-3}), indicating their clear impact on the content of the tested antioxidants in cell cultures. Ghorbanpour and Hadian [120] showed the significant influence of MWCTs (multi-walled carbon nanotubes) in the culture of the callus tissue of *Satureja khuzestanica* on the total flavonoid content in callus extracts for all MWCNT concentrations used. However, the analysis of the polyphenol content showed that its increase was observed only for some of the concentrations applied (50, 100, and 200 μg mL^{-1} MWCNTs). Their experiment further revealed that the maximum values for flavonoids and phenols were obtained using a MWCNT concentration of 100 μg·mL^{-1}. In the present study, the nanoparticle concentrations used in culture media were lower. Tian et al. [115] demonstrated the significant impact of Mn_2O_3NPs at the concentration 25 mg·dm^{-3} on the increase in the alkaloid content in *Atropa balladona* L. tissues.

Production of bioactive secondary metabolites is generally associated with plant defense mechanisms. However, the mechanism of action of nanoparticles and their influence on ROS activation and the production of secondary metabolites is still poorly understood. According to Saha and Gupta (2018) [121], who studied the influence of silver nanoparticles on the development of *Swertia chirata* in in vitro cultures, ROS generated in presence of AgNP triggered an array of antioxidative enzymes which later on balanced the ROS content in the treated plant system. These enzymes also stimulated the shoot proliferations and helped in maximization of generation of number of shoots per explant considered. According to the authors, the reduction of ethylene production due to AgNPs may have a stimulating effect on plant development. Amir et al., (2019) [80] suggest that NPs might act as signal compounds, those like in nature to the chemical elicitors and influence cellular growth and secondary metabolism. Upon exposure to application of NPs, plant cell goes through a series of events in a cascade manner, resulting in oxidative outburst and generation of reactive oxygen species (ROS) in the surrounding environment of the plant cell. The ROS in turn can damage cell membrane and nuclei. To cope with the intense stress situation and to scavenge the ROS, plants activate their

metabolic pathways including the notable mitogen-activated protein kinase (MAPK) pathway. MAPK activation propels the plant antioxidant elements to come in contact with ROS in a cascade fashion. Several studies have shown that nanoparticles, especially AgNPs, affect the up-regulation of genes related to the response to abiotic stress, including the production of enzymes such as SOD, CAT, AXP [122,123]. Nair and Chung, (2014) [124] when examining the toxicity of AgNPs on rice, found a significant up-regulation of SOD gene. In their opinion, induction of SOD gene in response to AgNPs stress might be to maintain the redox homeostasis of the cell.

Our results suggest that gold and silver nanoparticles added to in vitro culture media have a significant impact on the antioxidative potential (free radical scavenging capacity) and activation of antioxidative enzymes of in vitro true lavender cultures. The defence mechanism of lavender exposed to stress caused by AgNPs and AuNPs primarily consists of the APX-SOD enzymatic complex, with CAT remaining insignificant in this case. It can additionally be stated that AgNPs at the concentration of 5 mg·dm^{-3} result in the highest antioxidative activity of APX and SOD, and higher concentrations in the culture medium inhibit their activity. AgNPs have a more toxic impact on lavender plants cultivated in vitro than AuNPs. Silver and gold nanoparticles added to culture media have significant impacts on the total polyphenol contents in true lavender cultivated in vitro and its free radical scavenging capacity ABTS$^{\bullet+}$, however this depends on the applied nanoparticle concentration.

4. Materials and Study Methods

4.1. Plant Culture under In Vitro Conditions

The study material comprised *L. angustifolia* cultivar "Munstead" plants growing under in vitro conditions. The explants used for the experiment were single-node shoot fragments and apical shoot fragments with several leaves and the apical meristem. They were placed on the Murashige and Skoog (MS) media [125] with the addition of 2 mg dm^{-3} of kinetin and 0.2 mg dm^{-3} of indole-3-acetic acid [126] and 1, 2, 5, 10, 20, or 50 mg dm^{-3} of AgNPs or AuNPs. The medium pH was set at 5.7 using 0.1 M solutions of HCl and NaOH. The culture medium was hardened with 7 g·dm^{-3} of agar and was subject to a 20-min sterilization in an autoclave at 121 °C and a 1 ATM pressure. Propagation was carried out in jars of 200 mL volume, containing 20 mL of medium. Eight explants were placed in each culture vessel. The experiment was established in 10 replications for each type and concentration of the nanoparticles used. The jars were placed for 28 days in a growth chamber at a temperature of 24 °C and relative air humidity of 70–80%. The cultures were illuminated with fluorescent light at an intensity of 40 PAR (μE·m^{-2}·s^{-1}) for 16 h per day. After 28 days of culture, the study material was collected and subjected to analyses.

4.2. Nanoparticles

In the experiment, aqueous solutions of AuNPs measuring 24.2 ± 2.4 nm and AgNPs measuring 27.5 ± 4.8 nm were used. The aqueous suspensions were synthesized using the methods of Turkevich et al. [127] and Liu et al. [128] with modified synthesis conditions and two-stage microwave-convection heating. For this purpose, aqueous mixtures of 3.5 mM sodium citrate with 7.0 mM tetrachloroauric acid ($HAuCl_4$) and 7.0 mM silver nitrate ($AgNO_3$), respectively, were prepared. Once their spectra were plotted with a UV-Vis Epoch Microplate Spectrophotometer (BioTek, Winooski, VT, USA), the optical density of the fractions obtained was adjusted to a common DEV value using the spectra absorbance maxima (λ_{max} = 520 nm for gold colloid and λ_{max} = 445 nm for silver colloid). The similarities in the morphology, shape, and size of the synthesized and prepared AuNPs and AgNPs were assessed through analyzing their images obtained using a transmission electron microscope (TEM) JEM-2100 (JOEL ltd., Tokyo, Japan) (Figure 4).

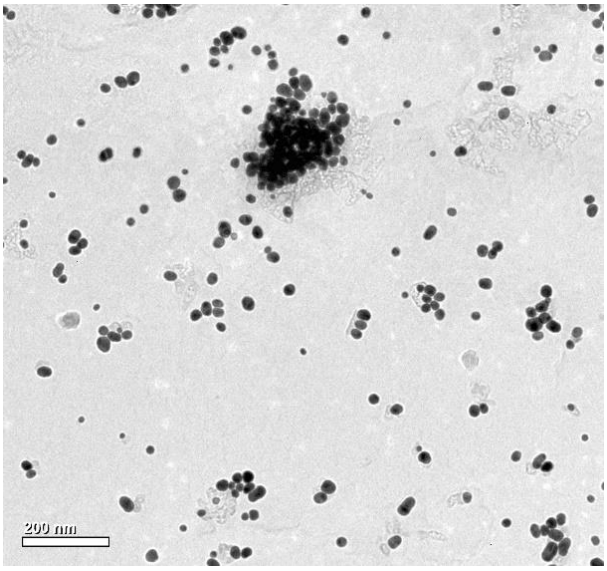

Figure 4. TEM image of AgNPs suspended in water; scale bar: 200 nm.

4.3. Antioxidant Enzymes Activity Assay

4.3.1. Sample Preparation

All the steps of the protein extraction were carried out at 4 °C on a cooled plate. Fresh leaf samples weighing approximately 0.2 g were previously powdered in liquid nitrogen using a cold mortar and pestle. The obtained powder was resuspended in 1 mL of cold 50 mmol L^{-1} phosphate buffer (pH 7.0) enriched with 1 mmol L^{-1} of phenylmethylsulfonyl fluoride, 2 mmol L^{-1} of EDTA, and 1% polyvinylpyrrolidone. The resulting solution was centrifuged at 15,000 rpm for 10 min at 4 °C. The extracts were immediately used for the determination of the enzyme activity or stored at −80 °C for further analysis.

4.3.2. Guaiacol Peroxidase Assay (POX)

The activity of POX was determined according to the protocol of Chance and Maehly [129], which was adapted to a microplate reader, by monitoring the rate of tetraguaiacol formation from guaiacol by an increase in absorbance at 470 nm (ε = 26.6 mM^{-1} cm^{-1}). One unit of POX is defined as the amount of enzyme that forms 1 µmol of tetraguaiacol per minute. The results were expressed in units per mg of the extracted protein.

4.3.3. Ascorbate Peroxidase Assay (APX)

The activity of APX was measured according to the protocol of Nakano and Asada [130] by monitoring the decrease in absorbance at 290 nm (ε = 2.8 mM^{-1} cm^{-1}). The measurements were performed using a microplate reader. One unit of APX is defined as the amount of enzyme that oxidizes 1 µmol of ascorbate per minute. The results were expressed in the units per mg of the extracted protein.

4.3.4. Dismutase Assay (SOD)

The total SOD activity was determined based on the inhibition of the photochemical reduction of nitroblue tetrazolium (NBT) according to the protocol of Beauchamp and Fridovich [131], which was adapted to a microplate reader. One unit of SOD is defined as the amount of enzyme that inhibits the NBT reduction by 50%. The results were expressed in units per mg of the extracted protein.

4.3.5. Catalase Assay (CAT)

The activity of CAT was determined according to the protocol of Li and Schellhorn [132] based on the rate of decomposition of hydrogen peroxide by measuring the decrease in absorbance at 240 nm ($\varepsilon = 43.6\ mM^{-1}\ cm^{-1}$). One unit of CAT is defined as the amount of enzyme that decomposes 1 µmol of hydrogen peroxide per minute. The results were expressed in units per mg of the extracted protein.

4.4. Total Phenol Content and Free-Radical ABTS$^{\bullet+}$ Scavenging Ability Assay

4.4.1. Tissue Extract Preparation

A total of 100 mg of tissue (previously frozen at −80 °C) was powdered in liquid nitrogen using a cooled laboratory mortar and pestle. The obtained powder was transferred to plastic tubes, mixed with 5 mL of cooled methanol, and left for 1 h in the dark. During extraction, the samples were mixed periodically. Then, the samples were centrifuged at 15,000 rpm and the obtained extract was used for further analysis.

4.4.2. Total Polyphenol Content Assay

The total content of polyphenolic acid in the tissue extracts was determined using Folin-Ciocalteu reagent according to the method of Anastasiadi et al. [133] which was modified to a microplate reader scale. The concentration of polyphenolic compounds was expressed as the mg of tannic acid equivalent (TEA) per 100 g of dry weight of sample (mg TAE/100 gDW).

4.4.3. Free Radical ABTS$^{\bullet+}$ Scavenging Ability Assay

The free radical scavenging activity was assessed in the tissue extracts using the cation radical discoloration of the 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt ABTS$^{\bullet+}$ assay adapted to a microplate reader scale, according to the method of Shi et al. [134]. Ascorbic acid (AAE) was used as a standard for calibration. The results were expressed in the µM of ascorbic acid equivalent per 100 g of dry weight of sample (µM AAE/100 gDW).

4.5. Statistical Analysis

The experiment was performed in three replications. An analysis of variance was performed, followed by Tukey's test ($p \leq 0.05$). Homogenous groups were labeled with successive letters of the alphabet.

Author Contributions: P.J. performed the experiments in in vitro cultures, analyzed the data, and wrote the manuscript; D.K. contributed to the revisions of the manuscript; W.P. developed, prepared, and standardized the solutions of nanoparticles. A.P. measured and analyzed the solutions of nanoparticles; R.D. manuscript editing and revision, enzymatic assay supervising. All the authors were responsible for processing information and manuscript writing. All the authors read and approved the final manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Cavanagh, H.M.A.; Wilkinson, J.M. Lavender essential oil: A review. *Healthc. Infect.* **2005**, *10*, 35–38. [CrossRef]
2. Verma, R.S.; Rahman, L.U.; Chanotiya, C.S.; Verma, R.K.; Chauhan, A.; Yadav, A.; Singh, A.; Yadav, A.K. Essential oil composition of *Lavandula angustifolia* Mill. Cultivated in mild hills of Uttarahand, India. *J. Serb. Chem. Soc.* **2010**, *75*, 343–348. [CrossRef]
3. Brailko, V.A.; Mitrofanova, O.; Lesnikowa-Sedoshenko, N.; Chelombit, S.; Mitrofanova, I.V. Anatomy features of *Lavandula angustifolia* Mill. and *Lavandula hybrida* rev. plants in vitro. *J. Agric. Food Chem.* **2017**, *63*, 111–117. [CrossRef]

4. Da Porto, C.; Decorti, D.; Kikic, I. Flavour compounds of *Lavandula angustifolia* L. to use in food manufacturing: Comparison of three different extraction methods. *Food Chem.* **2009**, *112*, 1072–1078. [CrossRef]
5. Hamad, K.J.; Al-Shaheen, S.J.A.; Kaskoos, R.A.; Ahamad, J.; Jameel, M.; Mir, S.R. Essential oil composition and antioxidant activity of *Lavandula angustifolia* from Iraq. *Int. Res. J. Pharm.* **2013**, *4*, 117–120.
6. Waithaka, P.; Gathuru, E.; Githaiga, B.; Kwoko, J. Making of perfumes from essential oils extracted from lavender plant collected from Egerton University, Main Campus Njoro, Kenya. *Afr. J. Biomed. Res.* **2016**, *2*, 35–40.
7. Landmann, C.; Fink, B.; Festner, M.; Dregus, M.; Engel, K.H.; Schwab, W. Cloning and functional characterization of three terpene sythases from lavender (*Lavandula angustifolia*). *Arch. Biochem. Biophys.* **2007**, *465*, 417–429. [CrossRef]
8. Mahmoudi, R.; Nostratpour, S. *Teuricum polium* L. essential oil: Phytochemical component and antioxidant properties. *Int. Food. Res. J.* **2013**, *4*, 1697–1701.
9. Gören, A.; TopÇu, G.; Bilsel, M.; AydoĞmuŞ, Z.; Pezzuto, J.M. The chemical contituents and biological activity of *Lavandula stocheas* ssp. stocheas. *Z. Nat. C* **2002**, *57*, 797–800.
10. Hajhashemi, V.; Ghannadi, A.; Sharif, B. Anti-inflammatory and analgesic properties of the leaf extracts and essential oil of *Lavandula angustifolia* Mill. *J. Ethnopharmacol.* **2003**, *89*, 67–71. [CrossRef]
11. Raut, S.J.; Karruppayil, M.S. A status review on the medicinal properties of essential oils. *Ind. Crops. Prod.* **2014**, *62*, 250–264. [CrossRef]
12. Prasad, A.; Shukla, S.P.; Mathur, A.; Singh, C.C.; Kumar, A.M. Genetic fidelity of long-term micropropagated *Lavandula officinalis* Chaix: An important aromatic plant. *Plant. Cell Tiss. Org.* **2015**, *120*, 803–811. [CrossRef]
13. Akhondzadeh, S.; Kashan, L.; Fotouhi, A.; Jarvandi, S.; Mobaseri, M.; Moin, M.; Khani, M.; Amir, H.J.; Baghalian, K.; Taghizadeh, M. Comparison of *Lavandula angustifolia* Mill. tincture and imipramine in the treatment of mild to moderate depression: A double-blind, randomized trial. *Prog. Neuropsychopharmacol. Biol. Psychiatry* **2003**, *27*, 123–127. [CrossRef]
14. Al-Qudah, T.; Shibli, R.A.; Alali, F.Q. In vitro propagation and secondary metabolites production in wild germander (*Teucrium polium* L.). *Vitr. Cell. Dev. Biol. -Plant* **2011**, *47*, 496–505. [CrossRef]
15. Frabetti, M.; Gutiérrez-Pesce, P.; Mendoza-de, G.E.; Rugini, E. Micropropagation of *Teucrium fruticans* L. an ornamental and medicinal plant. *Vitr. Cell. Dev. Biol. -Plant.* **2009**, *45*, 129–134. [CrossRef]
16. Gonçalves, S.; Romano, A. In vitro culture of lavenders (*Lavandula* spp.) and the production of secondary metabolites. *Biotechnol. Adv.* **2013**, *31*, 166–174.
17. Amoo, S.O.; Aremu, A.O.; Van Staden, J. In vitro plant regeneration, secondary metabolite production and antioxidant activity of micropropagated *Aloe arborescens* Mill. *Plant Cell Tiss. Org.* **2012**, *111*, 345–358. [CrossRef]
18. Jakovljević, D.Z.; Vasić, S.M.; Stanković, M.S.; Čomić, L.R.; Topuzović, M.D. Secondary metabolite content and in vitro biological effects of *Ajuga chamaepitys* (L.) Schreb subsp. *chamaepitys*. *Arch. Biol. Sci.* **2015**, *67*, 1195–1202.
19. Makowczyńska, J.; Sliwinska, E.; Kalemba, D.; Piątczak, E.; Wysokińska, H. In vitro propagation, DNA content and essential oil composition of *Teucrium scorodonia* L. ssp. *scorodonia*. *Plant Cell Tiss. Org.* **2016**, *127*, 1–13.
20. Vanisree, M.; Hsin-Sheng, T. Plant cell cultures—An alternative and efficient source for the production of biologically important secondary metabolites. *Int. J. Appl. Eng. Res.* **2004**, *2*, 29–48.
21. Shakeran, Z.; Keyhanfar, M.; Asghari, G.; Ghanadian, M. Improvement of atropine production by different biotic and abiotic elicitors in hairy root cultures of *Datura metel*. *Turk. J. Biol.* **2015**, *39*, 111–118. [CrossRef]
22. Rajak, A. Nanotechnology and its application. *J. Nanomed. Nanotechnol.* **2018**, *9*, 502. [CrossRef]
23. Das, M.; Shim, K.H.; An, S.S.A.; Yi, D. Review on gold nanoparticles and their applications. *J. Toxiol. Env.* **2012**, *3*, 193–205. [CrossRef]
24. Roco, M.C. Nanotechnology: Convergence with modern biology and medicine. *Curr. Opin. Biotechnol.* **2003**, *3*, 337–346. [CrossRef]
25. Nowack, B.; Bucheli, T.D. Occurrence, behavior and effects of nanoparticles in the environment. *Env. Pollut.* **2007**, *1*, 5–22. [CrossRef]
26. Rajmedevi, J.; Jeyasubramanian, K.; Marikani, A.; Rajakumar, G.; Rahuman, A.A. Synthesis and antimicrobial activity of copper nanoparticles. *Mater. Lett.* **2012**, *71*, 114–116.
27. Ahmed, J.; Gultekinoglu, M.; Edirsinghe, M. Bacterial cellulose micro-nano fibres for wound healing applications. *Biotechnol. Adv.* **2020**, *41*, 107549. [CrossRef]

28. Matharu, R.K.; Porwal, H.; Chen, B.; Ciric, L.; Edirsinghe, M. Viral filtration using carbon-based materials. *Med. Devices. Sens.* **2020**, *3*, e10107. [CrossRef]
29. Matharu, R.K.; Ciric, L.; Ren, G.; Edirsinghe, M. Comparative study of the antimicrobial effects of tungsten nanoparticles and tungsten nanocomposite fibres on hospital acquired bacterial and viral pathogens. *J. Nanomater.* **2020**, *10*, 1017. [CrossRef]
30. Panyala, N.R.; Pena-Mendez, E.M.; Havel, J. Gold and nano-gold in medicine: Overview, toxicology and perspectives. *J. Appl. Biomed.* **2009**, *7*, 75–91. [CrossRef]
31. Neira, T.R.; Carmora, E.; Recio, G.; Nesi, A.N.; Diaz, M.R.; Alberdi, M.; Rengel, Z.; Blancheteau, A.I. Metallic nanoparticles influence the structure and function of the photosynthetic apparatus in plants. *Plant. Psychiol. Biochem.* **2018**, *130*, 408–417. [CrossRef]
32. Alexis, F.; Pridgen, E.; Molnar, L.K.; Farokhzad, O.C. Factors affectin the clearance and biodistibution of polymeric nanoparticles. *Mol. Pharm.* **2008**, *5*, 505–515. [CrossRef]
33. Attarad, A.; Hira, Z.; Muhammad, Z.; Ihsan, U.H.; Abdul, R.P.; Joham, S.A.; Altaf, H. Sythesis, characterization, applications and challenges of iron oxide nanoparticles. *Nanotechnol. Sci. Appl.* **2019**, *9*, 49–67.
34. Jain, P.K.; Lee, K.S.; El-Sayed, I.H.; El-Sayed, M.A. Calculated absorption and acattering properties of gold nanoparticles of different size, shape, and composition: Applications in biological imaging and biomedicine. *J. Phys. Chem. B* **2006**, *110*, 7238–7248. [CrossRef]
35. Cheun-Yi, Y.; Ceran, B.; Rotello, V.M. Gold nanoparticles: Preparation, properties and applications in biotechnology. *Nanoscale* **2012**, *4*, 1871–1880.
36. Bhattacharya, R.; Mukherjee, P. Biological properties of "naked" metal nanoparticles. *Adv. Drug. Deliv. Rev.* **2008**, *11*, 1289–1306. [CrossRef]
37. Abdi, G.; Selehi, H.; Kosh-Khui, M. Noano silver: A novel nanomaterial for removal of bacterial contaminations in valerian (*Valeriana officinalis* L.) tissue culture. *Acta Physiol. Plant.* **2008**, *30*, 709–714. [CrossRef]
38. Mahna, N.; Vahed, S.Z.; Khani, S. Plant in vitro culture goes nano: Nanosilver-mediated decontamination of ex vitro explants. *J. Nanomed. Nanotechnol.* **2013**, *4*, 1. [CrossRef]
39. Wang, P.; Lombi, E.; Zhao, F.J.; Kopittke, P.M. Nanotechnology: A new opportunity in plant sciences. *Trends Plant. Sci.* **2016**, *21*, 699–721. [CrossRef]
40. Hussain, M.; Raja, N.I.; Mashwani, Z.; Iqbal, M.; Sabir, S.; Yasmeen, F. In vitro seed germination and biochemical profiling of *Artemisia absinthium* exposed to various metallic nanoparticles. *3 Biotech* **2018**, *130*, 408–417. [CrossRef]
41. Lee, W.M.; An, Y.J.; Yoo, H.; Kweon, H.S. Toxicity and bioavailibity of copper nanoparticles to the terrestrial plants mung been (Phaseolus radiatus) and wheat (Triticum aestivum): Plant agar test for water-insoluble nanoparticles. *Env. Toxicol. Chem.* **2008**, *27*, 1915–1921. [CrossRef] [PubMed]
42. Javed, R.; Yucesan, B.; Zia, M.; Gurel, E. Elicitation of secondary metabolites in callus cultures of Stevia rebaudiana Bertoni grown under ZnO and CuO nanoparticles stress. *Sugar Tech.* **2018**, *20*, 194–201. [CrossRef]
43. Asztemborska, M.; Steborowski, R.; Kowalska, J.; Bystrzejewska-Piotrowska, G. Accumulation of platinum nanoparticles by Sinapis alba and Lepidium sativum plants. *Water Air Soil Pollut.* **2015**, *226*, 126. [CrossRef] [PubMed]
44. Burman, U.; Saini, M.; Kumar, P. Effect of zinc oxide nanoparticles on growth and antioxidant system of chickpea seedlings. *Toxicol. Env. Chem.* **2013**, *95*, 605–612. [CrossRef]
45. Fraizer, T.P.; Burklew, C.E.; Zhang, B. Titanium dioxide nanoparticles affect the growth and microRNA expression of tobacco (*Nicotiana Tab.*). *Funct. Integr. Genom.* **2014**, *14*, 75–83. [CrossRef]
46. Sarmast, M.K.; Salehi, H. Silver nanoparticles: An influential element in plant nanobiotechnology. *Mol. Biotechnol.* **2016**, *58*, 441–449. [CrossRef]
47. Homaee, M.B.; Ehsanpour, A.A. Physiological and biochemical responses of potato (*Solanum tuberosum*) to silver nanoparticles and silver nitrate treatments under in vitro conditions. *Ind. J. Plant. Physiol.* **2015**, *20*, 353–359. [CrossRef]
48. Rani, P.U.; Yasur, J.; Loke, K.S.; Dutta, D. Effect of synthetic and biosynthesized silver nanoparticles on growth, physiology and oxidative stress of water hyacinth: Eichhornia crassipes (Mart) Solms. *Acta Physiol. Plant.* **2016**, *38*, 58. [CrossRef]
49. Arora, S.; Sharma, P.; Kumar, S.; Nayan, R.; Khanna, P.K.; Zaidi, M.G.H. Gold-nanoparticle induced enhancement in growth and seed yield of *Brassica Juncea*. *Plant. Growth Regul.* **2012**, *66*, 303–310. [CrossRef]

50. Nair, P.M.G.; Chung, I.M. Physiological and molecular level studies on the toxicity of silver nanoparticles in germinating seedlings of mung bean (*Vigna Radiata L.*). *Acta Physiol. Plant.* **2015**, *37*, 1719. [CrossRef]
51. Vecerova, K.; Vecera, Z.; Docekal, B.; Oravec, M.; Pompeiano, A.; Tríska, J.; Urban, O.; Pompiano, J. Changes of primary and secondary metabolites in barley plants exposed to CdO nanoparticles. *Environ. Pollut.* **2016**, *218*, 207–218. [CrossRef]
52. Golkar, P.; Moradi, M.; Garousi, A.G. Elicitation of stevia glycosides using salicylic acid and silver nanoparticles under callus culture. *Sugar Tech.* **2018**, *21*, 569–577. [CrossRef]
53. Jamshidi, M.; Ghanti, F.; Razaei, A.; Bemani, E. Change of antioxidant enzymes activity of hazel (*Corylus avellana* L.) cells by AgNPs. *Cytotechnology* **2014**, *68*, 525–530. [CrossRef]
54. Javed, R.M.; Usman, M.; Tabassum, S.; Zia, M. Effect of capping agents: Structural, optical and biological properties of ZnO nanoparticles. *Appl. Surf. Sci.* **2016**, *386*, 319–326. [CrossRef]
55. Lu, C.M.; Zhang, C.Y.; Wen, J.Q.; Tao, M.X. Research of the effect of nanometer materials on germination and growth enhancement of *Glycine max* and its mechanism. *Mater. Sci.* **2002**, *21*, 168–171.
56. Dimkpa, C.O.; McLean, J.E.; Latta, D.E.; Manangón, E.; Britt, D.W.; Johnson, W.P.; Boyanov, M.I.; Anderson, A.J. Cuo and ZnO nanoparticles: Phytotoxicity, metal speciation, and induction of oxidative stress in sand-grown wheat. *J. Nanopart. Res.* **2012**, *14*, 1–15. [CrossRef]
57. Shaw, A.K.; Hossain, Z. Impact of nano-Cuo stress on rice (*Oryza sativa* L.) seedlings. *Chemosphere* **2013**, *96*, 906–915. [CrossRef]
58. Gopinath, K.; Gowri, S.; Karthika, V.; Arumugam, A. Green synthesis of gold nanoparticles from fruit extract of *Terminalia arjuna* for the enhanced seed germination activity of Gloriosa superb. *J. Nanostructure Chem.* **2014**, *4*, 1–11. [CrossRef]
59. Ghosh, M.; Bandyopadhyay, M.; Mukherjee, A. Genotoxicity of titanium dioxide (TiO_2) nanoparticles at two trophic levels: Plant and human lymphocytes. *Chemosphere* **2010**, *81*, 1253–1262. [CrossRef]
60. Kumbhakar, D.V.; Datta, A.K.; Mandal, A.; Das, D.; Gupta, S.; Ghosh, B.; Halder, S.; Dey, S. Effectivity of copper and cadmium sulfide nanoparticles in mitotic and meiotic cells of *Nigella sativa* L. (black cumin)–can nanoparticles act as mutagenic agents? *J. Exp. Nanosci.* **2016**, *11*, 823–829. [CrossRef]
61. Pramanik, A.; Datta, A.K.; Gupta, S.; Ghosh, B.; Das, D.; Kumbhakar, D.V. Assessment of genotoxicity of engineered nanoparticles (cadmium sulphide-CdS and copper oxide-CuO) using plant model (*Coriandrum sativum* L.). *Int. J. Res. Pharm. Sci.* **2017**, *8*, 741–753.
62. Oukarroum, A.; Barhoumi, L.; Pirastru, L.; Dewez, D. Silver nanoparticle toxicity effect on growth and cellular viability of the aquatic plant *Lemna gibba*. *Env. Toxicol. Chem.* **2013**, *32*, 902–907. [CrossRef] [PubMed]
63. Jiang, H.S.; Qiu, X.N.; Li, G.B.; Li, W.; Yin, L.Y. Silver nanoparticles induced accumulation of reactive oxygen species and alteration of antioxidant systems in the aquatic plant *Spirodela polyrhiza*. *Env. Toxicol. Chem.* **2014**, *33*, 1398–1405. [CrossRef] [PubMed]
64. Gill, S.S.; Tuteja, N. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant. Physiol. Bioch.* **2010**, *48*, 909–930. [CrossRef]
65. Hatami, M.; Ghorbanpour, M. Defense enzymes activity and biochemical variations of *Pelargonium zonale* in response to nanosilver particles and dark storage. *Turk. J. Biol.* **2014**, *38*, 130–139. [CrossRef]
66. Chew, B.P.; Park, J.S. Carotenoid action on the immune response. *J. Nutr.* **2004**, *134*, 257S–261S. [CrossRef]
67. Mirzajani, F.; Askari, H.; Hamzelou, S.; Farzaneh, M.; Ghassempour, A. Effect of silver nanoparticles on *Oryza sativa L.* and its rhizosphere bacteria. *Ecotoxicol. Env. Saf.* **2013**, *88*, 48–54. [CrossRef]
68. Panda, K.K.; Achary, V.M.M.; Krishnaveni, R.; Padhi, B.K.; Sarangi, S.N.; Sahu, S.N.; Panda, B.B. In vitro biosynthesis and genotoxicity bioassay of silver nanoparticles using plants. *Toxicol. Vitr.* **2011**, *25*, 1097–1105. [CrossRef]
69. De, A.; Chakrabarti, M.; Ghosh, I. Evaluation of genotoxicity and oxidative stress of aluminium oxide nanoparticles and its bulk form in *Allium cepa*. *Nucleus* **2016**, *59*, 219–225. [CrossRef]
70. Rao, S.; Shekhawat, G.S. Phytotoxicity and oxidative stress perspective of two selected nanoparticles in *Brassica juncea*. *3 Biotech* **2016**, *6*, 244. [CrossRef]
71. Sharma, P.; Bhatt, D.; Zaidi, M.G.H.; Zaidi, M.G.H.; Saradhi, P.P.; Khanna, P.K.; Arora, S. Silver nanoparticle-mediated enhancement in growth and antioxidant status of *Brassica juncea*. *Appl. Biochem. Biotechnol.* **2012**, *167*, 2225–2233. [CrossRef]

72. Liu, F.; Jiang, H.; Ye, S.; Chen, W.P.; Liang, W.; Xu, Y. The Arabidopsis P450 protein CYP82C2 modulates jasmonate-induced root growth inhibition, defense gene expression and indole glucosinolate biosynthesis. *Cell Res.* **2010**, *20*, 539–552. [CrossRef] [PubMed]
73. Dai, Y.; Wang, Z.; Zhao, J.; Xu, L.; Yu, X.; Wei, Y.; Xing, B. Interaction of CuO nanoparticles with plant cells: Internalization, oxidative stress, electron transport chain disruption, and toxicogenomic responses. *Environ. Sci. Nano* **2018**, *5*, 2269–2281. [CrossRef]
74. Moharrami, F.; Hosseini, B.; Sharafi, A.; Farjaminezhad, M. Enhanced production of hyoscyamine and scopolamine from genetically trans-formed root culture of *Hyoscyamus reticulatus* L. elicited by iron oxide nanoparticles. *Vitr. Cell. Dev. Biol.-Plant* **2017**, *53*, 104–111. [CrossRef] [PubMed]
75. Zhang, B.; Zheng, L.P.; Li, W.Y.; Wang, J.W. Stimulation of artemisinin production in *Artemisia annua* hairy roots by Ag-SiO_2 core-shell nanoparticles. *Curr. Nanosci.* **2013**, *9*, 363–370. [CrossRef]
76. Sharafi, E.; Nekoei, S.M.K.; Fotokian, M.H.; Davoodi, D.; Mirzaei, H.H.; Hasanloo, T. Improvement of hypericin and hyperforin production using zinc and iron nano-oxides as elicitors in cell suspension culture of St John's wort (*Hypericum perforatum* L.). *J. Med. Plants By-Prod.* **2013**, *2*, 177–184.
77. Oloumi, H.; Soltaninejad, R.; Baghizadeh, A. The comparative effects of nano and bulk size particles of CuO and ZnO on glycyrrhizin and phenolic compounds contents in *Glycyrrhiza glabra* L. seedlings. *Indian J. Plant. Physiol.* **2015**, *20*, 157–161. [CrossRef]
78. Fazal, H.; Abbasi, B.H.; Ahmed, N.; Ali, S.S.; Shujait, A.S.; Akbar, F.; Kanwal, F. Correlation of different spectral lights with biomass accumulation and production of antioxidant secondary metabolites in callus cultures of medicinally important *Prunella vulgaris* L. *J. Photochem. Photobiol. B Bio.* **2016**, *159*, 1–7. [CrossRef]
79. Chung, I.; Rajakumar, G.; Thiruvengadam, M. Effect of silver nanoparticles on phenolic compounds production and biological activities in hairy root cultures of *Cucumis Anguria*. *Acta Biol. Hung.* **2018**, *69*, 97–109. [CrossRef]
80. Ali, A.; Mohamed, S.; Khan, M.A.; Raja, N.I.; Arif, M.; Kamil, A.; Mashwani, Z. Silver nanoparticles elicited in vitro callus cultures for accumulation of biomass and secondary metabolites in *Caralluma Tuberculate*. *Artif. Cells Nanomed. Biotechnol.* **2019**, *47*, 715–724. [CrossRef]
81. Kruszka, D.; Sawikowska, A.; Selvakesavan, R.K.; Krajewski, P.; Kachlicki, P.; Franklin, G. Silver nanoparticles affect phenolic and phytoalexin composition of *Arab Thaliana*. *Sci. Total Env.* **2020**, *716*, 135361. [CrossRef] [PubMed]
82. Jadczak, P.; Kulpa, D.; Bihun, M.; Przewodowski, W. Positive effect of AgNps and AuNps in in vitro cultures of *Lavandula angustifolia* Mill. *Tissue Organ. Cult.* **2019**, *139*, 191–197. [CrossRef]
83. Wesołowska, A.; Jadczak, P.; Kulpa, D.; Przewodowski, W. Gas chromatography mass spectrometry (GC-MS) analysis of essential oils from AgNps and AuNps elicited *Lavandula angustifolia* in vitro cultures. *Molecules* **2019**, *24*, 606. [CrossRef] [PubMed]
84. Akasaka-Kennedy, Y.; Yoshida, H.; Takathaka, Y. Efficient plant regeneration from leaves of rapeseed (*Brassica napus L.*): The influence of $AgNO_3$ and genotype. *Plant. Cell Rep.* **2006**, *24*, 649–654. [CrossRef]
85. Devi, G.H.; Suruthi, P.; Veerakumar, R.; Vinoth, S.; Subbaiya, R.; Chozhavendahn, S. A review on metallic gold and silver nanoparticles. *J. Pharm. Tech.* **2019**, *12*, 935–943. [CrossRef]
86. Aziz, S.G.G.; Aziz, S.G.G.; Akbarzadeh, A. Advances in silver nanotechnology: An update on biomedical applications and future perspectives. *Drug Res.* **2017**, *67*, 198–203. [CrossRef]
87. Colvin, V.L. The potential environmental impact of engineered nanomaterials. *Nat. Biotechnol.* **2003**, *21*, 1166–1170. [CrossRef]
88. Austin, L.A.; Mackey, M.A.; Dreaden, E.C.; El-Sayed, A.M. The optical, photothermal, and facile surface chemical properties of gold and silver nanoparticles in biodiagnostics, therapy, and drug delivery. *Arch. Toxicol.* **2014**, *88*, 1391–1417. [CrossRef]
89. Prociak, J.; Grabowska, A.; Chwastowski, J.; Majka, T.; Banach, M. Safety of the application of nanosilver and nanogold in topical cosmetic preparations. *Colloids Surf. B Biointerfaces* **2019**, *183*, 110416. [CrossRef]
90. Ovais, M.; Khalil, A.T.; Raza, A.; Ayaz, M.; Saravanan, M.; Ali, M.; Ahmed, I.; Shahid, M.; Shinwari, Z.K. Multifunctional theranostic applications of biocompatible green-synthesized colloidal nanoparticles. *Appl. Microbiol. Biotechnol.* **2018**, *102*, 4393–4408. [CrossRef]
91. Dhas, S.P.; Anbarasan, S.; Mukherjee, A.; Chandrasekaran, N. Biobased silver nanocolloid coating on silk fibers for prevention of post-surgical wound infections. *Int. J. Nanomed.* **2015**, *10*, 159–170.

92. Baygar, T. Characterization of silk sutures coated with propolis and biogenic silver nanoparticles (AgNPs); an eco-friendly solution with wound healing potential against surgical site infections (SSIs). *Turk. J. Med. Sci.* **2020**, *13*, 258–266.
93. Doughton, J.A.; Hofman, M.S.; Eu, P.; Hicks, R.J.; Williams, S. A first-in-human study of 68Ga-nanocolloid PET/CT sentinel lymph node imaging in prostate cancer demonstrates aberrant lymphatic drainage pathways. *J. Nucl. Med.* **2018**, *59*, 1837–1842. [CrossRef] [PubMed]
94. Rasilla, J.M.; Balbín, L.F.; Arboniés, J.C.; Elola-Olaso, A.M.; Delgado-Bolton, R.; Pereyra, L.I.; Rey, C.R.; Gutiérrez, L.L.; Maté, A.G.; Santamaría, J.M.R.; et al. SPECT-TAC: A new tool for localisation of sentinel lymph nodes in breast cancer patients. *Rev. Española Med. Nucl.* **2008**, *27*, 183–190.
95. Velmurugan, P.; Anbalagan, K.; Manosathyadevan, M.; Lee, K.J.; Cho, M.; Lee, S.M.; Park, J.H.; Oh, S.G.; Bang, K.-S.; Oh, B.T. Green synthesis of silver and gold nanoparticles using Zingiber officinale root extract and antibacterial activity of silver nanoparticles against food pathogens. *Bioprocess. Biosyst. Eng.* **2014**, *37*, 1935–1943. [CrossRef]
96. Carrillo-Inungaray, M.L.; Trejo-Ramirez, J.A.; Reyes-Munguia, A.; Carranza-Alvarez, C. Use of nanoparticles in the food industry: Advances and perspectives. In *Impact of Nanoscience in the Food Industry*; Academic Press: Cambridge, MA, USA, 2018; pp. 419–444.
97. Connor, E.E.; Mwamuka, J.; Gole, A.; Murphy, J.M.; Wyatt, M.D. Gold nanoparticles are taken up by human cells but do not cause acute cytotoxicity. *Small* **2005**, *1*, 325–327. [CrossRef]
98. Tiedemann, D.; Taylor, U.; Rehbock, C.; Jakobi, J.; Klein, S.; Kues, W.A.; Barcikowski, S.; Rath, D. Reprotoxicity of gold, silver, and gold-silver alloy nanoparticles on mammalian gametes. *Analyst* **2014**, *7*, 931–942. [CrossRef]
99. Sung, J.H.; Ji, J.H.; Park, J.D.; Song, M.Y.; Song, K.S.; Ryu, H.R.; Yoon, J.U.; Jeon, K.S.; Jeong, J.; Han, B.S.; et al. Subchronic inhalation toxicity of gold nanoparticles. *Part. Fibre Toxicol.* **2011**, *8*, 16. [CrossRef]
100. Pan, Y.; Neuss, S.; Leifert, A.; Fischler, M.; Wen, F.; Simon, U.; Schmid, G.; Brandau, W.; Jahnen-Dechent, W. Size-dependent cytotoxicity of gold nanoparticles. *Small* **2007**, *3*, 1941–1949. [CrossRef]
101. Sharma, V.K.; Siskova, K.M.; Zboril, R.; Gardea-Torresdey, J.L. Organic-coated silver nanoparticles in biological and environmental conditions: Fate, stability and toxicity. *Adv. Colloid Interface Sci.* **2014**, *204*, 15–34. [CrossRef]
102. Harish, K.K.; Nagasmay, V.; Himangshu, B.; Anuttam, K. Metallic nanoparticles: A review. *Biomed. J. Sci. Tech. Res.* **2018**, *4*, 3765–3775.
103. Mehrian, S.K.; Heidari, R.; Rahmani, F. Effect of chemical synthesis silver nanoparticles on germination indices and seedlings growth in seven varieties of *Lycopersicon esculentum* mill (tomato) plants. *J. Clust. Sci.* **2016**, *27*, 327–340. [CrossRef]
104. Barbasz, A.; Kreczmer, B.; Ocwieja, M. Effects of exposure of callus cells of two wheat varieties to silver nanoparticles and silver salt ($AgNO_3$). *Acta Physiol. Plant.* **2016**, *38*, 76. [CrossRef]
105. Iqbal, M.; Raja, N.I.; Ali, A.; Rashid, H.; Hussain, M.; Ejaz, M.; Iqbal, R.; Khan, U.A.; Shaheen, N.; Rauf, A.; et al. Effect of silver nanoparticles on growth of wheat under heat stress. *Iran. J. Sci. Technol. Trans. A Sci.* **2017**, *43*, 387–395. [CrossRef]
106. Kim, S.; Lee, I. Alteration of phytotoxicity and oxidant stress potential by metal oxide nanoparticles in *Cucumis sativus*. *Water Air Soil Pollut.* **2012**, *223*, 2799–2806. [CrossRef]
107. Gunjan, B.; Zaidi, M.G.H.; Sandeep, A. Impact of gold nanoparticles on physiological and biochemical characteristics of *Brarica juncea*. *J. Plant. Physiol. Biochem.* **2014**, *2*, 133.
108. Tripathi, D.K.; Singh, S.; Singh, S.; Srivastava, P.K.; Singh, V.P.; Singh, S. Nitric oxide alleviates silver nanoparticles (AgNps)-induced phytotoxicity in *Pisum sativum* seedlings. *Plant. Physiol. Biochem.* **2017**, *110*, 167–177. [CrossRef]
109. Miliauskas, G.; Venskutonis, P.R.; Beek, T.A. Screening of radical scavenging activity of some medicinal and aromatic plant extracts. *Food Chem.* **2004**, *85*, 231–237. [CrossRef]
110. Demissie, Z.A.; Sarker, L.S.; Mahmoud, S.S. Cloning and functional characterization of β-phellandrene synthase from *Lavandula angustifolia*. *Planta* **2011**, *233*, 685–696. [CrossRef]
111. Wornouk, G.; Demisse, Z.; Rheut, M.; Mahmoud, S. Biosythesis and therapuetic properties of lavandula essential oil constituent. *Planta Med.* **2011**, *77*, 7–15. [CrossRef]

112. Boudet, A.M. Evolution and current status of research in phenolic compounds. *Phytochemistry* **2007**, *68*, 2722–2735. [CrossRef] [PubMed]
113. Ainsworth, E.; Gillespie, K. Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin-Ciocalteu reagent. *Nat. Protoc.* **2007**, *2*, 875–877. [CrossRef] [PubMed]
114. Sanchezz-Rangel, J.C.; Benavides, J.; Heredia, J.B.; Cisneros-Zevallos, L.; Jacobo-Velazquez, D.A. The Folin-Ciocalteu assay revisited: Improvement of its specificity for total phenolic content determination. *Anal. Methods* **2013**, *5*, 5990–5999. [CrossRef]
115. Tian, H.; Ghorbanpour, M.; Kariman, K. Manganese oxide nanoparticle-induced changes in growth, redox reactions and elicitation of antioxidant metabolites in deadly nightshade (*Atropa Belladonna* L.). *Ind. Crop. Prod.* **2018**, *126*, 403–414. [CrossRef]
116. Krishnaraj, C.; Geneshan, J.E.; Rajan, R.; Abirami, S.M.; Mohan, N.; Kalaichelvan, P.T. Effect of biologically synthesized silver nanoparticles on *Bacopa monnieri* (Linn.) Wettst. Plant growth metabolism. *Process. Biochem.* **2012**, *47*, 651–658. [CrossRef]
117. Homaee, M.B.; Ehsanpour, A.A. Silver nanoparticles and silver ions: Oxidative stress responses and toxicity in potato (*Solanum tuberosum* L.) grown in vitro. *Hortic. Environ. Biotechnol.* **2015**, *57*, 544–553. [CrossRef]
118. Yasur, J.; Rani, P.U. Environmental effects of nanosilver: Impact on castor seed germination, seedling growth, and plant physiology. *Environ. Sci. Pollut. Res.* **2013**, *20*, 8636–8648. [CrossRef]
119. Jamshidi, M.; Ghanati, F. Taxanes content and cytotoxicity of hazel cells extract after elicitation with silver nanoparticles. *Plant. Physiol. Biochem.* **2017**, *110*, 178–184. [CrossRef]
120. Ghorbanpour, M.; Hadian, J. Multi-walled carbon nanotubes stimulate callus induction, secondary metabolites biosynthesis and antioxidant capacity in medicinal plant *Satureja khuzestanica* grown in vitro. *Carbon* **2015**, *94*, 749–759. [CrossRef]
121. Saha, N.; Gupta, S.D. Promotion of shoot regeneration of *Swertia chirata* by biosynthesized silver nanoparticles and their involvement in ethylene interceptions and activation of antioxidant activity. *Plant. Cell Tissue Organ. Cult.* **2018**, *134*, 89–300. [CrossRef]
122. García-Sánchez, S.; Bernales, I.; Cristobal, S. Early response to nanoparticles in the Arabidopsis transcriptome compromises plant defence and root-hair development through salicylic acid signaling. *BMC Genom.* **2015**, *16*, 341. [CrossRef] [PubMed]
123. Kaveh, R.; Li, Y.S.; Ranjbar, S.; Tehrani, R.; Brueck, C.L.; Van Aken, B. Changes in Arabidopsis thaliana gene expression in response to silver nanoparticles and silver ions. *Environ. Sci. Technol.* **2013**, *18*, 10637–10644. [CrossRef] [PubMed]
124. Nair, P.M.G.; Chung, I.M. Physiological and molecular level effects of silver nanoparticles exposure in rice (*Oryza sativa* L.) seedlings. *Chemosphere* **2014**, *112*, 105–113. [CrossRef]
125. Murashige, T.; Skoog, F. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plant.* **1962**, *15*, 473–497. [CrossRef]
126. Andrys, D.; Kulpa, D. In vitro propagation affects the composition of narrow-leaved lavender essential oils. *Acta Chromat.* **2018**, *30*, 225–230. [CrossRef]
127. Turkevich, J.; Stevenson, P.C.; Hillier, J. A study of the nucleation and growth processes in the synthesis of colloidal gold. *Discuss. Faraday Soc.* **1951**, *11*, 55–75. [CrossRef]
128. Liu, F.K.; Ker, C.J.; Chang, Y.C.; Ko, F.H.; Chu, T.C.; Dai, B.T. Microwave heating for the preparation of nanometer gold particles. *J. Appl. Physic.* **2003**, *42*, 4152–4158. [CrossRef]
129. Chance, B.; Maehly, A.C. Assay of catalase and peroxidases. *Method Enzym.* **1995**, *2*, 764–775.
130. Nakano, Y.; Asada, K. Hydrogen peroxide is scavenged by ascorbate specific peroxidase in spinach chloroplasts. *Plant. Cell Physiol.* **1981**, *22*, 867–880.
131. Beauchamp, C.; Fridovich, I. Superoxide dismutase: Improved assay and an assay applicable to acrylamide gels. *Anal. Biochem.* **1971**, *44*, 276–287. [CrossRef]
132. Li, Y.; Schellhorn, H.E. Rapid kinetic microassay for catalase activity. *J. Biomol. Tech.* **2007**, *18*, 185–187. [PubMed]
133. Anastasiadi, M.; Pratsinis, H.; Kletsas, D.; Skaltsounis, A.L.; Haroutounian, S.A. Bioactive non-coloured polyphenols content of grapes, wines and vinification by-products: Evaluation of the antioxidant activities of their extracts. *Food Res. Int.* **2010**, *43*, 805–813. [CrossRef]

134. Shi, F.; Jia, X.; Zhao, C.; Chen, Y. Antioxidant activities of various extracts from *Artemisisa selengensis* turcz (LuHao). *Molecules* **2010**, *15*, 4934–4946. [CrossRef] [PubMed]

Sample Availability: Samples of the compounds are not available from the authors.

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

DOI: 10.21005/AAPZ2020.56.4.01

FOLIA POMERANAE UNIVERSITATIS TECHNOLOGIAE STETINENSIS

Folia Pomer. Univ. Technol. Stetin., Agric., Aliment., Pisc., Zootech. 2020, 357(56)4, 5–18

Received 19 Jun 2020
Revised 21 Oct 2020
Accepted 31 Oct 2020

Paula JADCZAK, Danuta KULPA

Lavandula angustifolia PROPAGATED IN *IN VITRO* CULTURES ON MEDIA CONTAINING AgNPs AND AuNPs – AN ALTERNATIVE TO SYNTHETIC PRESERVATIVES IN COSMETICS

Department of Genetics, Plant Breeding and Biotechnology, West Pomeranian University of Technology, Szczecin, Poland

Abstract. We determined the preservation properties of *Lavandula angustifolia* propagated on media with gold or silver nanoparticles with a particle size of 13 and 30 nm. Cosmetic emulsions prepared by using lavender tissue that was propagated on media containing AuNPs and AgNPs showed increased preservative capacities when compared with the control ones. In the case of control cosmetic emulsions, which had no added plant tissues or dehydroacetic acid and benzoic acid (DHA BA), bacterial and fungal colonies appeared after the second week of the experiment. The addition of lavender tissue propagated on media without AuNPs or AgNPs protected the tasted samples from microbial contamination; in this case, bacterial contamination was detected after 4 weeks and fungal contamination after 6 weeks. The addition of lavender tissue propagated on medium containing AgNPs with a particle size of 13 nm at a concentration of 1 mg · dm^{-3} prolonged the time of detection of bacteria colonies to 8 weeks (0.9) and this result was close and comparable to the effect of DHA BA. Higher concentrations of AgNPs in the culture medium, as well as a larger particle diameter (30 nm), resulted in the decreased preservative capacity of plant tissues. The presence of AuNPs in the culture media showed a positive effect on the antimicrobial activity of lavender; however, to a lesser degree than in the case of AgNPs. Disintegrated fragments of lavender tissue propagated on media containing 1 mg · dm^{-3} AgNPs with particle size of 13 nm can be used to preserve short shelf life cosmetic emulsions.

Key words: cosmetic emulsion, metal nanoparticles, silver, gold, micropropagation, elicitation.

INTRODUCTION

The global cosmetic production is one of the most rapidly developing personal care industries. By the end of 2022, the global cosmetic market is expected to reap a profit of approximately $429.8 billion, registering a compound annual growth rate (CAGR) of 4.3% during the forecast period 2016–2022 (Allied Market Research 2016). The fulfillment of increasing market demand requires continuous multidimensional control, namely, to monitor toxic ingredients and microbial contamination (Halla et al. 2018). Inhibiting the growth of microorganisms is one of the most important aspects of production of cosmetics. Microbial contamination of a cosmetic product decreases its shelf life and increases the chances of potential infection to the consumer. The application of a contaminated product might cause

Corresponding author: Danuta Kulpa, Department of Genetics, Plant Breeding and Biotechnology, West Pomeranian University of Technology in Szczecin, Juliusza Słowackiego 17, 71-434 Szczecin, Poland, e-mail: Danuta.Kulpa@zut.edu.pl

irritation to the skin and/or damage the skin thereby threatening the consumer's health. Furthermore, it may contribute to the spread and development of various infections (Groot et al. 1995). Furthermore, microbial contamination of a cosmetic product leads to the decomposition of the active ingredients contained therein, which might decrease its therapeutic activity (Behravan et al. 2005). Modern-day cosmeceuticals contain water, which is the perfect environment for the microorganisms to grow. Microbial contamination in cosmetics and in personal care products might occur during the process of manufacturing or during their use by the customer. In general, these products are exposed to temperature variations. Furthermore, once a cosmetic product is opened, it is highly possible, until it is discarded, that the contamination is introduced or accelerated by consumer use (e.g. by dipping with nonsterile fingers and contact with nonsterile bodies) (Kerdudo et al. 2016). Therefore, it is impossible to mass-produce cosmetics free of preservatives.

According to the definition provided by the European Parliament and Council of the European Union, a preservative is a substance of natural origin or is synthetic, which when added to a cosmetic product inhibits the development of microorganisms during the production stage and prevents repeated contamination in the later stages of its use. Numerous studies have indicated that the presence of chemical preservatives in cosmetics cause allergies and skin diseases (Orton et al. 2004; Mario et al. 2012). Some of the synthetic preservatives available in the market are considered carcinogenic or mutagenic. Recent studies have confirmed that parabens cause breast cancer and affect the development of malignant melanoma (Golden et al. 2005). There are more than 12,000 synthetic chemicals that are used in the manufacture of cosmetics, among which less than 20% are considered completely safe (O'Dell et al. 2016). World Health Organization regulates the use of approved synthetic chemicals by the cosmetic industries thereby considerably reducing the number of chemicals being used during the production. Therefore, in recent years, there is an increasing demand for natural cosmetics that contain at least 95% of substances derived from natural origin (Fonseca-Santos et al. 2015).

Many companies prefer to use natural alternatives instead of synthetic chemicals in their cosmetics. Chemicals of plant origin act as active ingredients (e.g. moisturizers), excipients (e.g. surfactants), and additives (e.g. preservatives) in a single formula (Kusumawati et al. 2013; Kerdudo et al. 2016; Andrys et al. 2018). Because of the antimicrobial and antifungal nature of plant-based essential oils, cosmetics such as creams, gels, and ointments do not necessarily require addition of chemical preservatives if they already contain essential oils or those that contain a single compound as an active agent (Sticher et al. 2015).

Narrow leaf lavender (*Lavandula angustifolia*) (family *Lamiaceae*), originating from the Mediterranean region, has been successfully used in the cosmetic industry as an antimicrobial agent in various cosmetics and in personal care products (Upson et al. 2004). This ornamental and medicinal plant is considered one of the most valuable species because of its widespread use in the perfume, flavoring, cosmetics, and soap industries. Its use can be dated back to ancient Rome and Greece (Wornouk et al. 2011; Brailko et al. 2017). Apart from being used in aromatherapy, lavender is also used in the pharmaceutical industry and in medicine. Due to the characteristic aroma and antiseptic properties of the essential oil, it has been used in the production of cleaning agents (e.g. soaps and shampoos) and perfumes (Cavanagh et al. 2005). Lavender oil shows antimicrobial and antifungal activity; its effect has been

demonstrated in some of the antibiotic-resistant bacteria (Schwiertz et al. 2006). Lavender oil has been shown to be effective against *Staphylococcus aureus*, *Enterococcus faecalis* (Cavanagh et al. 2005), *Candida albicans* (D´auria et al. 2005), and *Botrytis cincerea* (Adam et al. 1998). Because of its antimicrobial property, lavender oil is used to alleviate skin inflammation caused by acne and psoriasis vulgaris. Furthermore, lavender oil has been used to treat wounds, burns, and purulent-states (Jopke et al. 2017).

The composition of the essential oil mainly depends on, among others, the plant's genotype, environmental conditions, and tissue propagation methods (Demissie et al. 2011). Tissue culture is a method of obtaining a high amount of tissues of medicinal plants for the production of secondary metabolites (Gonçalves et al. 2013). Through *in vitro* cultures, it is possible to produce genetically identical individuals that are free from contaminants, which are of particular importance. Medicinal plants propagated through *in vitro* techniques are characterized by a unique composition of essential oils than that of the plants cultivated on fields (Amoo et al. 2012; Jakowijević et al. 2015). The addition of elicitors (i.e. stress-inducing factors) to the media results in a considerable change in the composition of secondary metabolites, including essential oils (Wesołowska et al. 2019). The change in the composition of essential oils and other bioactive compounds may influence, among others, the change in the antioxidative or microbiological activity of essential oils isolated from them. *L. angustifolia* (Andrys et al. 2018), *Ocimum basilicum* (Bais et al. 2002; Złotek et al. 2016), *Dionaea muscipula* and *Dionaea campensis* (Królicka et al. 2008), and *Coleus blumeli* (Szabo et al. 1999) cultures have shown increased antimicrobial activity under the influence of elicitor in the culture media.

The latest, currently studied elicitors are metal nanoparticles (NPs) (Vanisree et al. 2004; Shakeran et al. 2015; Moharrami et al. 2017; Golkar et al. 2019). It has been proven that metal NPs are highly reactive toward plants, and due to their small size, they can easily penetrate the cell membrane and get accumulated intracellularly (Jamshidi et al. 2014). Wesołowska et al. (2019) demonstrated that the accumulation of nanoparticles in tissues of plants propagated through *in vitro* culture technique, depending on, among others, particle size and diameter, may contribute to increased antimicrobial activity.

Typically, essential oils of various plants are used as preservatives in cosmetics (Aburjai et al. 2003; Nostro et al. 2004; Herman et al. 2013). Fresh tissues and dried plants are far less commonly used as preservatives. This might be because of the contamination of the material growing in natural conditions, low content of secondary metabolites, and process issues associated with the usage, such as problems with disintegration due to the presence of high content of phloem. Sterile plant tissues without the thick epidermal tissue, plant tissue propagated through *in vitro* techniques, those with a high content of secondary metabolites may be an alternative to plants growing under natural conditions, as long as they provide a suitable level of sterility to the products. Therefore, in this study, we aimed to verify the preservative properties of disintegrated true lavender tissues propagated *in vitro* on media enriched with gold and/or silver nanoparticles (AuNPs and AgNPs, respectively) with a different particle size.

MATERIAL AND METHODS

In vitro cultures

Narrow leaf lavender (*Lavandula angustifolia* cultivar Munstead) constituted the plant material. Shoot explants were placed in the media with a mineral composition according to Murashige and Skoog (1962) containing 2 mg · dm^{-3} kinetin (KIN), 0.2 mg · dm^{-3} indole-3-acetic acid (IAA), and 1 or 10 mg · dm^{-3} AgNPs or AuNPs with the particle size of 13 or 30 nm (NPIN Poland). Lavender tissue grown on media that did not contain AuNPs or AgNPs constituted the control sample. The pH of the medium was set to 5.7 by using 0.1 M solutions of HCl and NaOH. To the media, 7 g · dm^{-3} of agar, 30 g · dm^{-3} of sucrose, and 0.1 g · dm^{-3} of inositol were added. Subsequently, the media were subjected to 20-minute sterilization in an autoclave at 121°C and a pressure of 1 atm. Tissue propagation was conducted in 200 mL glass jars containing 20 mL medium. At the culture initiation stage, jars with plants were placed in a growth chamber at a temperature of 24°C and relative humidity of 70–80%. The cultures were illuminated with fluorescent light at an intensity of 40 PAR ($\mu E \cdot m^{-2} \cdot s^{-1}$) for 16 h per day After 28 days of culture growth, the experiment was completed, and the tissues of the propagated plants were used to prepare cosmetic emulsions after disintegration in liquid nitrogen.

Preparation of cosmetic emulsions

Ingredients of the aqueous and oil phase were prepared in separate beakers. The oil phase consisted of 18 g lanolin, 12 g beeswax, 14 g shea butter, and 88 mL of jojoba oil. In order to prepare the aqueous phase, 6 g D-panthenol 75%, 6.4 g glycerin, and 68 mL water were added to a beaker. Both beakers with contents were tightly closed with aluminum foil and were placed in a water bath, where they were kept until all oil-phase ingredients were dissolved. The entire oil phase was added to the beaker containing aqueous phase which was placed in the water bath. Subsequently, the contents of the beaker were stirred for about 1 min, after which the beaker was removed from the water bath. Stirring was continued until the cream reached a temperature of 40°C.

The cosmetic emulsions were divided into 5 mL portions and were supplemented with the dehydroacetic acid and benzoic acid (DHA BA) as the chemical preservative or the material obtained from tissue propagation. During the study, 12 types of cosmetic emulsions were prepared (Table 1). To each of the variants, 0.1 g fresh plant tissue, which was ground in liquid nitrogen, was added, which constituted 2% plant material. The negative control contained 12.5 µL of DHA BA (Esent, Poland), containing 7% Dehydroacetic Acid (DHA) and 83% Benzyl Alcohol (BA) as the preservative, which constituted to 0.25%. The final cosmetic emulsions were placed in sterile Petri dishes and stored in a refrigerator maintained at 4°C. Emulsions without the addition of preservatives and plant tissues constituted the control samples. One of the control samples was stored in a refrigerator, and the other was stored at 24°C.

Microbial purity test

After a period of 4 weeks, 0.1 g of cosmetic emulsion was dissolved with 0.9 mL of saline using Vortex (IKA). Subsequently, 0.1 mL of the test material was placed on a microbiological

medium and a surface culture test was performed. For this experiment, Sabouraud medium with chloramphenicol and Brain Heart Infusion Agar medium (BHI) (Biomaxima SA, Poland) were used.

Table 1. Types of cosmetic emulsions and the preservatives added to them

Designation	Type and concentration of the preservative	Storage temperature [°C]
Control 24°C	None	24°C
Control 4°C	None	4°C
Control DHA BA	0.25% DHA BA	
Variant 1	2% of lavender tissue propagated on media without the addition of nanoparticles	
Variant 2	2% of lavender tissue propagated on media with addition of 1 mg · dm^{-3} AgNPs, size 13 nm	
Variant 3	2% of lavender tissue propagated on media with addition of 10 mg · dm^{-3} AgNPs, size 13 nm	
Variant 4	2% of lavender tissue propagated on media with addition of 1 mg · dm^{-3} AgNPs, size 30 nm	
Variant 5	2% of lavender tissue propagated on media with addition of 10 mg · dm^{-3} AgNPs, size 30 nm	
Variant 6	2% of lavender tissue propagated on media with addition of 1 mg · dm^{-3} AuNPs, size 13 nm	
Variant 7	2% of lavender tissue propagated on media with addition of 10 mg · dm^{-3} AuNPs, size 13 nm	
Variant 8	2% of lavender tissue propagated on media with addition of 1 mg · dm^{-3} AuNPs, size 30 nm	
Variant 9	2% of lavender tissue propagated on media with addition of 10 mg · dm^{-3} AuNPs, size 30 nm	

Explanations: AuNPs – gold nanoparticles, AgNPs – silver nanoparticles.

Each culture test was performed with six replicates. Cultures on the BHI medium were incubated at 37°C and the cultures on the Sabouraud medium were incubated at 25°C. The cultures were incubated for up to 24 h (bacteria) and 48 h (fungi). After this, the colonies were counted.

Statistical analyses

The results of all the experiments were statistically analysed using one-way analysis of variance. To evaluate the significance of the differences between treatments, Tukey's test was performed at $p = 0.05$.

RESULTS

Microbial contamination may occur during the manufacturing process (primary contamination) and/ or during consumer use (secondary contamination) (Mitsui T. 1997). Most cosmetic products are multi-use products that are also required to maintain low levels of contamination during consumer use, which means that their preservation systems need to be effective against contaminants that come in contact with the product after opening. An ideal preservative should protect the product from microbial contamination, both in its original closed packaging until use and in an open container throughout its use (Pitt et al. 2015). To estimate the level of microorganisms in a sample of a cosmetic product, it is required to select the appropriate conditions of each culture (e.g. culture medium, dilution, temperature, and period of incubation). These conditions encourage the microorganisms to grow, resulting in the inactivation of the preservative system present in the sample (Office of Regulatory Affairs, 2015). In this study, we investigated the preservative properties of true lavender tissue that was cultivated *in vitro* on media supplemented with AuNPs or AgNPs at a particle size of 13 and 30 nm and ground in liquid nitrogen. Tables 2 and 3 present the results of this analysis.

In this study, only the addition of synthetic preservative (DHA BA) resulted in complete inhibition of growth of bacterial and fungal colonies for up to 8 weeks of the experiment. The most rapid development of microorganisms was observed for control cosmetic emulsions, to which no plant tissues and DHA BA were added.

Table 2. Number of bacterial colonies in tested cosmetic emulsions per dish (±SD)

Cream preservation method			Storage temperature	Bacterial colony count/dish			
Preservative	Type and concentration of NPs in the medium			2 weeks	4 weeks	6 weeks	8 weeks
None			24°C	$0.6^{b} \pm 0.47$	$2.3^{b} \pm 0.47$	$4.5^{b} \pm 0.71$	$20.9^{b} \pm 0.96$
None			4°C	$5.2^{a} \pm 0.68$	$14.7^{a} \pm 1.37$	$35.4^{a} \pm 2.06$	$62.3^{a} \pm 1.97$
DHA BA				0^{c}	0^{c}	0^{e}	0^{f}
2% tissue of *in vitro* plant	Control medium without NPs			0^{c}	0^{c}	$3.5^{bcd} \pm 0.32$	$8.5^{c} \pm 0.75$
	13 nm AgNPs	1 mg · dm^{-3}		0^{c}	0^{c}	0^{e}	$0.9^{ef} \pm 0.69$
		10 mg · dm^{-3}		0^{c}	0^{c}	$1.4^{de} \pm 0.47$	$3.9^{de} \pm 1.07$
	30 nm AgNPs	1 mg · dm^{-3}		0^{c}	0^{c}	$1.8^{cde} \pm 0.68$	$4.5^{de} \pm 0.96$
		10 mg · dm^{-3}		0^{c}	0^{c}	$2.2^{bcde} \pm 0.37$	$4.9^{d} \pm 1.34$
	13 nm AuNPs	1 mg · dm^{-3}		0^{c}	0^{c}	$3.0^{bcd} \pm 0.57$	$7.1^{cd} \pm 1.21$
		10 mg · dm^{-3}		0^{c}	0^{c}	$2.9^{bcd} \pm 0.69$	$6.1^{cd} \pm 1.21$
	30 nm AuNPs	1 mg · dm^{-3}		0^{c}	0^{c}	$4.5^{b} \pm 0.74$	$.6.9^{cd} \pm 1.57$
		10 mg · dm^{-3}		0^{c}	0^{c}	$4.0^{bc} \pm 0.58$	$5.5^{d} \pm 1.21$
LSD $_{0,05}$				0.10	0.12	2.35	2.31

Explanations: NPs – nanoparticles, AuNPs – gold nanoparticles, AgNPs – silver nanoparticles, LSD – least significant difference, DHA BA – dehydroacetic acid and benzoic acid.

A significant effect of temperature on the appearance of bacterial colonies was observed in the case of samples collected from variants stored at 24°C than those stored at 4°C. After 8 weeks of storage at 24°C, the samples showed a three-fold greater count of bacterial colonies (62.3) than those stored at 4°C (20.9). However, storage temperature did not have any effect on the count of fungal colonies – in the case of variants stored at 24°C, 5.2 colonies were observed, whereas in the case of one stored at 4°C, 5.8 colonies were observed.

Table 3. Number of fungal colonies in tested cosmetic emulsions per dish (±SD)

Cream preservation method			Storage temperature	Fungal colony count/dish			
Preservative	Type and concentration of NPs in the medium			2 weeks	4 weeks	6 weeks	8 weeks
None			24°C	$0.2^{a} \pm 0.37$	$0.2^{b} \pm 0.37$	$0.3^{c} \pm 0.47$	$5.8^{a} \pm 2.19$
None			4°C	$0.2^{a} \pm 0.37$	$2.1^{a} \pm 1.34$	$4.5^{a} \pm 1.80$	$5.2^{a} \pm 1.34$
DHA BA				0^{b}	0^{b}	0^{d}	0^{e}
2% tissue of *in vitro* plant	Control medium without NPs			0^{b}	0^{b}	0^{d}	$2.5^{b} \pm 0.37$
	13 nm AgNPs	1 mg · dm^{-3}		0^{b}	0^{b}	0^{d}	$0.7^{e} \pm 0.47$
		10 mg · dm^{-3}		0^{b}	0^{b}	$0.2^{cd} \pm 0.37$	$0.9^{e} \pm 0.81$
	30 nm AgNPs	1 mg · dm^{-3}		0^{b}	0^{b}	$0.3^{bc} \pm 0.47$	$1.3^{de} \pm 1.37$
		10 mg · dm^{-3}		0^{b}	0^{b}	0^{d}	$1.1^{e} \pm 1.11$
	13 nm AuNPs	1 mg · dm^{-3}		0^{b}	0^{b}	$0.6^{b} \pm 0.47$	$2.9^{b} \pm 1.06$
		10 mg · dm^{-3}		0^{b}	0^{b}	0	$1.9^{cd} \pm 1.67$
	30 nm AuNPs	1 mg · dm^{-3}		0^{b}	0^{b}	0^{d}	$2.7^{b} \pm 2.67$
		10 mg · dm^{-3}		0^{b}	0^{b}	$0.2^{d} \pm 0.01$	$3.1^{b} \pm 2.41$
LSD $_{0,05}$				0.01	0.05	0.16	0.61

Explanations: NPs – nanoparticles, AuNPs – gold nanoparticles, AgNPs – silver nanoparticles, LSD – least significant difference, DHA BA – dehydroacetic acid and benzoic acid.

Addition of lavender tissue propagated on control media that did not contain AuNPs or AgNPs protected the samples against the development of bacteria for 4 and fungi for 6 weeks. After 8 weeks of storage, these variants showed 9.5 bacterial and 1.2 fungal colonies.

In the case of cosmetic emulsions supplemented with tissue-propagated lavender on media enriched with NPs, the appearance of bacterial colonies was observed after 6 weeks of storage. An exception was the variant containing lavender tissue propagated in medium containing 1 mg · dm^{-3} AgNPs with a particle size 13 nm; in this case only a few colonies were observed after 8 weeks of storage (0.9). This result is comparable to the variant preserved with synthetic preservative – DHA BA. In the remaining variants, i.e. lavender tissue propagated on media containing AuNPs or AgNPs, the count of bacterial colonies determined after 8 weeks of storage ranged between 3.9 and 7.1.

In the case of fungal colonies, lavender tissue propagated on media containing AuNPs or AgNPs, inhibited their development up to 4 weeks of storage (independently of the particle size and type). However, after 6 weeks of storage, the appearance of fungal colonies was detected in cosmetic emulsions containing lavender tissue propagated on media that did not contain AuNPs or AgNPs. Fungal colonies were also detected in cultures enriched with 10 mg · dm^{-3} AgNPs with a particle size of 13 nm, 1 mg · dm^{-3} with a particle size of 30 nm AgNPs, and in 10 mg · dm^{-3} AuNPs with a particle size of 13 and 30 nm (Table 3). After 8 weeks of incubation, fungal colonies were observed in samples collected from all the tested variants. The lowest colony count was determined for variants containing lavender tissue propagated on media containing 1 and 10 mg · dm^{-3} AgNPs with a particle size of 13 nm, which was only 0.7 and 0.9 colonies, respectively.

A comparison of antimicrobial activity of plant tissue propagated on media containing AgNPs and AuNPs showed that lavender tissues propagated on media containing AgNPs showed higher activity than that of AuNPs.

DISCUSSION

In this study, we conducted experiments to determine the possibility of using true lavender tissue propagated on media containing AuNPs or AgNPs as preservatives for cosmetic emulsions. According to our results, the tissue-propagated lavender demonstrated preservative properties. Tissue propagated in the absence of NPs ground in liquid nitrogen and added to cosmetic emulsion protected the cosmetic from the development of microorganisms for up to 4 weeks. Andrys et al. (2018) studied three cultivars of *L. angustifolia* and demonstrated that plants propagated in *in vitro* cultures are characterised by a comparatively higher antimicrobial and antioxidative activity than those cultivated in field conditions. This is probably linked to the content of essential oils in their tissues. Muyima et al. (2002) studied the essential oils of *L. officinalis* plants and demonstrated their efficiency in the reduction of microbiological contamination in an aqueous cream formulation. The bacterial population was markedly restricted up to day 7 of the experiments. Herman et al. (2013) compared the efficacy of synthetic versus essential oils toward inhibition of growth of microorganisms. Their results showed a higher inhibitory activity by essential oils against microorganisms than that of synthetic preservatives in cosmetic emulsion. They tested the essential oils obtained from *L. officinalis*, *Melaleuca alternifolia*, and *Cinnamonium zeylannicum* and methylparaben as the preservative. Our study provides further information on the possibility of using essential oils as preservatives instead of synthetic preservatives.

Lavender tissue propagated on media containing 1 mg · dm^{-3} AgNPs with a particle size of 13 nm was characterised by higher antimicrobial activity than that of control. The bacterial contamination was detected after 6 weeks of incubation, and the inhibition was found to be marked for up to 8 weeks. Kim et al. (2006) demonstrated that methyl jasmonate sprayed on *Ocimum basilicum* L. plants significantly increased the content of rosmarinic acid and caffeic acid, as well as it increased eugenol and linalool. These compounds show strong antimicrobial and antioxidant activity. Poulev et al. (2003) showed a powerful effect of elicitation of stress on increasing the antibacterial and antifungal activity of root extracts

of many plant species. Furthermore, Andrys et al. (2018) confirmed the possibility of using essential oil isolated from *L. angustifolia* elicited with jasmonic acid in *in vitro* cultures as a cosmetic emulsion preservative (Patent Andrys, Kulpa).

In our pervious study we showed that AuNPs and AgNPs have a significant effect on the composition and content of essential oil in true lavender propagated in *in vitro* cultures. AuNPs and AgNPs increased notably the content of antimicrobial compounds such as, borneol, γ-cadinene, caryophylleneoxide, τ-cadinol, cadalene, cis-14-nor-muurol-5-en-4-one, and bisabolol oxide A. The percentage increase in these compounds was dependent on the concentration of the nanoparticle used. For instant in case of τ-cadinol, there has been an increase in content up to 37%,for bisabolol oxide A up to 31%, for cis-14-nor-muurol-5-en-4-one up to 66%,and for γ-cadinene up to 22%. (Wesołowska et al. 2019).

Higher concentrations of AgNPs in the culture medium, as well as the particle size of 30 nm, resulted in the decreased preservative capacity of plant tissues used in our experiments. This result may be an outcome of two factors: On the one hand, bigger particle size, which did not allow to penetrate the cell membranes, prevented the accumulation of AgNPs in the tissues. On the other hand, excessive concentration of AgNPs in the culture medium produced excessive oxidative stress, which in turn exhausted the defensive capacities of lavender (it produced lower amounts of secondary metabolites).

The increased antimicrobial activity of lavender tissues propagated on culture media containing AgNPs and AuNPs may be associated with the presence of NPs in plant tissues, which penetrate the cell membranes. Domokos-Szabolcsy et al. (2012) and Lee et al. (2008) demonstrated that NPs penetrate the plant cells during the growth in the media in *in vitro* cultures. The antimicrobial activity of AgNPs and AuNPs is widely known in the literature (Rai et al. 2009; Gong et al. 2007; Zhang et al. 2015). AgNPs show positive effects on problematic skin suffering from acne because of its antimicrobial properties. It shows healing properties by accelerating wound healing, and it prevents the formation of scars (Gajbhiye et al. 2016). AgNPs is currently used as an additive in a range of beautifying face and body masks, as well as in products for improving skin conditions and in mouthwashes (Pardeike et al. 2009). The introduction of NPs along with plant tissues into a cosmetic product may be favorable – they can have a synergistic effect on the skin.

When considering the use of nanoparticles as elicitors or adding plant tissues grown on nutrients with them to cosmetics, careful attention should be paid to the health aspects associated with them. In a study by Asha Rani et al. (2009) it was found that AgNPs with a diameter penetrated inside human cells and induced DNA changes. However, the research was carried out by adding silver nanoparticles to the media in which the lung fibroblast cells (IMR-90) and human glioblastoma cells (U251) were multiplied, instead of treating living organisms with a protective layer with them. The toxicity of AuNPs towards *Arabidopsis thaliana* root cells was also indicated by Taylor et al. (2009).

Most of the published research results indicate the lack of toxicity of nanoparticles added to cosmetics. It is assumed that NPs are safe for the consumer in the cosmetic industry. At minimal and reasonable concentrations of NPs, there are no side effects on human health. According to a previous study, NPs in the current cosmetics do not penetrate the human skin, even in cases when the skin is damaged (Gajbhiye et al. 2016). Kokura et al. (2010) showed

that Ag nanoparticles are not able to penetrate human skin. However, when the barrier function of human skin is disrupted, Ag nanoparticles on the skin surface may penetrate the skin. It may be possible that 0.2% to 2% of Ag nanoparticles could penetrate the skin (0.002–0.02 ppm). At these levels Ag nanoparticles did not show any toxicity. Zang (2013) believes that the penetration of AuNPs depends on the particle size. Nanoparticles with a diameter less than 10 nm could reach the deeper layer of the *stratum corneum*, while NP larger than 40 nm could only reach 5–8 μm into the *stratum corneum*. Campbell et al. (2012), who stated that nanoparticles (20 to 200 nm) contacting intact or partially damaged skin cannot penetrate skin barrier and permeate to lower strata making them safe as cosmeceuticals.

The presence of AuNPs in culture media also had a positive effect on the antimicrobial activity of lavender; however, the effect was lesser than that of AgNPs. Lavender propagated on media containing AuNPs independently of the used concentration and particle size protected the cream from bacterial growth up to 4 weeks and fungal growth, depending on the particle diameter, for up to 4–6 weeks. This result is in-line with the results obtained by Wesołowska et al. (2019), who examined essential oils isolated from *L. angustifolia* tissue elicited with AuNPs and AgNPs determined that AgNPs has a stronger impact on the change of the composition of essential oils than AuNPs.

CONCLUSIONS

Narrow leaf lavender tissue propagated *in vitro* showed preservative properties. The addition of AuNPs and AgNPs to the culture media increases the preservative potential of lavender in various forms of cosmetic products; however, the effect depends on the particle size of the metal and its concentration in the medium. Disintegrated fragments of narrow leaf lavender propagated *in vitro* on media containing 1 mg · dm^{-3} AgNPs with a particle size of 13 nm can be used to preserve cosmetic emulsions, particularly those with short shelf life.

Author Contributions: Paula Jadczak and Danuta Kulpa designed and performed the experiments; Paula Jadczak analyzed the data and interpreted data; wrote the paper and Danuta Kulpa corrected the paper. All authors have read and agreed to the published version of the manuscript.
Funding: This research received no external funding.
Conflicts of Interest: The authors declare no conflicts of interest.

REFERENCES

Aburjai T., Natsheh F.M. 2003. Plants used in cosmetics. Phytother. Res. 17, 987–1000.

Adam K., Sivropoulou A., Kokkini S., Lanaras T., Arsenakis M. 1998. Antifungal activities of *Origanum vulgare* subsp. hirtum, *Mentha spicata*, *Lavandula angustifolia*, and *Salvia fruticosa* essential oils against human pathogenic fungi. J. Agric. Food. Chem. 46, 1739–1745.

Allied Market Research. 2016. https://www.alliedmarketresearch.com/cosmetics-market, access: 15.03.2020.

Amoo S.O., Aremu A.O., Van Staden J. 2012. *In vitro* plant regeneration, secondary metabolite production and antioxidant activity of micropropagated *Aloe arborescens* Mill. Plant. Cell. Tiss. Organ. Cult. 111, 345–358.

Andrys D., Adaszyńska-Skwirzyńska M., Kulpa D. 2018. Essential oil obtained from micropropagated lavender, its effect on HSF cells and application in cosmetic emulsion as a natural protective substance. Nat. Prod. Res. 32, 849–853.

Andrys D., Kulpa D., Grzeszczuk M., Białecka B. 2018. Influence of jasmonic acid on the growth and antimicrobial and antioxidant activities of *Lavandula angustifolia* Mill. propagated *in vitro*. Folia Hortic. 30, 3–13.

AshaRani P.V., Low Kah Mun G., Prakash Hande M., Valiyaveettil S. 2009. Cytotoxicity and Genotoxicity of Silver Nanoparticles in Human Cells. ACS Nan. 3(2), 279–290.

Bais H.P., Walker T. S., Schweizer H.P., Vivanco J.M. 2002. Root specific elicitation and antimicrobial activity of rosmarinic acid in hairy root cultures of *Ocimum basilicum*. Plant. Physiol. Bioch. 40, 983–995.

Behravan J., Bazzaz F., Malaekeh P. 2005. Survey of bacteriological contamination of cosmetic creams in Iran (2000). Int. J. Dermatol. 44, 482–485.

Brailko V.A., Mitrofanova O., Leśnikowa-Sedoshenko N., Chelombit S., Mitrofanova I.V. 2017. Anatomy features of *Lavandula angustifolia* Mill. and *Lavandula hybrida* rev. plants *in vitro*. J. Agric. For. 63, 111–117.

Campbell C.S.J., Contreras-Rojas L.R., Delgado-Charro M.B,. Guy R.H. 2012 Objective Assessment of Nanoparticle Disposition in Mammalian Skin after Topical Exposure. J. Controll. Rel.. 162, 201–207.

Cavanagh H.M.A., Wilkinson J.M. 2005. Lavender essential oil: a review. Aust. Infect. Control. 10, 35–38.

D´auria F.D., Tecca M., Strippoli V., Salvatore G., Battinelli L., Mazzanti G. 2005. Antifungal Activity of *Lavandula angustifolia* essential oil against *Candida albicans* yeast and mycelial form. Med. Mycol. 43, 391–396.

Demissie Z.A., Sarker L.S., Mahmoud S.S. 2011. Cloning and functional characterization of β-phellandrene synthase from *Lavandula angustifolia*. Planta 233, 685–96.

Domokos-Szabolcsy E., Marton L., Sztrik A., Babka B., Prokish J., Fari M. 2012. Accumulation of red elemental selenium nanoparticles and their biological effects in *Nicotinia tabacum*. Plant. Growth. Regul. 68, 525–531.

EU Patent. Andrys D., Kulpa D. 2019. Oil/ wather cosmetic emulsion PLA 41341615.

Fonseca-Santos B., Correa A.A., Chorilli M. 2015. Sustainability, natural and organic cosmetics: consumer products, efficacy, toxicological and regulatory considerations. Braz. J. Pharm. Sci. 51, 2175–9790.

Gajbhiye S., Sakharwade S. 2016. Solver Nanoparticles in Cosmetics. J. Cosm. Dermatol. Sci. App. 6, 48–53.

Golden R., Gandy J., Vollmer G. 2005. A Review of the Endocrine Activity of Parabens and Implications for Potential Risks to Human Health. Crit. Rev.Toxiol. 35, 435–458.

Golkar P., Moradi M., Garousi A.G. 2019. Elicitation of Stevia Glycosides Using Salicylic Acid and Silver Nanoparticles Under Callus Culture. Sugar. Tech. 4, 569–577.

Gonçalves S., Romano A. 2013. *In vitro* culture of lavenders (*Lavandula* spp.) and the production of secondary metabolites. Biotechnol. Adv. 31, 166–174.

Gong P., Li H., He X., Wang K., Hu J., Tan W., Ahang S., Yang X. 2007. Preparation and antibacterial activity of Fe3O4Ag nanoparticles. Nanotechnol. 18, 604–11.

Groot C., White I.R. 1995. Textbook of Contact Dermatitis, 2 ed., Berlin, Springer-Verlag, 461.

Halla N., Fernandes I.P., Heleno S.A., Costa P., Boucherit-Otmani Z., Boucherti K., Rodrigues A.E., Ferreria I.C.F.R., Barrerio M.F. 2018. Cosmetics Preservation: A Review on Present Strategies. Molecules 23, 1571.

Herman A., Herman A.P., Domagalska B.W., Młynarczyk A. 2013. Essential oils and herbal extracts as antimicrobial agents in cosmetic emulsion. Indian. J. Microbiol. 53, 232–237.

Jakowijević D.Z., Sava M. V., Stanković Čomić L., Topuzović M.D. 2015. Secondary metabolite content and *in vitro* biological effects of *Ajuga chamaepitys* (L.) *Schreb Subsp Chamaepitys*. Arch. Biol. Sci. Belgrade 67,1195–1202.

Jamshidi M., Ghanti F., Razaei A., Bemani E. 2014. Change of antioxidant enzymes activity of hazel (*Corylus avellana* L.) cells by AgNPs. Cytoteh. 68, 525–530.

Jopke K., Sanders H., White-Traut R. 2017. Use of Essential Oils Following Traumatic Burn Injury: Acase Study. J. Pedriat. Nurs. 34, 72–77.

Kerdudo A., Burger P., Merck F., Dingas A., Rolland Y., Michel T., Fernandez X. 2016. Development of a natural ingredient- Natural preservative: A case study. Comptes. Rendus. Chimie. 19, 1077–1098.

Kim H.-J., Chen F., Wang X., Rajapakse N.C. 2006. Effect of Methyl Jasmonate on Secondary Metabolites of Sweet Basil (*Ocimum basilicum* L.). J. Agr. Food. Chem. 54, 2327–2332.

Kokura S., Handa O., Takagi T., Ishikawa T., Naito Y., Yoshikawa T. 2010. Silver Nanoparticles as a Safe Preservative for Use in Cosmetic. Nanomed. Nanotech. Biol. Med. 6, 570–574.

Królicka A., Szpitter A., Gilgenast E., Romanik G., Kaminski M., Lojkowska E. 2008. Stimulation of antibacterial naphthoquinones and flavonoids accumulation in carnivorous plants grown *in vitro* by addition of elicitors. Enzyme. Microbial. Technol. 42, 216–221.

Kusumawati I., Idrayanto G. 2013. Natural Antioxidants in Cosmetics. Stud. Nat. Prod. Chem. 40, 485–505.

Lee W., An Y., Yoon H., Kweon H. 2008. Toxicity and bioavailability of copper nanoparticles to the terrestrial plants mung bean (*Phaseolus radiatus*) and wheat (*Triticum awstivum*): plant uptake for water insoluble nanoparticles. Environ. Toxicol. Chem. 27, 1915–21.

Mario P., Carvalho R., Amoro C., Santos R., Cardoso J. 2012. Contact allergy to methylchoroisothiazoline/ methylisothiazolinone (MCI/MI): findings from a Contact Dermatitis Unit. Cutan. Ocul. Toxicol. 31, 151–153.

Mitsui T. (ed.). 1997. Preservation of cosmetics in New Cosmetic Science. Amsterdam, Elsevier, 199–208.

Moharrami F., Hosseini B., Sharafi A., Farjaminezhad M. 2017. Enhanced production of hyoscyamine and scopolamine from genetically trans-formed root culture of *Hyoscyamus reticulatus* L. elicited by iron oxide nanoparticles. In Vitro. Cell. Dev. Biol. Plant. 53, 104–111.

Murashige T., Skoog,F. 1962. A revised medium for rapid growth and bio assays with Tobacco Tissue Cultures. Physiol. Plantarum. 15, 473–497.

Muyima N.Y.O., Zulu G., Bhengu T. Popplewell D. 2002. The potential application of some novel essential oils as natural cosmetic preservatives in an aqueous cream formulation. Flavour. Fragr. J. 17, 258–266.

Nostro A., Cannatelli M. A., Morelli I., Musolino A. D. Scuderi F. Pizzimenti F., Alonzo V. 2004. Efficiency of *Calamintha officinalis* essential oil as preservative in two topical product types. J. Appl. Microbiol. 97, 395–401.

O'Dell L.E., Sullivan A., Periman L.M. 2016. Beauty does not have to hurt. Adv. Ocul. Care. 7/8, 42–47.

Office of Regulatory Affairs. 2015. Pharmaceutical Microbiology Manual, vol. ORA.007. Office of Regulatory Affairs, Silver Spring, Meryland, USA.

Orton D.I., Wilkinson J.D. 2004. Cosmetic Allergy. Incidence, Diagnosis, and Management. Am. J. Clin. Dermatol. 5, 327–337.

Pardeike J., Hommoss A., Müller R.H. 2009. Lipid nanoparticles (SLN, NLC) in cosmetic and pharmaceutical dermal products. Int. J. Pharm. 366, 170–184.

Pitt T.L., McClure J., Parker M.D., Amezquita A., McClure P.J. 2015. *Bacillus cereus* in personal care products: Risk to consumers. Int. J. Cosmet. Sci. 37, 165–174.

Poulev A., O'Neal J.M., Logendra S., Pouleva R.B., Timeva V., Garvey A.S., Raskin I. 2003. Elicitation, a New Window into Plant Chemodiversity and Phytochemical Drug Discovery. J. Med. Chem. 46, 2542–2547.

Rai M., Yadav A., Gade A. 2009. Silver nanoparticles as a new generation of antimicrobials. Biotechnol. Adv. 27, 76–83.

Schwiertz A., Duttke C., Hild J. 2006. *In vitro* activity of essential oils on microorganisms isolated from vaginal infections. Inter. J. Aromather. 16, 169–174.

Shakeran Z., Keyhanfar M., Asghari G., Ghanadian M. 2015. Improvement of atropine production by different biotic and abiotic elicitors in hairy root cultures of *Datura metel*. Turk. J. Biol. 39, 111–118.

Sticher O., Heilmann J., Zündorf I. 2015. Hänsel & Sticher Pharmakognosie-Phytopharmazie, 10nd ed. Stuttgart, Germany, Wissenschaftliche Verlagsgesellschaft Press, 673.

Szabo E., Thelen A., Petersen M. 1999. Fungal elicitor preparations and methyl jasmonate enhance rosmarinic acid accumulation in suspension cultures of *Coleus blumei.* Plant. Cell. Rep. 18, 485–489.

Taylor A.F., Rylott E.L., Anderson C.W.N., Bruce N.C. 2014. Investigating the Toxicity, Uptake, Nanoparticle Formation and Genetic Response of Plants to Gold. PLoS ONE 9(4), e93793.

Upson T., Andrews S. 2004. The genus *Lavandula*.1nd ed., Portland, Oregon, Timber Press.

Vanisree M., Hsin-Sheng T. 2004. Plant Cell Cultures. An Alternative and Efficient Source for the Production of Biologically Important Secondary Metabolites. Int. J. Appl. Sci. Eng. 2, 29–48.

Wesołowska A., Jadczak P., Kulpa D., Przewodowsk W. 2019. Gas Chromatography-Mass Spectrometry (GC-MS) Analysis of Essential Oil from AgNPs and AuNPs Elicited *Lavandula angustifolia in vitro* Cultures. Molecules. 24, 606.

Wornouk G., Dermissie Z., Rheault M., Mahmoud S. 2011. Biosynthesis and therapeutic properties of *Lavandula* essential oil constituents. Planta. Med. 77, 7–15.

Zhang M. 2013. Au Natural Nanoparticles Sun Protection. Cosmet. Toiletries. 128, 440.

Zhang Y., Dasari T.P.S., Deng H., Yu H. 2015. Antimicrobial Activity of Gold Nanoparticles and Ionic Gold. J. Environ. Sci. Helath, C. Environ. Carcinog. Ecotoxicol. Rev. 33, 286–327.

Złotek U., Michalak-Majewska M., Szymanowska U. 2016. Effect of jasmonic acid elicitation on the yield, chemical composition, and antioxidant and anti-inflammatory properties of essential oil of lettuce leaf basil (*Ocimum basilicum* L.). Food. Chem. 15, 1–7.

Lavandula angustifolia NAMNAŻANA W KULTURACH *IN VITRO* NA POŻYWKACH ZAWIERAJĄCYCH AgNP i AuNPs – ALTERNATYWNE ROZWIĄZANIE DLA SYNTETYCZNYCH KONSERWANTÓW W KOSMETYKACH

Streszczenie. Określono właściwości konserwujące lawendy wąskolistnej namnażanej na pożywkach z nanocząstkami złota lub srebra o rozmiarach cząsteczek 13 i 30 nm. Emulsje kosmetyczne, przygotowane przy użyciu lawendowej tkanki namnażanej na podłożach zawierających AuNPs i AgNPs, wykazały zwiększone właściwości konserwujące w porównaniu

z emulsjami kontrolnymi. W przypadku kontrolnych emulsji kosmetycznych, które nie zawierały dodatku tkanek roślinnych ani kwasów dehydrooctowego i benzoesowego (DHA BA), po drugim tygodniu trwania doświadczenia zaobserwowano pojawianie się kolonii bakteryjnych i grzybowych. Dodatek tkanki lawendy namnażanej na pożywkach bez nanocząstek chronił badane próbki przed zanieczyszczeniem mikrobiologicznym; w tym przypadku kontaminacja bakteryjna została zaobserwowana po 4 tygodniach, a grzybowa po 6 tygodniach. Dodatek tkanki lawendy, namnażanej na pożywce zawierającej AgNPs o wielkości cząstek 13 nm, w stężeniu 1 mg · dm^{-3} wydłużył czas pojawiania się kolonii bakteryjnych do 8 tygodni (0,9); wynik ten był bliski i porównywalny z efektem DHA BA. Wyższe stężenia AgNPs w pożywkach, a także większa średnica cząstek (30 nm) spowodowały zmniejszenie aktywności konserwujących tkanek roślinnych. Obecność AuNPs w mediach hodowlanych wykazała pozytywny wpływ na aktywność antybakteryjną lawendy, jednak w mniejszym stopniu niż w przypadku AgNPs. Rozdrobnione fragmenty tkanki lawendy, namnażane na pożywkach zawierających 1 mg · dm^{-3} AgNP, o wielkości cząstek 13 nm, mogą być stosowane do zachowania emulsji kosmetycznych w krótkim okresie trwałości.

Słowa kluczowe: emulsja kosmetyczna, nanocząstki metalu, srebro, złoto, mikropropagacja, elicytacja.