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Using silver nanoparticles to increase the accumulation of withanolide compounds in cultured callus of *Withania somnifera* L.

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ABSTRACT

Background: *Withania somnifera* L. is a famous medicinal plant that has been utilized in Global folk medicine for a long time. The leaves and roots of the plant are rich in withanolide constituents, which are primarily responsible for their therapeutic effectiveness. Objective: This study focuses on the differences in the content of withanolide compounds and biomass in *W. somnifera* plants under different treatments with silver nanoparticles (Ag-NPs). Results: The leaves of the field plant (*in vivo* cultures) showed a rise in content of 27-OH-withanolide, withaferine-A, and withastramonolide. While the leaves and roots in the *in vitro* cultures revealed a high content of withanolide-B and withanone. The high content of withanolide-A was mainly observed in callus cultures. Moreover, the findings showed a considerable reduction in callus biomass across different concentrations of Ag-NPs (1, 2, and 3 mg/L). Conversely, there was a noteworthy rise in the production of withanolides. Specifically, withastramonolide and withanolide-A exhibited significant increases at 2 mg/L Ag-NPs, showing 121% and 15% increases, respectively, compared to the control. Additionally, several other withanolide compounds, including 27-OH-withanolide, withaferin-A, withanone, and withanolide-B, accumulated significantly at 3 mg/L Ag-NPs, with percentage increases ranging from 67% to 277%. Additionally, the study evaluated the DPPH scavenging activity in Ag-NPs-treated samples, showing a substantial 64.4% increase at 3 mg/L, which suggests the presence of elevated active components or improved antioxidants in this treatment. Conclusion: The findings provide insights into the variations in withanolide content under different culture systems and demonstrate the potential of Ag-NPs treatment to enhance withanolide yield and antioxidant activity in *Withania* plant cultures.

Keywords: Withanolide compounds, Ag-NPs, callus cultures, DPPH

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INTRODUCTION

The ancient Indian and Unani medical systems have valued *Withania somnifera* L. for its medicinal properties for over 3,000 years (1). *W. somnifera* is known for its steroidal lactones called withanolides, which are naturally occurring C-28 steroidal lactone triterpenoids arranged on an ergostane structure with approximately 40 known structures. The formation of a six-membered lactone ring entails the oxidation of carbon atoms at positions C22 and C26 (2).

Withanolide compounds exhibit pharmacological activity and are believed to contribute to the plant's properties, including anti-cancer effects, relief from low back pain, and muscle strengthening (3). Additionally, they possess

immunomodulatory, diuretic, anti-inflammatory, hypnotic, and anxiolytic properties, as well as adaptogenic and cytotoxic effects, and exhibit antitussive and sedative influences (4). Secondary metabolites (SM) like alkaloids, withanolides, tannins, sitoindosides, flavonoids, and glycosides are found in different amounts in all parts of *W. somnifera* (5). The quantity of these metabolites depends on several factors, including the plant's development rate, location, and environmental conditions (2).

Only the Solanaceae family is known to contain 580 naturally occurring withanolides (6). Withaferin-A, withanolide-A, D, E, and F, as well as withalongolide-A and B, all of which have the potential to be therapeutic withanolide compounds (7). Recently, researchers quantified withanoside VII, dihydrowithaferin A, viscosalactone B, and 27-hydroxywithanone in *W. somnifera* using UHPLC−PDA and mass fragmentation (8).

Callus culture techniques can be used to produce beneficial plant SM, including withanolides, in a controlled and scalable way (9). By altering the growing conditions and introducing external stimuli, such as biotic or abiotic stress, the accumulation of target chemicals in cultured plant cells can be significantly enhanced (10).

W. somnifera has successfully undergone callus induction from various plant parts, including leaves, stems, roots, epicotyls, and hypocotyls, using Murashige and Skoog (MS) medium amplified with several combinations of plant growth regulators (PGRs) (11). Among these explants, the leaf has demonstrated the most favorable products in terms of generating a valuable callus mass and a desirable texture (12, 13).

Nanoparticles (NPs) have emerged as promising elicitors in plant tissue culture systems for several medicinal plants (14). Their unique physicochemical characteristics, such as a large surface area, reactivity, and the ability to penetrate biological systems, make them attractive for enhancing the production of bioactive compounds (15). The ability of silver nanoparticles (Ag-NPs) to alter plant physiology and increase the generation of bioactive chemicals was particularly well-established (16). Researchers in various kinds of culture systems have often used low NPs concentrations. For instance, Ag-NPs at strengths ranging from 30 to 90 µg/L in *Caralluma tuberculata* caused an increase in callus fresh weight, along with increased levels of phenolics and flavonoids, and boosted activities of the antioxidant enzymes (17). Similarly, in *Chrysanthemum* cultures, Ag-NPs at concentrations ranging from 5 to 20 mg/L induced genetic and biochemical variations (18).

In *W. somnifera* culture systems, the application of copper oxide nanoparticles (CuO-NPs) at a concentration of 1 mg/L for 15 and 20 days resulted in increased quercetin and gallic acid content in the shoot and root parts (19). While Singh *et al*. (20) demonstrated that synthesized Zn: Ag NPs, used in different ratios, exerted varied impacts on the photosynthesis rate, transpiration, as well as the content of withanolides and carbohydrates.

The objective of this study was to find out how adding Ag-NPs to the culture medium changed the amount of callus biomass and the content of withanolide compounds in *W. somnifera* callus cultures. For pharmaceutical and nutraceutical uses, understanding how the effects of Ag-NPs affect callus development and the accumulation of withanolides could help investigators develop better methods for producing these valuable compounds.

METHODOLOGY

The main steps of the methodology are summarized in a graphical abstract represented in Figure. 1.

Figure (1). The graphical abstract shows the main steps of the methodology

2.1 Plant Material

The mature and dried seeds of *W. somnifera* were sourced from the Plant Genetic Resources Division, Botany Directorate, Abu-Ghraib, Iraq. To overcome seed dormancy, the seeds were soaked in a solution of 600 mg/L gibberellic acid (GA_3) at room temperature for 24 hours (11). Subsequently, the soaked seeds were divided into two groups for establishment as *in vivo* and *in vitro* plants. Seeds subjected to GA_3 treatment were sown in September, with the objective of cultivating in vivo plants after a 6-month growth period. Simultaneously, another group of seeds underwent sterilization using the method described by AL-Alwani and Mohammed (21), which involved the use of 70% ethanol (EtOH) and 2% sodium hypochlorite. The sterilized seeds were grown in MS medium to develop *in vitro* plants.

2.2 Establishment of Callus Cultures

To initiate callus formation, sterilized leaves from three-month-old *in vitro* seedlings were cut into uniform pieces and placed onto MS medium provided with 0.5 mg/L each of NAA and TDZ (22). All cultures were then incubated in a growth chamber incubator (Sanyo, Japan) under dark conditions at a temperature of 25 ± 1 °C. The established callus was sub-cultured every four weeks to maintain its growth and viability.

2.3 Characterization of Ag-NPs by SEM

The silver nanoparticles (Ag-NPs) have been obtained from Nanjing Nano Technology Co., Ltd., China. The scanning electron microscope (SEM, InspectTM F50, Spain) was used to verify the morphology and dimensions of the NPs. The ultrasonicated Ag-NPs in ethanol were applied onto a SEM sample holder and subsequently dried using air.

2.4 Effect of Ag-NPs on Callus Development and Bioactive Compounds Production

Approximately 200 mg of compact yellow calli obtained from *in vitro* leaf explants were cultured at an initial weight on MS medium containing the same combination of PGRs used for callus induction, along with varying concentrations of Ag-NPs (0, 1, 2, and 3 mg/L). The pH of all treatments was adjusted to a range of 5.7-5.8 and sterilized by autoclave at 121 ºC for 20 minutes (23).

2.5 Biomass Measurement

To assess the impact of Ag-NPs on the growth of callus, calli that were 30 days old from each treatment were subjected to weighing in order to determine the final fresh weight (FW) and dry weight (DW). The measurements were conducted using a sensitive balance (Kern, Germany). The calli were

then subjected to a drying process in a controlled laboratory environment at 45 °C for 24 hours to measure their weight in a dry state.

2.6 Extraction of Withanolide Compounds by HPLC

A comparative investigation of certain withanolide compounds was conducted using different plant segments. Samples were obtained from both leaf and root sections of mature plants during the flowering stage, 6 months after cultivation in field conditions (*in vivo* cultures). Likewise, leaf and root segments from *in vitro* cultures were examined after three months of growth. The study also analyzed extracts from callus cultures, contrasting those untreated with elicitors to those treated with Ag-NPs as an elicitor.

The extraction procedure was conducted as per (24). Each sample of 100 mg of dry material was subjected to reflux and sonication in 50 mL of 80% methanol for 45 minutes at room temperature. Each extract was subjected to vacuum concentration. The extracts were dissolved in methanol and then passed through a 0.22 µm Millipore filter.

2.7 Quantification of Withanolide Compounds by HPLC

To examine the withanolides, High-performance liquid chromatography (HPLC) was adopted using a Shimadzu-10 system equipped with a UV and diode array detector (DAD). The column employed had a particle size of 3 mm and was a C8 type with dimensions of 50 x 4.6 mm. The flow rate was 1 mL/min. The mobile phase consisted of two components: solvent A, which was 0.01M orthophosphoric acid, and solvent B, which was acetonitrile. A linear gradient program of solvent B from 0% to 100% over a period of 15 min achieved the chromatographic separation.

The concentrations of withanolide compounds in the samples were estimated following the method described by Taha (25), using the formula:

Concentration of sample ((µg)/ml)=(Area of sample)/(Area of standard) x Conc. of standard x dilution factor

Six standards of withanolide compounds, namely withanolide-A, withanolide-B, withaferin-A, withanone, 27-OH-Withanolide-A, and 27-OH-Withanolide-B, were obtained from Sigma-Aldrich Company, USA. Each standard was individually dissolved in methanol to achieve a final concentration of 25 mg/L.

2.8 DPPH Scavenging Activity

The free radical scavenging activity of the samples was evaluated using the 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) method, following the procedure outlined by Frezzini *et al*. (26). In brief, ten mg of each dry sample was soaked in 5 mL of 96% EtOH for 4 hours. The suspension was then filtered using a syringe filter with a 0.45 µm pore size (PTFE). A DPPH reagent at a concentration of 0.1 mM was made by dissolving 0.0039 g of DPPH in 100 mL of 96% EtOH using a vortex mixer (Stuart). Next, 1.5 mL of each sample extract was mixed with 1.5 mL of the DPPH solution using a vortex mixer for 15 seconds. Subsequently, the mixture was allowed to rest in the dark at ambient temperature for a duration of 30 minutes. The measurement of absorbance at a wavelength of 517 nm was conducted using a spectrophotometer (OPTIMA SP-300). The degree of decolorization, indicating the percentage reduction of DPPH, was calculated using the equation:

Where A0 = the absorbance of blank (EtOH + DPPH), and As = the Absorbance of the sample (sample + DPPH).

2.9 Statistical Analysis

The data generated from the experiment underwent analysis of variance (ANOVA) and subsequent use of the Duncan least significant difference (LSD) post hoc test, using a completely randomized design (CRD). The software IBM SPSS Statistics 26 was used to perform statistical analysis. A statistical difference was adopted at a probability level of ≤ 0.05.

RESULTS

3.1 Content of Withanolide Compounds Under Different Systems of Cultures

Table 1 provides information about the quantities of different compounds found in various *Withania* plants under different culture conditions. When analyzing the *in vivo* plants that grow naturally in their environment, it was observed that *in vivo* leaves contained the highest concentrations of 27-OH-withanolide, withaferine-A, and withastramonolide compounds, measuring 65.4, 129.0, and 173.8 µg/mL, respectively. On the other hand, the leaves in *in vitro* cultures revealed the highest concentration of withanolide-B (75.1 µg/mL) while *in vitro* roots exhibited the highest concentration of withanone (90.3 µg/mL). Interestingly, the callus cultures, which measured 121.1 µg/mL of Withanolide-A.

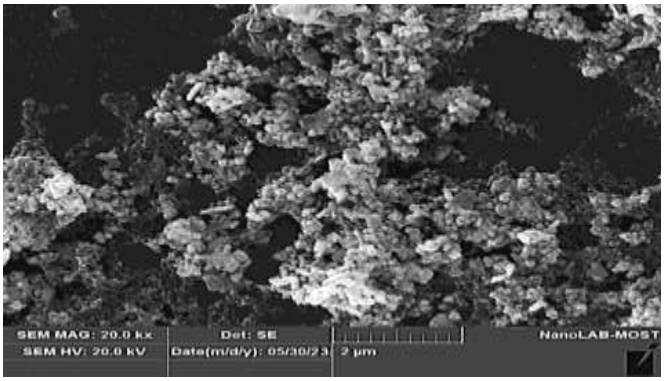
The same table shows that the total content of withanolides was highest in leaves, particularly in vivo cultures (518.9 µg/mL), while the lowest value was recorded in *in vivo* roots (343.3 µg/mL).

Table (1). Concentration of withanolide compounds (µg/mL) in different parts of *in vivo* and *in vitro* cultures of *W. somnifera*.

Withanolide compounds	*Different parts of W. somnifera*				
	***In vivo* leaves**	***In vitro* leaves**	***In vivo* roots**	***In vitro* roots**	**Callus culture**
27-OH-Withanolide	**65.4 a**	28.2 b	14.0 d	26.8 b	20.3 c
Withaferine-A	**129.0 a**	98.6 b	39.6 d	75.9 c	98.6 b
Withastramonolide	**173.8 a**	107.2 b	68.2 e	97.6 c	88.1 d
Withanolide-A	54.6 c	107.1 b	105.6 b	118.9 a	**121.1 a**
Withanone	32.8 e	79.4 b	67.3 c	**90.3 a**	46.5 d
Withanolide-B	63.1 b	**75.1 a**	48.4 c	31.2 d	27.4 d
Total content	518.9 A	495.6 B	343.3 E	441.0 C	402.2 D
There is no significant difference between the values that share the same letter inside a row.					

3.2 Characterization of Ag-NPs

The imaging of scanning electron microscope (SEM) at a magnification of 20 kx reveals that the Ag-NPs are nearly sphere-like in shape and have an average diameter of around 50 nm, as depicted in Fig. 2.



Figure(2) The scanning electron microscopy (SEM) image depicts the morphology and dimensions of Ag nanoparticles (Ag-NPs).

3.3 Effect of Ag-NPs on the weights of Callus cultures and total yield of withanolides

In our study, the effects of different Ag-NP treatments on Withania plant cultures were investigated, and their impact on fresh weight (FW), dry weight (DW), and total withanolide yield was analyzed (Table 2 and Figure 3). The control treatment exhibited an FW of 714.1 ± 140 mg and a DW of 70.5 ± 14.7 mg, while the yield of withanolides in the control callus was 402.2 µg/mL.

Three different concentrations of Ag-NPs (1, 2, and 3 mg/L) were applied to assess their impact on the *Withania* callus

cultures. All treatments of Ag-NPs resulted in significantly decreased fresh and dry weights compared to the control. Notably, the highest concentrations of Ag-NPs (3 mg/L) significantly enhanced the yield of withanolides, which resulted in a great total mean (665.9 µg/mL). The size and morphology of callus cultures under NPs stress are illustrated in Figure 3.

Table (2) Effects of Ag-NPs treatments on fresh weight (FW), dry weight (DW), and withanolide yield in *W. somnifera* cultures.

Treatments	*FW (mg)*	*DW (mg)*	*Total withanolides (µg/ml)*
Control	714.1 ± 140 a	70.5 ± 14.7 a	402.2 c
1mg/L Ag NPs	552.3 ± 64.9 b	54.4 ± 10.1 b	561.6 b
2 mg/L Ag NPs	556.5 ± 75.1 b	56.1 ± 9.9 b	661.7 a
3 mg/L Ag NPs	550.1 ±82.9 b	53.3 ± 8.0 b	665.9 a
Data shown are the mean ± Standard Deviation (n = 15 for weight). "Means followed by the same letter inside a column are not significantly different at P ≤ 0.05 according to Duncan LSD post hoc analysis (ANOVA)"			

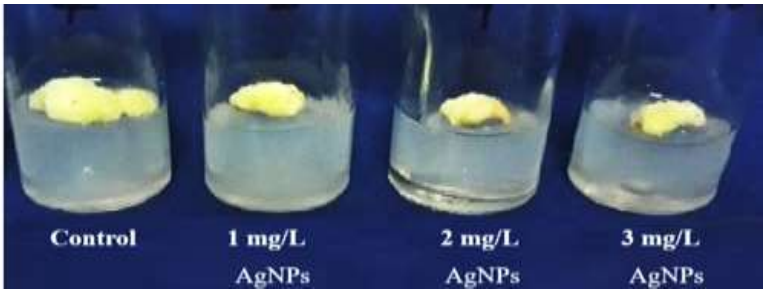
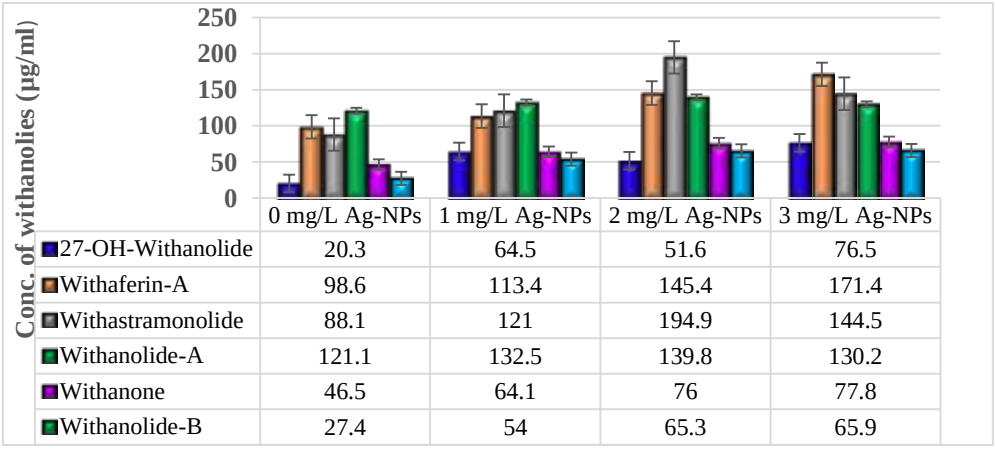


Figure (3) The investigation evaluates the morphology of callus cultures of *W. somnifera* subsequent to exposure to varying doses of Ag-NPs.

3.4 Effect of Ag-NPs treatments on the content of Withanolide compounds

The quantification and qualification of certain SMs are illustrated in Table 3 and Figure 4. The substantial increase in the concentration of studied withanolide compounds following treatment with Ag-NPs demonstrates a clear dose-dependent response and the efficacy of Ag-NPs as an elicitor. Notably, withastramonolide and withanolide-A exhibited significant increases at a concentration of 2 mg/L Ag-NPs, with measurements of 194.9 µg/mL (121% increase) and 139.8 µg/mL (15% increase), respectively. Additionally, at 3 mg/L of Ag-NPs, there was a substantial accumulation of several withanolide compounds, including 27-OH-withanolide, withaferin-A, withanone, and withanolide-B, with significant content values of 76.5 µg/mL, 171.4 µg/mL, 77.8 µg/mL, and 65.9 µg/mL (Figure 4), corresponding to percentage increases of 277%, 74%, 67%, and 14% (Table 3).



	0 mg/L Ag-NPs	1 mg/L Ag-NPs	2 mg/L Ag-NPs	3 mg/L Ag-NPs
27-OH-Withanolide	20.3	64.5	51.6	76.5
Withaferin-A	98.6	113.4	145.4	171.4
Withastramonolide	88.1	121	194.9	144.5
Withanolide-A	121.1	132.5	139.8	130.2
Withanone	46.5	64.1	76	77.8
Withanolide-B	27.4	54	65.3	65.9

Figure(4) Concentration of withanolide compounds (µg/mL) in callus cultures of *W. somnifera* treated with different doses of Ag-NPs.

Table (3) Percentage increase of withanolide compounds compared to the control treatment *in W. somnifera* callus cultures after being treated with Ag-NPs.

Withanolide compounds	*1 mg/L(Ag-NPs)*	*2 mg/L(Ag-NPs)*	*3 mg/L(Ag-NPs)*
27-OH-Withanolide	218 %	154 %	277 %
Withaferin-A	15 %	47 %	74 %
Withastramonolide	37 %	121 %	64 %
Withanolide-A	9 %	15 %	8 %
Withanone	38 %	63 %	67 %
Withanolide-B	97 %	138 %	141 %

3.5 Effect of Ag-NPs Treatments on DPPH Scavenging Activity

Figure 5 displays the radical scavenging activity (DPPH•) percentage inhibition values for NPs treatments. The inhibition percentages exhibited a statistically significant similarity when subjected to different Ag-NP treatments, ranging from 0 to 2 mg/L, with recorded values of 57.8%, 57.9%, and 59.2%, respectively. Nevertheless, a significant elevation in inhibitory activity is detected at a concentration of 3 mg/L of Ag-NPs, resulting in a value of 64.4%. The results of this study indicate that an increased concentration of Ag-NPs demonstrates a greater capacity for scavenging radicals. This observation may be due to a larger quantity of active components or better antioxidant capabilities found in the sample.

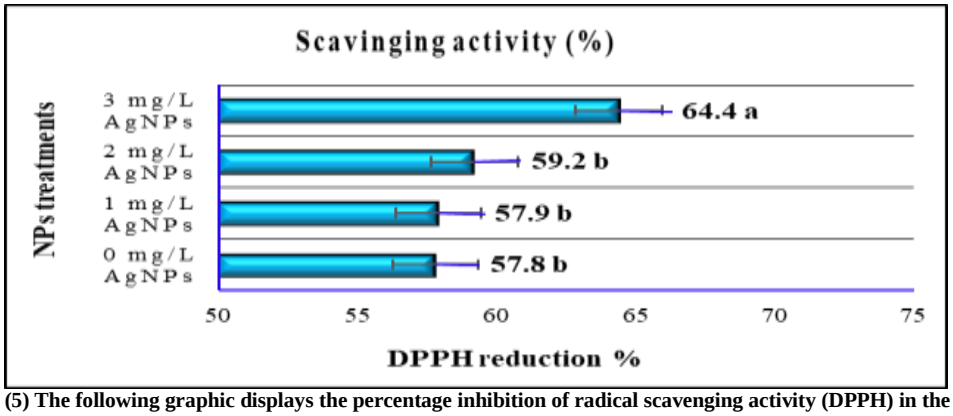


Figure (5) The following graphic displays the percentage inhibition of radical scavenging activity (DPPH) in the methanolic extract of *W. somnifera* callus cultures.

DISSCUSION

The findings of this study provide valuable insights into how withanolide levels change in various growing systems and how treating with Ag-NPs affects these compounds, as well as the antioxidant properties and biomass of callus cultures. The present study successfully demonstrated the achievement of callus formation from leaf explants through cultivation on MS medium supplemented with an optimal combination of PGRs. Previous studies have indicated that many *W. somnifera* explants do not naturally possess sufficient levels of endogenous hormones required for callus formation **(**11**).** Therefore, Adil *et al*. (2019) **(**9**)** suggested supplementing the culture medium with a suitable mixture of auxins and cytokinins to ensure the development of calluses with desirable characteristics, such as weight, size, and texture. The findings obtained by the present study regarding the withanolide content in different parts of the *Withania* plant under various culture conditions align with those of previous studies. The leaves contained the highest concentration of SMs, particularly in *in vivo* cultures, compared to other parts and culture systems. This can be attributed to the fact that more than 25% of withanolide biosynthesis occurs in the plastids **(**27**).**

Additionally, the distribution of most secondary metabolites varies depending on the type of compound and the plant's stage of development. For instance, Singh *et al*. (28) reported that withaferin-A is the major compound found in leaves, whereas withanolide-A is the principal metabolite present in roots. These observations align with the hypothesis proposed by Sabir *et al*., suggesting that withanolide-A synthesis is likely linked to roots and their development (29).However, in vitro culture systems exhibited the highest concentrations of other compounds, such as withanone in roots *in vitro* cultures, and withanolide-A in callus cultures. This suggests the potential of in vitro systems as alternative sources to produce these compounds, which is consistent with the findings of several studies highlighting the effectiveness of these systems in enhancing the accumulation of active withanolide compounds (13).

The results also indicate that treating callus cultures with different concentrations of Ag-NPs had a significant effect on the weights of the callus compared to the control treatment. Notably, the control treatment of callus showed moderate levels of withanolide yield, but a significant increase was detected when treated with Ag-NPs. Intriguingly, the highest yield was observed at a concentration of 3 mg/L Ag-NPs. These results indicate that Ag-NPs act as elicitors, inducing a dose-dependent response in the formation of withanolide compounds in the callus cultures.

Previous investigations have shown diverse responses in callus cultures treated with nanoparticles. Positive effects, such as an increase in callus growth, have been observed in callus cultures of *Fagonia indica* treated with Ag-NPs (30). Also, the frequency of callus induction was enhanced in *Panicum virgatum* explants treated with ZnO-NPs (23). While the adverse effects of a decline in callus growth have been reported in cultures of *Rapeseed* (31) and *Stevia rebaudiana* (32) after exposure to Zn-NPs. The impact of nanoparticle treatment may reach a specific threshold, beyond which callus growth is compromised and antioxidant activities are reduced. This can lead to the onset of oxidative stress and a reduction in the plant's natural antioxidant capacity. The production of reactive oxygen species (ROS) plays a crucial role in nanoparticle-induced oxidative stress (33). The triggers of the ROS signaling network can stimulate the expression of defensive pathways. Furthermore, this phenomenon might result in alterations in DNA methylation and histone modification, hence inducing epigenetic modifications (34). Additionally, (35) illustrated that NPs cause oxidative stress by the activation of Ca^{2+} through the induction of Ca^{2+} permeable pores and the direct oxidation of apoplastic L-ascorbic acid. NPs can penetrate tissue junctions and cellular membranes, causing structural damage and DNA mutations (36). The NPs can also disrupt cell wall permeability, affect cellular respiration, and interact with proteins and compounds in DNA, leading to cellular death (37). The varied effects observed in nanoparticle-treated plants can be attributed to several factors such as nanoparticle concentration, type, duration of exposure, plant species, plant part, the specific growth and cultural system employed (38). Despite the varying influences of NPs on plant growth, extensive research has demonstrated their ability to significantly stimulate the production of secondary metabolite compounds and enhance antioxidant properties, which are integral to plant defense mechanisms (39). This was also observed in the current study. Similar findings have been reported in other studies. For instance, the total content of phenolics and flavonoids increased with elevation in DPPH free radical scavenging activity, which was achieved under treatments of ZnO-NPs and CuO-NPs for callus cultures of *Stevia rebaudiana* (32). While application of ZnO-NPs and TiO2-NPs showed the highest action of phenylalanine ammonia lyase (PAL) with a rise of total phenols concentration in *Linum usitatissimum* cell suspension cultures (40), and high accumulation of α-tocopherol was improved in *Argania spinosa* suspension cultures by TiO_2-NPs and silicon dioxide SiO_2-NPs feeding in medium (41). It is noteworthy to mention that Ag-NPs at varying concentrations are widely utilized in plant growth systems to promote the production of significant active compounds. For example, high production of flavonoids obtained in *Dodonaea viscosa* callus cultures (42). A significant increase in glycoside content was also detected in the callus of *Stevia rebaudiana* (43). Abiotic elicitors are often thought of as components with strong antioxidant potential due to their capacity to boost resistance to free radicals produced by oxidative stress (44).

CONCLUSION

Although most withanolide compounds were at their highest concentration in the field plant, the valuable compounds (Withanolide-A, Withanone, and Withanolide-B) were present at a high concentration in the various in vitro cultures. These results encourage researchers to conduct further studies that enhance the accumulation of these compounds.

This research demonstrates that the use of Ag-NPs in the culture medium at appropriate concentrations results in a decrease in callus development, while the content of beneficial secondary metabolites with antioxidant activity increases significantly. These results suggest that nanoparticles may act as abiotic stimulants, triggering metabolic processes associated with stress, which in turn support the biosynthesis of bioactive substances.

Nevertheless, the particular processes operating these reactions are still undetermined; the current study contributes to our understanding of optimizing elicitation strategies for the enhanced production of valuable compounds in plant-in-vitro systems.

Future studies can focus on elucidating the underlying molecular mechanisms involved in the elicitation process induced by Ag-NPs and further optimize the Ag-NPs treatment conditions for maximal withanolide yield.

CONFLICT OF INTEREST

"The authors declare that they have no conflicts of interest."

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استخدام جزيئات الفضة النانوية لزيادة تراكم المركبات الوذانوليدية في الكالس المستنبت من *Withania somnifera* L.

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الخلاصة

الخلفية: *Withania somnifera* L هو نبات طبي مشهور والذي تم استخدامه في الطب الشعبي العالمي لفترة طويلة من الزمن. اوراق وجذور النبات غنية بالمكونات الوذانوليدية، والتي في الغالب المسؤولة عن فاعليتها العلاجية. الهدف من الدراسة: تقييم الاختلافات في محتوى المركبات الوذانوليدية و الكتلة الحيوية لنبات *W. somnifera* تحت معاملات مختلفة من دقائق الفضة النانوية. النتائج: اظهرت اوراق نبات الحقل (المزارع الحية) ارتفاعاً في محتوى 27 OH-withanolide و withaferine-A وwithastramonolide. بينما اظهرت اوراق وجذور المزارع المختبرية محتوى مرتفعاً من withanolide-B و withanone. علاوة على ذلك، اظهرت النتائج اختزالاً كبيراً في الكتلة الحيوية للكالس عند التراكيز المختلفة لدقائق الفضة النانوية (1، 2، 3 ملغم/لتر). على العكس من ذلك، وُجِدَ ارتفاعاً ملحوظاً في انتاج الوذانوليدات. بالاخص، withastramonolide وwithanolide-A اظهرا زيادة معنوية عند التركيز 2 ملغم/لتر من الفضة النانوية بنسبة زيادة 121% و 15% على التوالي، مقارنة مع السيطرة. بالاضافة الى ذلك، بعض المركبات الوذانوليدية الاخرى والتي تضم withaferin-A ،-OH-withanolide27، withanone وwithanolide-B ، تراكمت معنويا عند 3 ملغم/لتر من الفضة النانوية، بنسبة زيادة تراوحت بين 67% الى 277%. فضلاً عن ذلك، قيمت الدراسة النشاط المماسح للجذور الحرة باستخدام DPPH في العينات المعاملة بالفضة النانوية، وقد اظهرت زيادة جوهرية 64.4% عند 3 ملغم/لتر، مقترحةً ارتفاعاً في العناصرالفعالة او تحسناً في مضادات الاكسدة في هذه المعاملة. الاستنتاج: زودت النتائج رؤى على التنوع في المحتوى الوذانوليدي تحت انظمة زراعة مختلفة، واظهرت احتمالية تحسين معاملة الفضة النانوية في انتاجية الوذانوليدات والفعالية المضادة للاكسدة في مزارع نبات *Withania*.

الكلمات المفتاحية: المركبات الوذانوليدية ، Ag-NPs، مزارع الكالس، DPPH