In Vitro Comparison of Silver Nanoparticles (Agnps) and Silver Nitrate as Growth Enhancers and Contamination Reducers in Tissue Culture of Date Palm (cv. Sewi) on the Germination Stage

Lewaa, L. M.[1*], Mohamed, A. M.[1] and Malhat, M. H. Mervat[2]

[1]Central laboratory for Date Palm Research and Development, Agricultural Research Center, Giza 12619, Egypt.

[2]Agricultural Genetic Engineering Research Institute (AGERI), Tissue culture and transformation dept., Agric., Res. Center (ARC), Giza, Egypt

Abstract

Date palm (*Phoenix dactylifera* L.) is one of the strategic plants in Egypt and the Middle East.Date palm (Phoenix dactylifera L.) is a crucial crop in arid and semi-arid regions. Tissue culture, a modern propagation technique, is often hindered by contamination. This study investigated the potential of silver nanoparticles (AgNPs) and silver nitrate ($AgNO_3$) to enhance date palm tissue culture by controlling contamination and promoting growth. AgNPs were synthesized and characterized, exhibiting an absorption peak at 405 nm and a particle size of 43.8 nm. The minimum inhibitory concentration (MIC) of AgNPs and $AgNO_3$ against Bacillus subtilis was determined. AgNPs at 100 mg/L exhibited the highest antimicrobial activity.AgNPs and $AgNO_3$ were applied to date palm immature female inflorescence explants at various concentrations. AgNPs at 50 mg/L significantly increased embryo and shoot formation. Biochemical analysis revealed that $AgNO_3$ at 50 and 100 mg/L elevated total phenol and IAA levels, while AgNPs at 100 mg/L increased gibberellins. Antioxidants were significantly higher in explants treated with 25 mg/L AgNPs.Genetic stability analysis using ISSR-PCR confirmed that the treatments did not induce significant genetic alterations.These findings highlight the potential of AgNPs and $AgNO_3$ as effective agents for improving date palm tissue culture efficiency and quality.

Keywords: Date palm (*Phoenix dactylifera* L.), sliver nanoparticles, AgNPs, silver nitrate, tissue culture, genetic stability

[*]**Corresponding author:** LoayLewaa@agr.asu.edu.eg

Introduction

Date palm (*Phoenix dactylifera* L.) considered a cornerstone of agriculture in many arid and semi-arid regions worldwide for millennia, particularly in the Middle East and North Africa. Its economic importance is multifaceted, spanning food production, trade, and cultural significance (Marinova *et al.*, 2011). This importance is rooted in its versatile products, adaptability to harsh environments, and role in supporting livelihoods (Soomro and Shaikh, 2023). Date fruits are a staple food in many countries that provide essential nutrients such as carbohydrates, fiber, vitamins, and minerals. They are a particularly important source of energy and nutrition for people living in regions with limited agricultural resources (Ghnimi *et al.,* 2017). It can be processed

into various products, including dry dates, jam, syrup, chutney, date bars, butter, candy, concentrated juice, relish, date paste, pickles, and date sugar, expanding their culinary applications and nutritional value. (Oladzad *et al.,* 2021). Vegetative propagation of date palms using offshoots and tissue culture technique (a modern technique) is generally used in economic propagation protocol (Elaziem and Mahfouz 2023). Many problems are faced in the propagation of date palms in the tissue culture technique. Contaminations (including bacteria and fungi) are considered the major problem facing this technique (Abdel-Karim, 2017). Female immature inflorescence explant culture is considered one of the sources of vegetative propagation to avoid problems that face the shoot tip explant like a high percentage of contamination (Malhat *et al.,* 2019). Nanotechnology offers a promising solution to these challenges (Manimaran, 2015). Silver nanoparticles (AgNPs) and silver nitrate ($AgNO_3$) are potent antimicrobial agents that can enhance tissue culture success rates by inhibiting microbial growth (Ruttkay-Nedeoky *et al.,* 2017). Silver ion has a broad spectrum range of anti-microbes (bacteria and fungi). It is used for controlling bacterial growth in many fields (Jung *et al.,* 2008). AgNPs was used as supplemented in media of tissue culture as an anti-contaminant (Alfarraj *et al.,* 2023). This study aims to investigate the efficacy of AgNPs and $AgNO_3$ in promoting germination and reducing contamination during the tissue culture of date palm (cv. Sewi). By comparing the effects of these silver-based agents, we seek to optimize the efficiency and reliability of date palm propagation.

Materials and methods

The Tissue Culture Lab was the site of this investigation at the Agricultural Genetic Engineering Research Institute (AGERI) and Central Laboratory for Research and Development of Date Palm (CLPRD), Agricultural Research Center, Ministry of Agriculture, Giza, Egypt.

2.1. Source of date explants

Immature female inflorescences (5-7cm) were collected in late January to early February from a farm at Abu Suwir, Ismailia Governorate, Egypt with a geographic location 30°29'37.3"N 32°11'20.6"E from mature palm (Sewi cultivar) with age about 15 years. Inflorescences were cultured on MS medium as mentioned by Murashige and Skoog (1962). According to Malhat *et al.,* (2019), MS media was supplemented with 100 mg/l Glutamine, 100 mg/l Myo inositol, 1mg/l Biotein, 5mg/l Thiamin HCl, 40g/l sucrose, and 6 g/l agar. The pH was adjusted to 5.7-5.8. Inflorescence explants were grown from initiation, and maturation to germination stages to be ready in this study.

2.2. Silver nanoparticles (AgNPs) synthesis

Silver nanoparticles (AgNPs) were synthesized according to Šileikaitė *et al.,* (2006) as follows**:** 50 ml of 1mM $AgNO_3$ (prepared in deionized water) was boiled on the heater. Then, a solution of sodium citrate (1% w/v) was added by dropping to the boiled $AgNO_3$ in the presence of stirring. The previous process continued till the color of the mixture

solution converted to a light yellowish. The mixture was lifted from the heater and left to cool at room temperature. Finally, the cooled mixture was centrifuged and the pellet was collected and was weighted and resuspended in deionized water to obtain a solution with a concentration of 100 mg/ml.

2.3. AgNPs characterization

2.3.1. Optical characterization of AgNPs

The absorbance of synthesized AgNPs was assayed as mentioned by Alfarraj *et al.,* (2023). The absorbance was carried out using a UV/Visible spectrophotometer (Orion-Aquamate 8000 UV/VIS) at a wavelength range from 300 to 500nm with an interval wavelength of 5 nm.

2.3.2. Particle size analysis of AgNPs

The synthesized AgNPs were diluted using deionized water. Then, the particle size analysis of AgNPs was measured at the Nanotechnology and Advanced Materials Central Lab. (NAMCL), Agriculture Research Center (ARC) using a laser particle-size analyzer (Zeta sizer nano series- Nano ZS-ZEN3600, Malvern, UK).

2.4. Effect of AgNPs and silver nitrate on the bacterial contamination growth

2.4.1. Bacterial contamination source

Identified isolate of *Bacillus subtilis* which was dicussed by Lewaa *et al.,* (2023) as a bacterial contamination in date palm was kindly obtained from the Dept. of Central Lab's Date Palm Pests and Diseases, Central Lab. for Date Palm, Agric. Res. Center, Giza, Egypt.

2.4.2. AgNPs and silver nitrate versus *B.subtilis*

To study the effect of AgNPs and silver nitrate on *B.subtilis* and reach to minimum inhibitory concentrations (MIC) that can inhibit *B.subtilis* growth. Three different concentrations of each AgNPs and silver nitrate (25, 50, and 100 mg/l) were added to flasks that containing 50 ml of nutrient broth media. Each concentration was separately with 3 replicates to each substance and then the flasks were autoclaved. Afetr sterilization, each concentration was inoculated with 1 ml of of *B.subtilis* ($1x10^8$cfu/ml) and incubated at 37ºc/24hrs/150 rpm. After incubation, the bacterial growth was measured on a spectrophotometer (Orion-Aquamate 8000 UV/VIS) at OD_{600}. This test was carried out according to Dove *et al.,* (2023).

2.5. Effect of AgNPs and silver nitrate on the growth of somatic embryos.

In the germination stage, clusters of somatic embryos (3-4 embryos) were used as explant materials. The explants were cultured in jars containing 20 ml of MS media with added AgNPs (with concentrations 25, 50, and 100 mg/l) and another MS media with added silver nitrate (with concentrations 25, 50, and 100 mg/l). The treated jars were incubated in a controlled growth chamber at 27±2ºC. Clusters were cultured for three subcultures

(one-month intervals). Finally, the growth and development of somatic embryos from the germination and multiplication stages were noticed and recorded.

2.6. Biochemical changes and genetic stability on treated explants

2.6.1. Biochemical changes

Three replicas of each treatment were used to assess the biochemical change. To 1gm of each treatment sample, ten milliliters of 80% ethanol were applied. Using a pestle and mortar, each sample was homogenized, and it was then stored in the dark at 4ºC for 24 hours. The sample was then centrifuged at 13000xg/5min, and the supernatant was moved to the test for total amino acids, total phenol, total indols, antioxidants, flavonoids, indol acetic acid (IAA), and gibberellins (GA3).

2.6.1.1. Total phenol

As mentioned by Ainsworth and Gillespie (2007) and modified by Patel *et al.,* (2010), the total phenol contents were estimated as follows: One ml of the previously extracted sample was put into a tube and 5 ml of dH2o and about 500µl of Folin-Ciocalteu reagent (F-C) were added and the mixture was vortexed for 5min. Then, 1.5 ml of sodium carbonate (20%) was added and the total volume was completed up to 10 ml. In the dark room, the tubes were incubated at room temperature for 2 hrs. Finally, the results were recorded using wavelength 765 nm on a spectrophotometer (Orion AquaMate 8000).

2.6.1.2. Total indol

The following method of estimating the total indol contents was used as mentioned by Selim *et al.* (1978): After transferring one milliliter of the aliquot sample to a tube, 4 milliliters of para di-amino benzoate (PDAB) were added. The tubes were then incubated at 37ºC for one hour. Samples were read at 530 nm using the Orion AquaMate 8000 spectrophotometer.

2.6.1.3. Total amino acids

According to Mc. Grath (1972), after adding one milliliter of the extracted sample and four milliliters of deionized water to fill the remaining space, one milliliter of ninhydrin was added as a reagent. At 100ºC for 15 minutes, the tubes were covered and placed in the water. Following a submersion of the tubes in cooled water, 1 milliliter of 50% ethanol was added. At last, samples were red on the Orion AquaMate 8000 spectrophotometer at wavelength 570 nm.

2.6.1.4. Gibberellins (GA3)

2.6.1.4.1. Gibberellins extraction

Gibberellins were extracted as mentioned by Sayed *et al.,* (2019) as following: The pH of the obtained filtrate was adjusted to 2.8 using HCl (1N) and extracted using ethyl acetate (3 times when the aqueous phase was discarded each time). The ethyl acetate phase was collected and GA3 began ready to be assayed.

2.6.1.4.2. Gibberellins assaying

The method of gibberellins estimation was carried out according to Udagwa and Kinoshita (1961) as follows: About 300µl of collected ethyl acetate phase was mixed well with 1ml of HCl (10N) in a test tube and 1ml of Folin- Ciocalteau reagent was added followed by 3ml H_2O and the mixture was vortexed well. After being submerged in a bath of boiling water for five minutes, the test tubes were allowed to cool to room temperature. Finally, samples were red at wavelength 750 nm on the spectrophotometer (Orion AquaMate 8000).

2.6.1.5. Antioxidant activity

The activity of antioxidants in extracted samples was evaluated using 2, 2'diphenylpicrylhydrazyl (DPPH). The activity of antioxidants was estimated according to Burits and Bucar (2000) as follows:

One ml of the extracted sample was added to the test tube with 1 ml of 0.004% (w/v) of DPPH (dissolved in 95% methanol) and the mixture was incubated for 30 min at room temperature. After that, samples were red on the Orion AquaMate 8000 spectrophotometer at 517nm and compared to DPPH (as blank) using quercetin as a positive control. The percentage activity of antioxidants (%I) was calculated according to the formula:

$$I\% = (A_{blank} - A_{sample} / A_{blank}) \times 100$$

While I%: The antioxidant activity as a percentage

A_{blank}: the positive control's absorbance

A_{sample}: the tested sample's absorbance

2.6.1.6. Total flavonoids

Total flavonoids were estimated according to Christel *et al.,* (2000) as follows: One ml of extracted sample was added to the test tube with 1 ml of 2% aluminum chloride (dissolved in methanol) and 0.2 ml of 1M of potassium acetate. After 30 min of incubation at room temperature, the mixture was red on Orion AquaMate 8000 spectrophotometer at 430nm.

2.6.1.7. Indol acetic acid (IAA)

Estimation of indol acetic acid (IAA) was performed as described by Glickmann and Dessaux (1995) as follows: One ml of extracted sample was mixed with 1 ml of salkowski reagent (12 g/l of $Fecl_3$, 7.9M H_2SO_4) in a test tube and the mixture was incubated in darkness for 30min. At wavelength 530nm on Orion AquaMate 8000 spectrophotometer, the mixture was red to estimate IAA.

2.6.2. Genetic stability

The treated explants in the multiplication stage with AgNPs and silver nitrate were tested for stability on the level of genetics by using fingerprinting for the treated samples compared with control using inter simple sequence repeat (ISSR)-PCR.

2.6.2.1. DNA extraction

Plant tissue's DNA was extracted according to Arif *et al.,* (2010) as follows: One hundred milligrams of plant tissue was grounded in mortar with 500µl of lysis buffer (100ml/pH 8.0 containing 0.4g Na2EDTA, 1.21g tris-base, 8.12g NaCl, 2.0g CTAB, and 2.0g PVP). In a 1.5 ml Eppendorf tube, the grounded tissue was transferred and kept in a water-bath at 60ºc/ 30min. After that, samples were centrifuged at 9500xg/5min and the supernatant was transferred to a new Eppendorf. An equal volume of mixture chloroform: isoamyl-alcohol (24:1) was added to the transferred supernatant and vortexed well and the samples were centrifuged at 9500xg/5min. The aqueous phase was transferred to new Eppendorf and 10% of the transferred volume was added from 3M of potassium acetate and 500µl of cool isopropanol was added and mixed gently and the samples were centrifuged at 11500xg/10min. The supernatant was discarded and 500µl of cool ethanol was added and samples were centrifuged at 7000xg/5min. The pellet was resuspended in 100µl of sterilized dH2O and kept at 4ºC until use.

2.6.2.2. Fingerprinting assays

Fingerprinting for the treated plant's DNA was carried out using ISSR primers (10 primers) as shown in table (1). The PCR reaction (25µl) containing 2 µl of DNA (~250-400ng), 2 µl of each primer (10 p.mol), 12.5µl of TaqMan (OmniPCR, BIO-HELIX), and 6.5µl of dH_2O. The program of PCR amplification was carried out using MJ-Research thermo-cycler (PTC-200) was 94ºc/5min, 35 cycles consisting of 94ºc/45sec, 50.5ºc/45sec, and 72ºc/90 s., and 72 ºC/7min. Electrophoresis was carried out using agarose (1.2%) and the samples were electrophoresed at 120V/30min and using ethidium bromide stain (1mg/ml) to stain the gel.

2.6.2.3. Analysis of data

Amplified fragments of ISSR-PCR were scored as present (1) or absent (0) and only clear fragments were scored. Using Past program (version 4.03), a matrix of similarity using Jaccard's coefficient was used to analyze the obtained data.

Table (1): List of ISSR primer sequences used in PCR

No.	Name of primer	Sequence 5'-3'	
1	ISSR-1	AGAGAGAGAGAGAGAGYC	Abouseada *et al.,* (2023)
2	ISSR-2	AGAGAGAGAGAGAGAGYG	
3	ISSR-3	ACACACACACACACACYT	
4	ISSR-4	ACACACACACACACACYG	
5	ISSR-5	GTGTGTGTGTGTGTGTYG	
6	ISSR-6	CGCGATAGATAGATAGATA	
7	ISSR-7	GACGATAGATAGATAGATA	
8	ISSR-8	AGACAGACAGACAGACGC	
9	ISSR-9	GATAGATAGATAGATAGC	
10	ISSR-10	GACAGACAGACAGACAAT	

2.7. Statistical analysis of data

All collected data were statistically examined utilizing SPSS version 23 and one method of analysis of variance (ANOVA), following Snedecor and Cochran (1980).

Results

Spectroscopy characterization

UV/Visible spectrophotometer was used to record the absorbance spectrum for synthesized AgNPs at wavelength range from 300 to 500nm with interval wavelength 5nm and determination of the peak. The obtained results as shown in fig (1) showed that the maximum absorbance at 405nm.

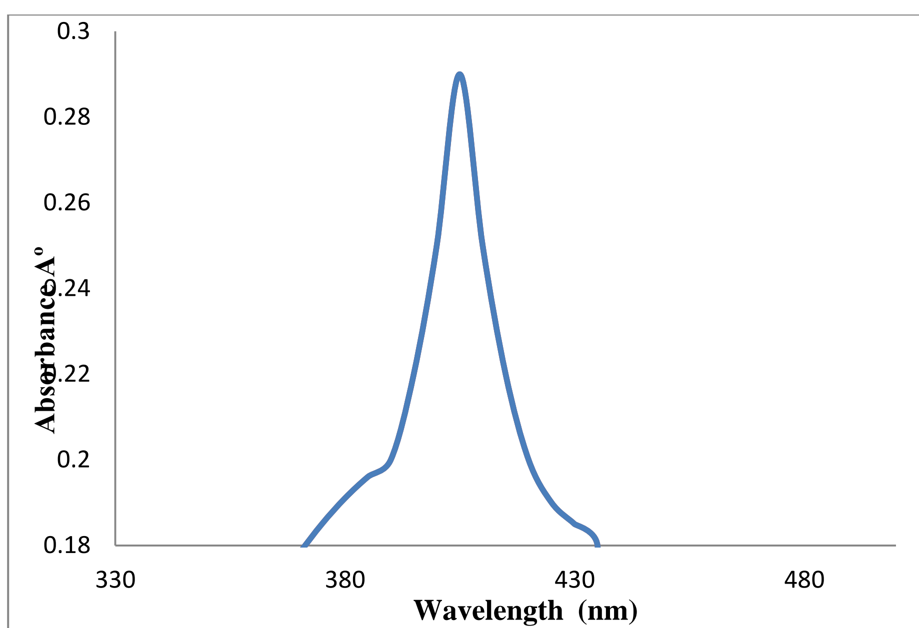

Fig. (1): Absorbance spectrum of synthesized AgNPs

Particle size analysis

The size of AgNPs was measured using particle zeta sizer analysis using deionized water as a dispersant with a refractive index: of 1.330 and with viscosity: of 0.8872cP. From the results of zeta sizer analysis as shown in Fig (2), the average of synthesized AgNPs diameter was 43.82nm.

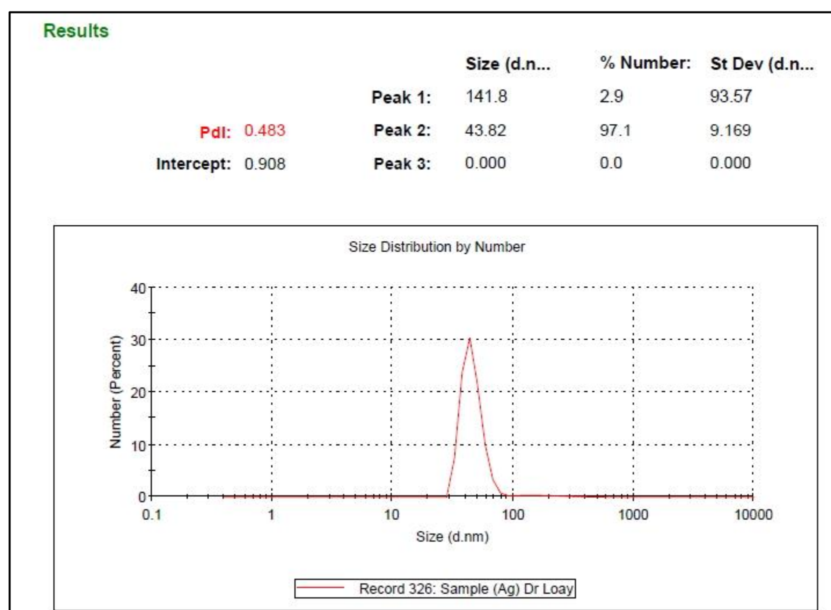

Fig. (2): Particle size analysis of AgNPs

Effect of AgNPs and silver nitrate on bacterial growth

The effectiveness of AgNPs and silver nitrate (with different concentrations) in reducing the bacterial growth (*B.subtilis*) as a contaminant bacterium was evaluated by inoculation of *B.subtilis* inoculum in nutrient broth medium supplemented with several concentrations and after 24 hrs from incubation at 37ºc, the bacterial growth mass was evaluated at OD_{600} on a spectrophotometer. MIC was recognized as the lowest concentration which inhibited the bacterial growth and measured by ≤0.1 at OD_{600} The result as shown in Fig (3) mentioned that each increase in AgNPs and silver nitrate concentration corresponds to a decrease in bacterial growth until reaching a concentration of 100 mg/l of AgNPs which is considered the MIC of bacterial growth because it gave the lowest concentration of bacterial growth ($OD_{600} \leq 0.1$).

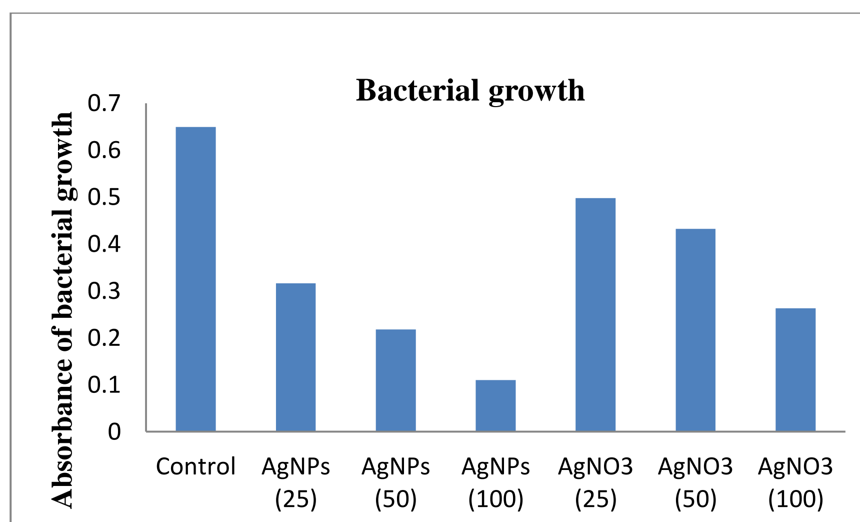

Fig (3): Effect of AgNPs and silver nitrate on bacterial growth

Effect of AgNPs and silver nitrate on the germination stage

Growth of embryos and shoots (after 3 subcultures) from the germination stage on MS media which was treated with different concentrations of AgNPs and silver nitrate (25, 50, and 100mg/l) was noticed and illustrated in Fig (4). Results as shown in table (2) and in fig (5) referred to the treatments with different concentrations of AgNPs were better than the treatments with different concentrations of silver nitrate. In addition to that, 50mg/l of AgNPs was more significant on the number and growth of embryos and shoot formation compared with all treatments.

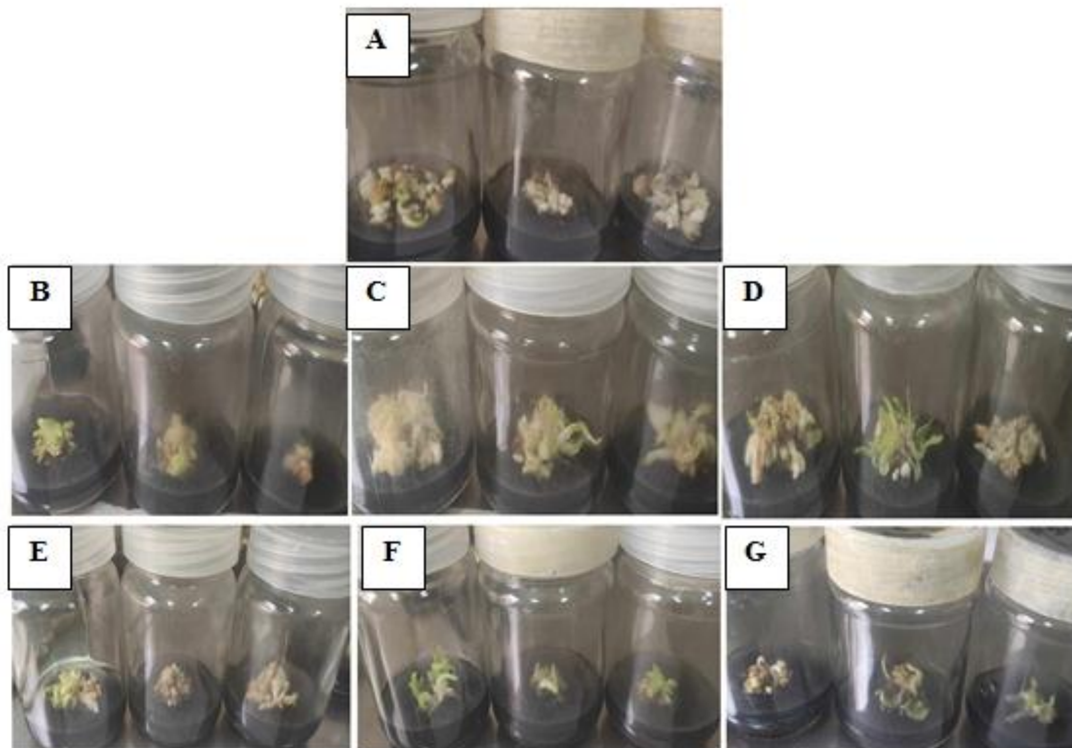

Fig. (4): Effects of different concentrations of AgNPs and silver nitrate on germination Stage A) Control, B) AgNPs (25 mg/l), C) AgNPs (50 mg/l), D) AgNPs (100 mg/l), E) AgNO3 (25 mg/l), F) AgNO3 (50 mg/l), G) AgNO3 (100 mg/l)

Table (2): Effect of AgNPs and silver nitrate concentrations on the number of embryos and shoots during the germination stage.

Treatment	No. of embryos	No. of shoots
Control	14.67^{b}	2.33^{a}
AgNPs (25)	10.3^{ab}	4.1^{a}
AgNPs (50)	26^{c}	13.33^{c}
AgNPs (100)	14.67^{b}	7.67^{b}
$AgNO_3$ (25)	5^{a}	3.67^{a}
$AgNO_3$ (50)	3^{a}	4.33^{ab}
$AgNO_3$ (100)	2.67^{a}	2.33^{a}
P-value	0.01	0.01
LSD	5.4	2.1

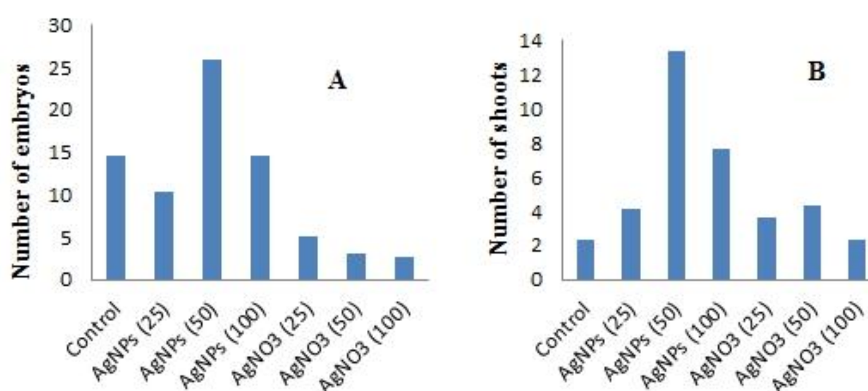

Fig (5): Effect of AgNPs and silver nitrate concentrations A) on embryo number B) on shoot number

Biochemical changes

Some of the biochemical (total phenol, total indol, total amino acid, gibberellins, anti-oxidants, total flavonoids, and indol acetic acid) in the treated explants with different concentrations with AgNPs and silver nitrate were assayed and recorded in Table (3) and illustrated in Fig (6). Data showed that there is significant diversity in total phenol in all treatments excepting the treated explants with a concentration of AgNPs 50 and 100 mg/l and also the concentration of AgNO3 50 and 100 mg/l and there was the high concentration of total phenol when the low concentration was in control data showed there is no significant between each both treatments. Total indol was estimated in all treatments. The results showed significant differences between the treatments but there was non-significant between the treatment of 50mg/l of AgNPs and control and also between $AgNO_3$ with concentration 25 and AgNPs 50, and 100 mg/l and the high level of total indol was in the treatment of $AgNO_3$ with concentration 100mg/l and the low level was in the control sample and treatment of AgNPs 50mg/l. Estimation of total amino acid was carried out. Data showed that the low concentration of amino acid was in $AgNO_3$ with 25mg/l and the high concentration was in control and $AgNO_3$ 100mg/l. While data showed that there is no significant between AgNPs 25 and 50mg/l and between AgNPs 50 and 100mg/l. The obtained data referred to the level of gibberellins (GA3) was different in all treatments and the high level was in AgNPs 100mg/l and the low level was in 50 mg/l of $AgNO_3$. Anti-oxidants in the treatments were evaluated. Results showed that there was no significant between control and $AgNO_3$ 50 and 100 mg/l while there was a significant between all treatments except AgNPs 25mg/l, and $AgNO_3$ 25mg/l. The high level of anti-oxidants was in AgNPs 25mg/l, and $AgNO_3$ 25mg/l (91.1%) and the low level was in AgNPs 100mg/l (66.83%). Total flavonoids were determined and the results showed that there is no significant between all concentrations of AgNPs and $AgNO_3$. Data showed that the high level of total indol acetic acid (IAA) was in $AgNO_3$ 50 and 100mg/l (0.0092 mg/g) and the low level was in control, AgNPs 25mg/l, and $AgNO_3$ 25mg/l (0.0029mg/g).

Table (3): Biochemical changes on treated explants.

Treatments	Total phenol mg/g	Total indol mg/g	Total amino acid mg/g	GA3 mg/g	Anti-oxidants %	Total flavonoids mg/g	Indol acetic acid mg/g
Control	15.08[a]	0.01[a]	0.34[d]	24.54[d]	83.17[c]	1.3[a]	0.0029[a]
AgNPs (25)	47.82[d]	0.05[d]	0.09[ab]	21.23[c]	91.17[d]	1.78[a]	0.0029[a]
AgNPs (50)	28.46[c]	0.02[ab]	0.19[bc]	23.73[d]	68.67[b]	1.24[a]	0.0071[b]
AgNPs (100)	30.12[c]	0.03[c]	0.28[cd]	29.17[e]	66.83[a]	1.29[a]	0.0071[b]
$AgNO_3$ (25)	18.06[b]	0.03[bc]	0.03[a]	19.61[bc]	91.17[d]	0.99[a]	0.0029[a]
$AgNO_3$ (50)	53.35[e]	0.06[d]	0.17[bc]	13.36[a]	84.25[c]	1.26[a]	0.0092[c]
$AgNO_3$ (100)	52.80[e]	0.09[e]	0.39[d]	19.11[b]	83[c]	1.9[a]	0.0092[c]
P-value	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**
LSD	**2.15**	**0.012**	**0.11**	**3.1**	**2.15**	**0.96**	**0.0016**

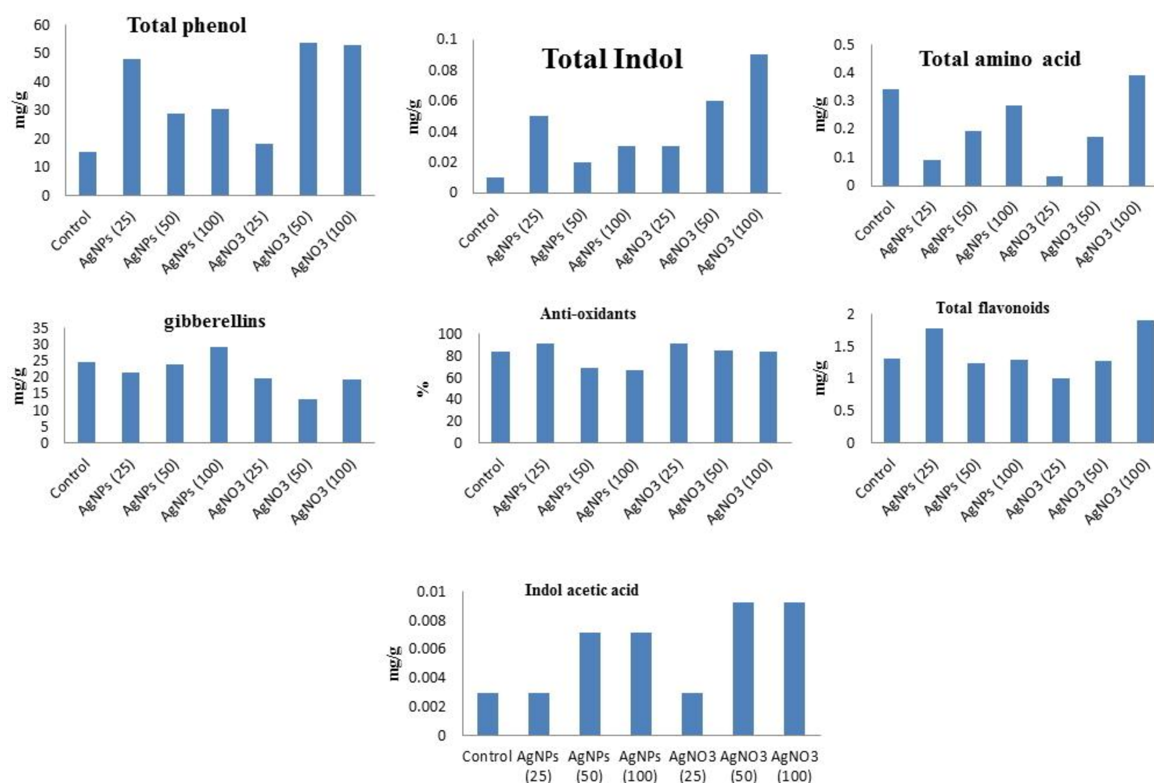

Fig (6): Biochemical changes on treated explants

Genetic stability

Genetic stability on the treated samples with several concentrations of AgNPs and silver nitrate was carried out using ISSR-PCR using 10 ISSR primers and the result of PCR fragments on electrophoresed gel for each primer was recorded and the similarity and distance indices were calculated. From the obtained results of the ISSR-PCR test, as

presented in Fig (7), there was no change in the results of the fragments formed in the PCR gel, and therefore the similarity result was stable at 100% in all treated samples.

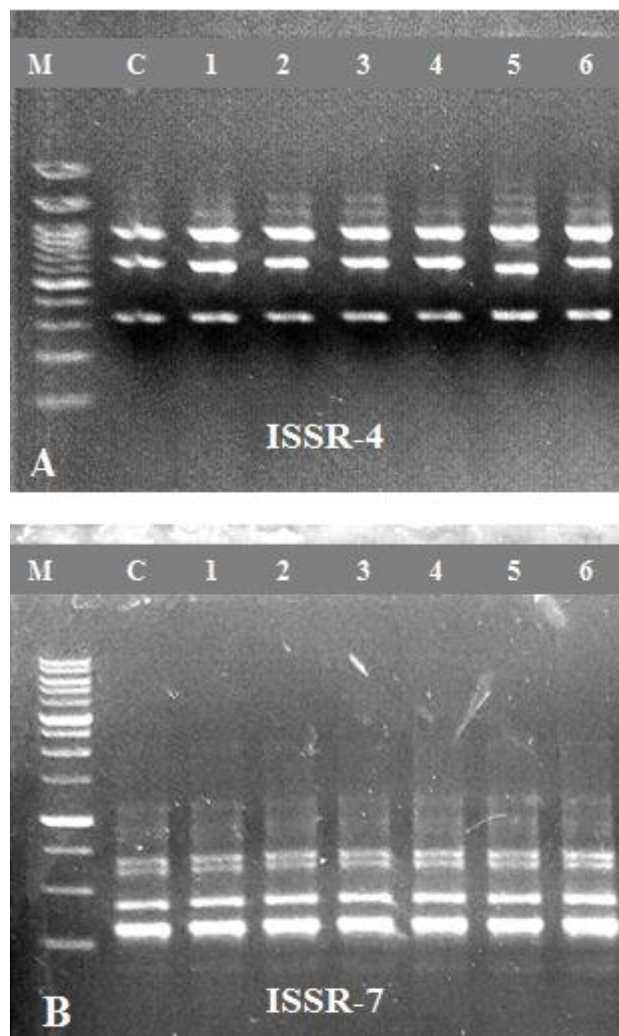

Fig. (7): Gel profile for treated samples with several concentrations of AgNPs and silver nitrate using ISSR-PCR technique using ISSR-4 primer in (A) and ISSR-7 (B). M: Marker (1Kb), C: control sample, 1: AgNPs (25mg/l), 2: AgNPs (50mg/l), 3: AgNPs (100mg/l), 4: $AgNO_3$ (25mg/l), 5: $AgNO_3$ (50mg/l), and 6: $AgNO_3$ (100mg/l)

Discussion

Many methods were documented to prepare silver nanoparticles like chemical and physical methods. The chemical method depends on reducing agents such as sodium citrate, hydrazine hydrate, and sodium hydroxide. In this study silver nanoparticles (AgNPs) were prepared using sodium citrate. The prepared solution of AgNPs was stopped based on the color of the solution (light yellowish). Each nanoparticles metals get several colors based on the type, shape, and size of synthesized nanoparticles. In the case of silver nanoparticles, the color reflected from AgNPs formed was light yellowish and

stopped before it turned yellow to brown to get the shape, and size of AgNPs in tiny range. This preparation method agreement with Zhang *et al.,* (2011) and Tessema *et al.,* (2023) absorption and size of AgNPs from exposure to wavelength in the range 300 to 500nm with interval wavelength 5nm measured by nano-sizer were carried out. The results observed that the maximum absorbance to the synthesized AgNPs was 405nm and the size of the particles was 43.8nm. In this case, the peak's range which formed from the absorbance to AgNPs had to lie between 400-420nm depending on the size, purity, and concentration of AgNPs (Christopher *et al*., 2011). Particle radius in theoretical calculations ranged from 5 nm to 100 nm based on the preparation method and purity. As described by Guzmán *et al.,* (2009), AgNPs was prepared using hydrazine hydrate as a reducing agent and the maximum absorption band was at 418nm and the range of particle size was between 8-50nm. Also, Kumar *et al.,* (2017) reported that the absorption peak for AgNPs was at 411 nm with a size of 20 ± 2 nm Alfarraj *et al.,* (2023) noted that absorption' maximum of synthesis AgNPs was at 415nm and the size of AgNPs was in range 21-26nm. On the other hand, Šileikaitė *et al.,* (2006) referred to the absorption's peak of synthesized AgNPs that was at 445nm while the particle size of AgNPs was about 240-400nm. Minimum inhibitory concentrations (MIC) of AgNPs and silver nitrate for *B.subtilis* as contamination bacteria were evaluated by incubation of the nutrient broth supplemented with several concentrations of AgNPs and silver nitrate (25, 50, and 100mg/l) and inoculated with *B.subtilis* ($1x10^8$cfu). The culture was measured at OD_{600} and the results which get ≤ 0.1 consider MIC. From the obtained results, AgNPs with a concentration of 100 mg/l get 0.1 at OD_{600}. Kumar *et al.,* (2017) referred to MIC of synthesized AgNPS against *staphylococcus aureus* (MRSA), *E.coli*, *pseudomonas aeruginosa*, and *klebsiella pneumoniae* was estimated and found that a concentration of 10ml/l from AgNPs consider MIC for all previous bacteria. Dove *et al.* (2023) mentioned that MIC of AgNPs was estimated for *E.coli* and the results showed that MIC was 3µl/l. Also Alfarraj *et al.,* (2023) mentioned that AgNPs was used as an antibacterial agent for *Bacillus pumilus* as a contaminant bacterium in tissue culture of *rumex nervosus* plants. Results showed that a concentration of 40mg/l of AgNPs was MIC for the contaminant bacterium. Synthesized AgNPs and silver nitrate were used as supplements in MS media for date palm explants in the germination stage with several concentrations (25, 50, and 100 mg/l). After 3 subcultures (with 1-month intervals) the number of embryos and shoot formation was observed and the results showed that the concentration of 50 mg/l of AgNPs was significantly increased in embryos and shoots. Alfarraj *et al.,* (2023) represented that 40mg/l of AgNPs was the best concentration for *rumex nervosus* plants in tissue culture. Genetic stability of treated explants with several concentrations from AgNPs and silver nitrate (25, 50, and 100mg/l) was assayed using ISSR-PCR technique using 10 inter simple sequence repeat (ISSR) primers. From the obtained results, the genetics of treated explants didn't change compared with the control explant. In agreement with Al-Mayahi, (2022), the genetic stability of date palm explants (cv. Barhee) were assayed after treating the explants with the combination of chitosan and thidiazuron. Genetic stability was assayed using random amplified polymorphic DNA (RAPD) using RAPD-PCR and the results referred to the genetic stability of explants didn't change

compared with control. Also Arafa *et al.,* (2023a) mentioned that iron nanoparticles (FeNPs) was used with different concentrations (0.5, 1, 2, 4 mg/l) to enhance the growth of explants of date palm (*cv* Bartmoda) in tissue culture technique and the genetic stability of treated explants was assayed by using 7 RAPD primers. RAPD-PCR technique was used and the results represented that the genetic stability of explants didn't change comparing with control. Arafa *et al.,* (2023b) tested the genetic stability of an explant of date palm (cv. Zaghloul) that was produced from tissue culture and compared with the mother plant using ISSR-PCR technique. Results showed that there are no differences in similarity between explants and mother plants.

Conclusion

In conclusion, the study demonstrated that silver nanoparticles (AgNPs) and silver nitrate (AgNO3) effectively enhance the tissue culture efficiency of date palm (*Phoenix dactylifera* L.) by controlling microbial contamination and promoting growth. AgNPs at 50 mg/L significantly improved embryo and shoot formation, while higher concentrations of AgNO3 elevated phenolic and IAA levels. Additionally, treatments did not induce genetic alterations, indicating the safety of these agents for improving tissue culture practices. These findings suggest that AgNPs and AgNO3 can be valuable tools in the propagation of date palms, contributing to better crop production in challenging environments.

References

Abdel-Karim, A. H. (2017). Identifying and controlling contamination of date palm tissue cultures. In *Date Palm Biotechnology Protocols Volume I: Tissue Culture Applications* (pp. 165-174).

Abouseada, H. H., Mohamed, A. S. H., Teleb, S. S., Badr, A., Tantawy, M. E., Ibrahim, S. D., & Ibrahim, M. (2023). Genetic diversity analysis in wheat cultivars using SCoT and ISSR markers, chloroplast DNA barcoding and grain SEM. *BMC Plant Biology, 23*(1), 193.

Ainsworth, E. A., & Gillespie, K. M. (2007). Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin–Ciocalteu reagent. *Nature Protocols, 2*(4), 875-877.

Alfarraj, N. S., Tarroum, M., Al-Qurainy, F., Nadeem, M., Khan, S., Salih, A. M., & Perveen, K. (2023). Biosynthesis of silver nanoparticles and exploring their potential of reducing the contamination of the in vitro culture media and inducing the callus growth of *Rumex nervosus* explants. *Molecules, 28*(9), 3666.

Al-Mayahi, A. M. W. (2022). In vitro propagation and assessment of genetic stability in date palm as affected by chitosan and thidiazuron combinations. *Journal of Genetic Engineering and Biotechnology, 20*(1), 165.

Arafa, R. N., Elsayh, S. A., El-Din, I. M., El-Habashy, S., Ahmed, E. A., Afifi, E. H., & Gaber, M. F. (2023a). Impact of iron nanoparticles application on date palm in-vitro regeneration stability. *Plant Cell Biotechnology and Molecular Biology, 24*(3-4), 89-107.

Arafa, R. N., Elsayh, S. A., Abdalgaleel, M. M., El-Habashy, S., Ahmed, E. A., Afifi, E. H., & AA, D. H. (2023b). The genetic stability of date palm shoots regenerated from leaves explant. *International Journal of Environment, Agriculture and Biotechnology, 8*(5).

Arif, I. A., Bakir, M. A., Khan, H. A., Ahamed, A., Al Farhan, A. H., Al Homaidan, A. A., & Shobrak, M. (2010). A simple method for DNA extraction from mature date palm leaves: impact of sand grinding and composition of lysis buffer. *International Journal of Molecular Sciences, 11*(9), 3149-3157.

Burits, M., & Bucar, F. (2000). Antioxidant activity of *Nigella sativa* essential oil. *Phytotherapy Research, 14*, 323-328.

Christel, Q. D., Gressier, B., Vasseur, J., Dine, T., Brunet, C., Luyckx, M., Cazin, J. C., Bailleul, F., & Trotin, F. (2000). Phenolic compounds and antioxidant activities of buckwheat hulls and flour. *Journal of Ethnopharmacology, 72*, 35–42.

Christopher, D., Alee, K. S., & Rao, D. N. (2011). Synthesis and characterization of silver nanoparticles produced by laser ablation technique in aqueous monomer solution. *J. Trends Chem, 2*(1).

Dove, A. S., Dzurny, D. I., Dees, W. R., Qin, N., Nunez Rodriguez, C. C., Alt, L. A., & Czyż, D. M. (2023). Silver nanoparticles enhance the efficacy of aminoglycosides against antibiotic-resistant bacteria. *Frontiers in Microbiology, 13*, 1064095.

Elaziem, A., & Mahfouz, T. (2023). In vitro propagation of date palm (*Phoenix dactylifera* L.) "Lakramat" date palms in Aswan Governorate. *Egyptian International Journal of Palms, 3*(1), 1-20.

Ghnimi, S., Umer, S., Karim, A., & Kamal-Eldin, A. (2017). Date fruit (*Phoenix dactylifera* L.): An underutilized food seeking industrial valorization. *NFS Journal, 6*, 1-10.

Glickmann, E., & Dessaux, Y. (1995). A critical examination of the specificity of the Salkowski reagent for indolic compounds produced by phytopathogenic bacteria. *Applied and Environmental Microbiology, 61*, 793-796.

Guzmán, M. G., Dille, J., & Godet, S. (2009). Synthesis of silver nanoparticles by chemical reduction method and their antibacterial activity. *International Journal of Chemical and Biomolecular Engineering, 2*(3), 104-111.

Jung, W. K., Koo, H. C., Kim, K. W., Shin, S., Kim, S. H., & Park, Y. H. (2008). Antibacterial activity and mechanism of action of the silver ion in *Staphylococcus aureus* and *Escherichia coli*. *Applied and Environmental Microbiology, 74*(7), 2171-2178.

Kumar, M., Bala, R., Gondil, V. S., Jain, D. V. S., Chhibber, S., Sharma, R. K., & Wangoo, N. (2017). Efficient, green and one pot synthesis of sodium acetate functionalized silver nanoparticles and their potential application as food preservative. *BioNanoScience, 7*, 521-529.

Lewaa, L. M., Othman, B. A., Metwaly, A. M., & El Dougdoug, K. A. (2023). Application of Bacilli phages for controlling *Bacillus subtilis* associated date palm plantlets malformation in tissue culture. *Egyptian International Journal of Palms, 3*(2), 15-37. https://doi.org/10.21608/esjp.2023.370487

Malhat, M. M., El-Wakeel, H., El-Hamid, A., Khalil, S. S., & Hassan, M. M. (2019). Direct embryogenesis and indirect organogenesis of date palm (*Phoenix dactylifera* L.) cv. Sewi using immature female inflorescences. *Arab Universities Journal of Agricultural Sciences, 27*(1), 737-747.

Manimaran, M. (2015). A review on nanotechnology and its implications in agriculture and food industry. *Asian Journal of Plant Science Research, 5*(7), 13-15.

Marinova, E., De Meyer, M., Van Loon, G., & Willems, H. (2011). Plant economy and land use in Middle Egypt during the late Antique/early Islamic period – archaeobotanical analysis of mud bricks and mud plasters from the area of Dayr al Barsha. In *Windows on the African Past: Current Approaches to African Archaeobotany* (pp. 119).

McGrath, R. (1972). Protein measurement by ninhydrin determination of amino acids released by alkaline hydrolysis. *Analytical Biochemistry, 49*, 95-102.

Murashige, T., & Skoog, F. (1962). A revised medium for rapid growth and bio assays with tobacco tissue cultures. *Physiologia Plantarum, 15*(3).

Oladzad, S., Fallah, N., Mahboubi, A., Afsham, N., & Taherzadeh, M. J. (2021). Date fruit processing waste and approaches to its valorization: A review. *Bioresource Technology, 340*, 125625.

Patel, A., Patel, A., Patel, A., & Patel, N. M. (2010). Estimation of flavonoid, polyphenolic content and in vitro antioxidant capacity of leaves of *Tephrosia purpurea* Linn. (Leguminosae). *International Journal of Pharma Sciences and Research, 1*(1), 66-77.

Ruttkay-Nedeoky, B., Krystofova, O., Nejdl, L., & Adam, V. (2017). Nanoparticles based on essential metals and their phytotoxicity. *Journal of Nanobiotechnology, 15*(1), 33.

Sayed, A. E. A., Hassan, E. A. E. T., El-Tobgy, K. M. K., & Ramadan, E. M. (2019). Characterization of endophytic bacteria associated with some medicinal plants. *Arab Universities Journal of Agricultural Sciences, 27*(5), 2513-2526.

Selim, H. H., Fayek, M. A., & Sewidan, A. M. (1978). Reproduction of Bircher apple cultivar by layering. *Annals of Agricultural Science Moshtohor, 9*, 157-165.

Šileikaitė, A., Prosyčevas, I., Puišo, J., Juraitis, A., & Guobienė, A. (2006). Analysis of silver nanoparticles produced by chemical reduction of silver salt solution. *Materials Science, 12*(4), 287-291.

Snedecor, G. W., & Cochran, W. G. (1980). *Statistical Methods* (7th ed.). Iowa State University Press.

Soomro, A. H., Marri, A., & Shaikh, N. (2023). Date palm (*Phoenix dactylifera* L.): A review of economic potential, industrial valorization, nutritional and health significance. In *Neglected Plant Foods of South Asia: Exploring and Valorizing Nature to Feed Hunger* (pp. 319-350).

Tessema, B., Gonfa, G., Hailegiorgis, S. M., Prabhu, S. V., & Manivannan, S. (2023). Synthesis and characterization of silver nanoparticles using reducing agents of bitter leaf (*Vernonia amygdalina*) extract and tri-sodium citrate. *Nano-Structures & Nano-Objects, 35*, 100983.

Udagwa, K., & Kinoshita, S. (1961). A colorimetric determination of gibberellin A3. Part II. *Journal of the Agricultural Chemical Society of Japan, 35*(3), 219-223.

Zhang, Z., Chen, H., Xing, C., Guo, M., Xu, F., Wang, X., & Tang, J. (2011). Sodium citrate: A universal reducing agent for reduction/decoration of graphene oxide with gold nanoparticles. *Nano Research, 4*, 599-611.

مقارنة معملية بين جزيئات الفضة النانوية ونترات الفضة كمعززات للنمو و مقللة للتلوث في زراعة الأنسجة لنخيل التمر (صنف سيوي) في مرحلة الإنبات

لؤي لواء منصور[١] ، عبد الرحمن متولي محمد[١] ، مرفت محمد حسن ملهط [٢]

[١]قسم أمراض وآفات نخيل البلح- المعمل المركزي للأبحاث وتطوير نخيل البلح، مركز البحوث الزراعية، الجيزة

[٢]معهد بحوث الهندسة الوراثية الزراعية- قسم زراعة الانسجة والتحول الوراثي، مركز البحوث الزراعية، الجيزة

الملخص العربي

يُعد نخيل التمر احد النباتات ذات الاهمية الاستراتيجية في مصر ومنطقة الشرق الاوسط، كما تُعد تقنية زراعة الانسجة احدى الطرق الحديثة المستخدمة في مجال اكثار نخيل التمر. ويعتبر التلوث في معامل زراعة الانسجة من اكبر المشاكل التي تواجه هذه التقنية. تعتبر تقنية النانوتكنولوجي هي واحدة من التقنيات التي تعمل على حل هذه المشكلة كما أن لها دور في تعزيز واكثار النباتات. في هذه الدراسة تم استخدام الازهار المؤنثة الغير ناضجة من نخيل التمر (صنف السيوي) كمنفصل نباتي في زراعة الانسجة. بالاضافة الى ذلك تم استخدام جزيئات الفضة النانوية ونترات الفضة لدراسة الفرق بينهما في التأثير على زيادة الاعداد والنمو والتطور للأجنة الجسدية و التلوث. تم تحضير جزيئات الفضة النانوية ودراسة منحنى طيف الامتصاص وكانت قمة المنحنى عند ٤٠٥ نانومتر كما تم تقدير قياس حجم الجزيئات وكانت بقطر ٤٣.٨ نانومتر. كل من جزيئات النانو و نترات الفضة تم استخدامهما بتركيزات مختلفة (٢٥، ٥٠ و١٠٠ مجم/لتر) في مزارع الانسجة وتم دراسة أقل تركيز مثبط لنمو بكتيريا *B.subtilis* كميكروب ملوث في بيئات زراعة الانسجة وذلك عند القراءة عند طيف امتصاص ٦٠٠ وكانت افضل نتيجة موجودة من التركيزات كمثبطات للبكتيريا عند تركيز ١٠٠ مجم/لتر من جزيئات الفضة النانوية. وفي حالة استخدم نفس التركيزات السابقة كمعززات لنمو الاجنة في مرحلة الانبات فكانت افضل تركيزات مستخدمة لزيادة نمو وتكوين الاجنة و الافرع بشكل معنوي كانت باستخدام تركيز ٥٠مجم/لتر من جزيئات الفضة النانوية. تم دراسة بعض التغيرات الكيميائية على النباتات نتيجة المعاملة بالتركيزات المختلفة من جزيئات الفضة النانوية و نترات الفضة، كانت تقدير الفينولات الكلية وحمض الاندول استيك اسيد (IAA) في أعلى مستوياتها مع نترات الفضة بتركيز ٥٠ و ١٠٠ مجم/لتر بينما كانت في اقل مستوياتها في النبات الكنترول وكذلك تركيز ٢٥مجم/لتر من نترات الفضة. وفي حالة تقدير كل من الاندولات الكلية والاحماض الامينية الكلية كانت في اعلى مستوياتها عند استخدام نترات الفضة بتركيز ١٠٠مجم/لتر وكانت في اقل مستوياتها في النبات الكنترول و في نترات الفضة بتركيز ٢٥ مجم/لتر. وأيضا عند قياس تركيز الجبريللين كانت في اعلى مستوياتها عند تركيز ١٠٠ مجم/لتر من جزيئات الفضة النانوية واقل مستوياتها عند ٥٠ مجم/لتر من نترات الفضة. في حالة قياس مضادات الاكسدة، فقد زادت بشكل معنوي في النباتات المعاملة بتركيز٢٥مجم/لتر من جزيئات الفضة النانوية بينما قلت في حالة استخدام تركيز ١٠٠ مجم/لتر من جزيئات الفضة النانوية. بينما في حالة تقدير الفلافونويد، فقد لوحظ انه لا يوجد تغيير معنوي في جميع المعاملات. تم أيضا دراسة مدى الثبات الوراثي على النباتات التي تم معاملتها بالتركيزات المختلفة سابقة الذكر من جزيئات الفضة النانوية و نترات الفضة وذلك باستخدام نقنية تفاعل البلمرة المتسلسل (PCR) باستخدام بادئات من النوع التكرار التسلسلي البيني البسيط (ISSR) وتم فيها استخدام عدد ١٠ بادئات مختلفة. ومن خلال النتائج التي تم التحصل عليها فقد تم الوصول انه لايوجد أي تغيير حدث للمدى الوراثي وأن درجة الثبات الوراثي للنباتات لم تتغير مقارنة مع النبات الكنترول.

الكلمات الدالة: نخيل التمر، جزيئات الفضة النانوية، نترات الفضة، زراعة الانسجة، الثبات الوراثي