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Different Responses in Micropropagation of Three Avocado Cultivars using Silver and Zinc Nanoparticles

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Abstract

In recent years, nanomaterials have drawn considerable interest in different areas of science, including plant tissue culture. A critical step for successful micropropagation is preventing or avoiding microbial contamination. This problem can be described as a bottleneck when using single node culture during the establishment stage of different woody plants. In this study, the influence of (40 and 80 mg/l) of both silver and zinc oxide (Ag and ZnO) nanoparticles (NPs) during establishment and micropropagation stages of avocado "cv." Hass, Fuerte and Red were examined by incorporating AgNPs and ZnONPs into cultural media. Also, *in vitro* minimal inhibitory concentration (MIC) for both types was studied on various bacterial and fungal strains. The obtained results showed a significant difference between various types and concentrations of nanoparticles on microbial contamination. In relation to how nanoparticles avocado affect tissue culture, the experiments showed that, AgNPs were more effective in increasing all studied parameters compared with ZnONPs. Moreover, adding 40 mg/l of silver nanoparticles to either establishment or multiplication media resulted in the greatest number of shoots/explant, number of leaves/explant and shoot length/explant compared with 80 mg/l. Biometric parameters for shoot multiplication were affected positively by adding 40 mg/l AgNPs to woody plant multiplication medium (WPM). To our knowledge, there are no previous investigations utilizing both types of nanomaterials with avocado cultures *in vitro*. Our results suggested that, culture media supplemented with AgNPs reflected positively for reducing contamination and the enhancement of avocado micropropagation in vitro.

Key words: Silver, Zinc, Nanotechnology, Micropropagation, Contamination, Avocado.

1. Introduction

Nanotechnology, a relatively new branch of science, has more and more attention lately and is predicted to revolutionize a wide range of agricultural fields including tissue culture [1]. Nanoparticles and nanomaterials are increasingly being viewed as "new antibiotics." Silver nanoparticles (AgNPs) are a new non-toxic material that have a good capability for eliminating fungal, bacterial and virus contamination without adverse effects on plant growth and development [2]–[10].Silver nanoparticles (AgNPs) have numerous applications in plant biotechnology. For instance, it has a unique biological activities to be able to control different types of pathogens such as bacteria, virus and fungus, without affecting the development and functionality of plants [11], [12]. Additionally, it has been described that, it increases the survival of plant shoots growing *in vitro* [5]–[8], [10], and may improve the growing of plants and increase shoot multiplication rate *in vitro* when added to the media [13]–[16].

On the other hand, zinc oxide (ZnO) stands out as a promising antimicrobial agent due to its photocatalytic activity [17], high penetration capability, and relative safety for multicellular organisms, especially when compared to other methods used for sterilizing explants [18]. Zinc oxide nanoparticles (ZnONPs) has strong antibacterial action against a wide range of bacteria and fungi [19]–[22], although, research is being conducted on the bactericidal characteristics of ZnO, until now. Zinc oxide exhibits enhanced antimicrobial activity as particle size is reduced to the nanometer scale, allowing ZnONPs to interact with the cell surface and/or nucleus during cellular penetration [23]. Regarding to the effects of on plant tissue culture, [24], stated that the using of ZnONPs caused a positive influence in growth and a significant enhancement of *steviol glycosides*of *Stevia rebaudiana*.

Plant tissue culture is an essential component of plant biotechnology, and advancements in this approach have a significant impact on progress in other biotechnological fields. The basic goal of plant tissue culture is to produce plants (or plant parts) aseptically on the suitable kind of nutrient medium based on the concept of totipotency [25]. For producing pathogen-free, true-to-type cultivars in large quantities, micropropagation has been documented as tool for genetic improvement and germplasm conservation in different species like avocado [26] and olive [6].

Explants in tissue culture are particularly vulnerable to microbial contamination, which poses a severe risk to plant tissue culture procedure. The most important step in plant tissue culture is explant sterilization, and success rates are greater when

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using an optimized procedure [27]. Explants need to be treated with disinfectants for a certain amount of time and at an appropriate concentration, in order to eliminate or kill the contaminants, however, without side effects on the biological activity of explants [28], [29].

Avocado (*Persea americana* Mill.), With a history dating back around 10,000 years, was produced from tropical trees known for their pear-shaped, blackish-green fruits, which are prized for their high nutritional value, creamy texture, and distinctive taste [30]–[32]. Avocado is a highly nutritious fruit containing essential nutrients like carbohydrates, proteins, and fats, as well as a wide range of vitamins (A, B, C, D, E, and K) and minerals with a unique flavor and healthy oil [33]. Additionally, avocado oil is widely used in the cosmetics industry due to its therapeutic properties, enhancing the economic value of the avocado industry. To conserve genetic stability during propagation, two approaches can be used: vegetative propagation or seeds obtained from controlled pollination [34]. However, in open-pollinated plants like avocado, vegetative propagation is the only method that can maintain genetic uniformity during propagation [35]. Clonal rootstock propagation of avocado remains a significant challenge and is a critical step in the avocado propagation process. With the advent of micropropagation techniques, efforts have focused on developing an industry-appropriate tissue culture method for avocado clonal propagation. Besides, the direct application of micropropagation as a mass propagation tool for economically important plants has attracted significant attention for several reasons, including its independence from climatic variations, high multiplication rate, and minimal space requirements under well-controlled, pest and disease-free conditions [33]. Expanding in growing avocado acreage is a welcome need. Geographically, the best place to grow avocados is Egypt. With its land, climate, and proximity to the Middle East and European markets, no other nation will be able to compete it. Therefore, there is a strong need for advancement in avocado-applied research and the use of cutting-edge techniques. Thus, our goals of this work are; 1- to test minimal inhibitory concentration (MIC) for AgNPs and ZnONPs against various bacterial and fungal strains, 2- to examine the positive effect of using AgNPs and ZnONPs in improving *in vitro* growing and multiplication of three avocado cultivars "cv." Hass, Red and Furete.

2. Materialsand Methods

a. Plant material

Actively growing shoots of avocado "cv." Hass, Red and Furete were collected as plant materials from three years- old potted trees. Stem nodal segments, approximately 2 cm in length, were excised from the shoots and used as explants. The explants were first washed under running tap water with a drop of detergent, then rinsed under tap water for about an hour. Surface sterilization was performed by immersing the explants in 70% ethanol for five minutes, followed by 20% (v/v) Clorox bleach solution (5.25% sodium hypochlorite) for 20 minutes with gentle agitation. After this, the explants were rinsed three times with sterile double-distilled water, then immersed in a 0.1% (w/v) sterile mercuric chloride solution for three minutes, followed by six rinses with sterile distilled water. and finally, the explants were immersed in H_2O_2 16.6% for a few seconds.

b. Nanoparticles characterization

Two chemically synthesized nanoparticles were used (Alex Biotechnology):

a- Silver nanoparticles < 4-20 nm size of the particles.

b- Zinc oxide nanoparticles, 30-40 nm size of the particles.

Transmission electron microscopy (TEM) was used to characterize the studied nanoparticles. TEM images were employed to determine particle size, size distribution, and shape. This imaging technique provides a fast and automated solution for image analysis.

c. In vitro antibacterial and antifungal assay

This experiment aimed to determine the minimum inhibitory concentration (MIC) of the nanoparticles used in the study. Antimicrobial activity was assessed using the agar diffusion method as described by [36], [37]. The compounds were tested against reference strains, including *Staphylococcus aureus* (ATCC 25923), *Bacillus subtilis* (ATCC 6633), *Escherichia coli* (ATCC 25922), and *Salmonella enterica* (ATCC 25566), as well as yeast; *Candida albicans* (ATCC10321), *Candida tropicalis* (ATCC750), and fungi (*Fusarium oxisporium* and *Aspergillus niger*). The bacterial and yeast strains were obtained from the American Type Culture Collection, while fungal isolates were sourced from the culture collection of the Department of Chemistry of Natural and Microbial Products. Thiophenicol (Thiamphenicol Sanofiaventis, France) and Treflucan (Fluconazole, Egyptian International Pharmaceutical Industries Company EIPICO) were used as positive controls for antibacterial and antifungal activities, respectively, at a concentration of 100 µg/disk, while dimethyl sulfoxide (DMSO) served as a negative control. Each nanoparticle was dissolved in DMSO at concentrations of 1000, 800, and 600 µg/5 µl and applied to paper disks (5 mm diameter) made from blotting paper. These disks were placed on inoculated agar plates and incubated at 30 °C for 24 hours for bacteria and at 28 °C for 72 hours for fungi. The inhibition zones were measured and compared with the control treatments.

D. Avocado tissue culture media and conditions

After surface sterilization avocado explants were cultured during establishment stage on WPM [38] supplemented with 0,5 mg/l BAP +0,5mg/l IBA +1g/l PVP +20 gm / l sucrose +7 gm/l agar for only one month then they were transferred to multiplication medium (WPM supplement with 2.0 mg/l BAP +0,2mg/l IBA +1g/l PVP 30 gm / l sucrose +7 gm/l agar) for three consecutive months. The medium pH was adjusted to 5.7 before being autoclaved at 121°C for 20 minutes. Avocado explants were cultivated in a growth room with a light intensity of 2500– 3000 lux, a photoperiod of 16/8 h light/dark and a constant temperature of 25 ± 2 C°.

e. Nanoparticles addition

The different nanoparticle types (AgNPs &ZnONPs) were added to both of the above mentioned, establishment and multiplication, WPM media as follows. 1- Control (no nano-particles), 2- 40mg/l AgNPs, 3- 80 mg/l AgNPs, 4- 40 mg/l ZnONPs, and 5- 80 mg/l ZnONPs.

f. Data Recorded

Four weeks later after cultured on establishment medium, the average number of shoot/explant, number of leaves/explant and shoot length (cm) were recorded. After three successive subcultures average number of shoot/explant, number of leaves/explant and shoot length (cm) were recorded. Subculture was done every 30 days.

g. Statistical Analysis

This study was conducted using a two-way factorial experiment arranged in a completely randomized design (CRD) with three replicates, each consisting of five glass jars. Analysis of variance (ANOVA) was employed to determine statistical differences, and the significance of differences between means was assessed using Duncan's multiple range test at $p < 0.05$, using Genstat (21st Edition) software.

3. Results

a. Characterization of nanoparticles:

Characterization of nanoparticles was done with transmission electron microscope (TEM). The size, shape, and distribution of the nanoparticles were ascertained by examining the transmission electron microscopy images.Rapid automated image analysis is provided by Imaging Direct. The diameter of whole particles has been used to calculate the size of AgNPs. It is evident that the AgNPs are semi-regularly distributed, mainly spherical, and vary in size as seen in Fig. 1.a. Also, ZnONPs are mainly spherical shape and different size (Fig.1.b).

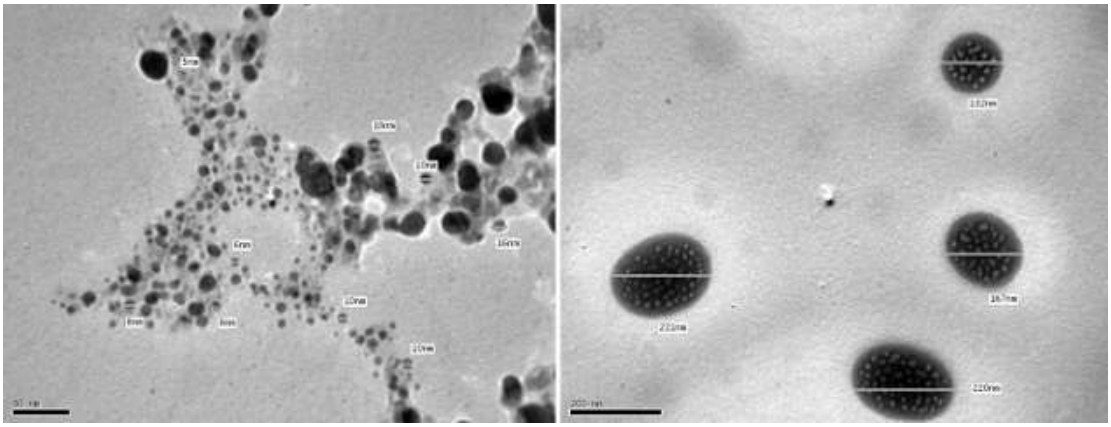

Fig. (1): Transmission Electron Microscope (TEM) of a) silver (AgNPs) and b) zinc oxide (ZnONPs) nanoparticles.

b. Antimicrobial activity: MIC measurement

Silver and zinc oxide nanoparticles were evaluated for their minimal inhibitory concentration (MIC). The two compounds were evaluated at the final concentrations; 6.25, 12.5, and 25 µg. The lowest concentration showing inhibition zone around the disk was taken as the MIC (tables 1&2).The antimicrobial activity of AgNPs and ZnONPs samples has been performed by adopted cup plate method, the results showed in table (2). Both types showed killing activity against all tested microbes (Fig. 2). The obtained results demonstrated that there was a substantial difference between the different types and concentrations of nanoparticles on microbial contamination with regard to the variance in inhibitory potential of the tested nanoparticles on in vitro microbial contamination. When compared to the negative control plates, the concentration of AgNPs at 10 mg/1 showed the lowest value of microbial contamination in the tissue culture media. Selenium nanoparticles had the lowest rating, and chitosan nanoparticles were rated in the second position.

Table (1). Nanoparticles used and its concentrations.

Nanoparticles	Concentration
Silver	1000 ug
Silver	800 ug
Silver	600 ug
Zinc oxide	1000 ug
Zinc oxide	800 ug
Zinc oxide	600 ug
Control	Positive Control

Table (2). Antimicrobial characterization of silver (AgNPs) and zinc oxide (ZnONPs) nanoparticles. The inhibition zone diameter (IZD) was measured using the agar diffusion technique and expressed in millimeters (mm). Positive controls, thiophenicol and Treflucan, were utilized at a 100 µg/disk concentration.

Pathogenic	**The inhibition zone diameter (IZD)**							
	Bacteria				**Yeast**		**Fungi**	
Extract	*B. subtilis*	*S. aureus*	*E. coli*	*S.enterica*	*C. albicans*	*C.tropicalis*	*F.oxysporium*	*A.niger*
Silver 1000ug	1.5	2.1	1.5	1.6	1.4	2.2	2.0	2.1
Silver 800 ug	1.2	1.6	1.3	1.6	1.0	1.4	1.7	1.2
Silver 600 ug	1.1	1.4	1.1	1.6	0.9	1.0	1.2	0.9
Zinc 1000 ug	1.2	1.1	1.1	1.0	0.9	1.2	1.2	N.A
Zinc 800 ug	0.9	0.8	0.9	0.9	0.7	0.9	0.9	N.A
Zinc 600 ug	0.8	0.7	0.7	N.A	N.A	0.8	0.8	N.A
Control	2	1.9	2.3	2.4	1.2	0.9	1.4	N.A

(N.A.= No activity).

3.3 Responses in Tissue culture

a. Hass "cv."

i. Establishment stage

In "cv." Hass, it was obvious that, the addition of AgNPs to WPM medium had a significant effect on average number of shoots, average number of leaves/explant, and shoot length compared with ZnNPs. Silver nanoparticle (AgNPs) at 40 mg/L recorded the highest number of shoots/explant and number of leaves/explant, and shoot length/explant. On the contrary, higher concentrations of both AgNPs and ZnNPs (80 mg/L) recorded the lowest value of all parameters, compared with control (Table 3).

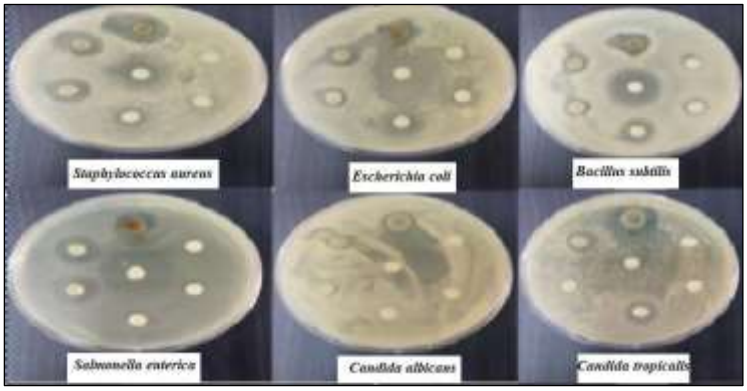

Fig. (2): Antimicrobial characterization results of silver (AgNPs) and zinc oxide (ZnONPs) nanoparticles.

ii. Multiplication stage

When Hass explants were cultured on WPM medium supplemented with AgNPs at 40 mg/l gave the greatest number of shoots /explant followed by 80mg/l while control treatment came at the last rank in this respect (Table 4). As for the effect of number of subcultures, it was obvious that, avocado explants "cv." Hass planted for three subcultures scored the greatest number of shoots /explants followed by second subculture. Interaction between different treatments and the three subcultures it was clear that, avocado explants treated with AgNPs at 40 mg/l gained the greatest number of shoots/explant after the third subculture.

According to the obtained results, avocado average number of leaves was significantly affected by the type of nanoparticles and their concentrations, as AgNPs at 40 mg/l scored the greatest number of leaves compared with ZnONPs (Table 4). Moreover, the greatest number of leaves was recorded after the third subculture. As for the interaction, it was clear that avocado explants treated with 40 mg/l AgNPs scored the highest number of leaves after the third subculture. The tallest explants were obtained when WPM medium was supplemented with 40 mg/l of AgNPs compared with the rest of other treatments (Table 4). While increasing nanoparticles concentration to 80 mg/l resulted in shorter explants, with more decreasing with ZnONPs. Here, increasing the number of subculture gave the tallest avocado explants. The tallest avocado explants were found after three subcultures following treatment with 40 mg/l of AgNPs, indicating a significant interaction.

Table (3). The effect of silver (AgNPs) and zinc oxide (ZnONPs) nanoparticles and their concentrations on average number of leaves/explant and shoot length /explants of Avocado "cv." Hass during establishment stage.

Treatments	No. of shoots/explant	No. of leaves/explant	Shoot length/explant (cm)
Control	2.739D	4.85D	1.7C
Ag40mg/l	5.6A	9.667A	4.6A
Ag80mg/l	4.567B	7.833B	3.217B
Zn40mg/l	3.417C	5.717C	1.944C
Zn80mg/l	1.583E	2.983E	1.0D

Means followed by different letters are significantly different at $p \leq 0.05$

Table (4). The effect of silver (AgNPs) and zinc oxide (ZnONPs) nanoparticles and their concentrations on average number of shoots/explant, average number of leaves/explant and shoot length /explants of avocado "cv." Hass during three successive subcultures.

Treatments	No. of shoots/explant			Means	No. of leaves/explant			Means	Shoot length/explant (cm)			Means
	Sub1	Sub2	Sub3		Sub.1	Sub.2	Sub.3		Sub.1	S2ub	Sub.3	
Control	2h	2.917efgh	3.efg	2.739D	2.25gh	6e	6.3e	4.85D	0.5h	1.5fg	3.1d	1.7C
Ag40mg/l	3.5def	5.3c	8a	5.6A	5ef	11b	13a	9.667A	2.5de	4c	7.3a	4.6A
Ag80mg/l	3efgh	4de	6.7b	4.567B	3.5fgh	9c	11b	7.833B	1.25fgh	3d	5.4b	3.217B
Zn40mg/l	2.5fgh	3.25efg	4.5cd	3.417C	2.75gh	6.5de	7.9cd	5.717C	0.833gh	1.75ef	3.25cd	1.944C
Zn80mg/l	0.5i	2h	2.25gh	1.583E	2h	3.75fg	3.2gh	2.983E	0.5h	0.5h	2.0ef	1D
Means	2.3C	3.49B	4.95A		3.1C	7.25B	8.28A		1.117C	2.15B	4.21A	

Means followed by different letters are significantly different at $p \leq 0.05$

b. Fuerte "cv."

i. Establishment stage

The greatest number of shoots/explant were obtained with AgNPs compared with ZnONPs, while control treatment came in between. No significant differences were obtained between the concentrations of nanoparticles. The greatest number of leaves as well as the tallest explant were obtained with 40 mg/l AgNPS followed by 80 mg/l. It seems that, Fuerte "cv." responded negatively for ZnONPs with both concentrations as it scored the lowest number of leaves and gained the shortest explants (Table 5).

***ii.* Multiplication stage**

Results in Table (6) demonstrated that, the greatest number of shoots were obtained with 40 mg/l AgNPs followed by control treatment while ZnONPs came at the last rank in this respect. As long as the number of subcultures increased the number of shoots/explant increased. It was clear that, there is a significant interaction between nanoparticles types, concentrations and increasing the number of subcultures, as fuerte explants scored the highest number of shoots after the third subculture when adding AgNPs at 40 mg/l to WPM multiplication medium.

In contrast to 80 mg/l, it was evident that AgNPs at 40 mg/l scored the highest number of leaves/explant and the tallest explants. In this regard, ZnONPs (particularly 80 mg/l) had the lowest ranking, with the control treatment falling in the middle. Our findings showed that Fuerte explants grew taller and had more leaves after the third subculture. Regarding the interaction, it was found that, after three consecutive subcultures, 40 mg/l was the best treatment, producing the most leaves and the tallest explant (Table 6).

c. Red "cv."

i. Establishment stage

The results demonstrated that red "cv." explants treated with 40 mg/l AgNPs had the highest number of shoots, number of leaves, and the tallest explants, with 80 mg/l following suit. Meanwhile, ZnONPs treatment got the last rank with insignificant differences between their concentrations except for shoot length as 80 mg/l resulted in the shortest explant (Table 7). Control treatment scored the second rank among AgNPs and ZnONPs.

***ii.*Multiplication stage:**

The highest number of shoots/explant was produced by adding 40 mg/l AgNPs to the multiplication medium, followed by 80 mg/l of avocado "cv." Red. No significant differences were found between the other treatments (Table 8). An increase in subcultures is correlated with an increase in the number of shoots. Red explants treated with 40 mg/l AgNPs gained the greatest number of shoots following the third subculture, indicating a significant and observable interaction.

Silver nanoparticles proved to be the best type of nanoparticle compared with zinc oxide nanoparticles, as AgNPs resulted in, the greatest number of leaves and the tallest Red explants after treated with 40 mg/l followed by 80 mg/l AgNPs compared with other treatments (Table 8). By increasing the subcultures, number of leaves and height of shoots were increased in Red explants. After the third subculture, a significant interaction revealed that 40 mg/l of AgNPs is the optimal treatment, producing the most leaves and the tallest explant.

Table (5). The effect of silver (AgNPs) and zinc oxide (ZnONPs) nano-particles and their concentrations on number of shoots/explant, average number of leaves/explant and shoot length /explants of avocado "cv." Fuerte during establishment stage**.**

Treatments	No. of shoots/explant	No of leaves/explant	Shoot length/explant (cm)
Control	3.0 B	5.9 C	2.049 C
Ag40mg/l	5.767 A	9.867 A	5.667 A
Ag80mg/l	5.267 A	7.4 B	4.939 B
Zn40mg/l	1.378 C	4.033 D	2.166 C
Zn80mg/l	1.378 C	3.2 E	1.278 D

Means followed by different letters are significantly different at $p \leq 0.05$

Table (6). The effect of silver (AgNPs) and zinc oxide (ZnONPs) nanoparticles and their concentrations on average number of shoots/explant, average number of leaves/explant and shoot length /explants of avocado "cv." Fuerte during three successive subcultures.

Treatments	No. of shoots/explant			Means	No. of leaves/explant			Means	Shoot length/explant (cm)			Means
	Sub1	Sub2	Sub3		Sub.1	Sub.2	Sub.3		Sub.1	S2ub	Sub.3	
Control	2.0 def	3.0 cde	4.0 bc	3.0 B	3.0 gh	6.5 d	8.2 c	5.9 C	0.767 g	1.88 ef	3.5 d	2.049 C
Ag40mg/l	3.5 bcd	4.8 b	9.0 a	5.767 A	5.0 ef	9.6 bc	15.0 a	9.867 A	1.5 fg	4.5 c	11.0 a	5.667 A
Ag80mg/l	3.0 cde	4.0 bc	8.8 a	5.267 A	3.9 fg	8.3 c	10.0 b	7.4 B	1.25 fg	3.9 cd	9.677 b	4.939 B
Zn40mg/l	1.0 f	1.5 ef	1.633 ef	1.378 C	2.3 h	4.2 efg	5.6 de	4.033 D	0.667 g	1.03 fg	4.8 c	2.166 C
Zn80mg/l	0.833 f	1.2 f	2.0 def	1.378 C	2.0 h	3.3 gh	4.3 efg	3.2 E	0.5 g	0.833 g	2.5 e	1.278 D
Means	2.067 C	2.9 B	5.107 A		3.24 C	6.38 B	8.62 A		0.937 C	2.429 B	6.293 A	

Means followed by different letters are significantly different at $p \leq 0.05$

Table (7). The effect of silver (AgNPs) and zinc oxide (ZnONPs) nanoparticles and their concentrations on number of shoots/explant, average number of leaves/explant and shoot length /explants of avocado "cv." Red during establishment stage.

Treatments	No. of shoots/explant	No. of leaves/explant	Shoot length/explant (cm)
Control	2.25 C	3.583 C	1.25 C
Ag40mg/l	10.028 A	11.378 A	5.161 A
Ag80mg/l	6.167 B	8.433 B	3.606 B
Zn40mg/l	1.717 C	3.35 CD	1.406 C
Zn80mg/l	1.75 C	2.378 D	0.794 D

Means followed by different letters are significantly different at $p \leq 0.05$

Table (8). The effect of silver (AgNPs) and zinc oxide (ZnONPs) nanoparticles and their concentrations on average number of shoots/explant, average number of leaves/explant and shoot length /explants of avocado "cv." Red during three successive subcultures.

Treatments	No. of shoots/explant			Means	No. of leaves/explant			Means	Shoot length/explant (cm)			Means
	Sub1	Sub2	Sub3		Sub.1	Sub.2	Sub.3		Sub.1	S2ub	Sub.3	
Control	2.0 d	2.25 d	2.5 d	2.25 C	3.25 fg	3.5 fg	4.0 fg	3.583 C	0.75 gh	0.9 gh	2.1 de	1.25 C
Ag40mg/l	2.5 d	8.583 b	19.0 a	10.028 A	6.5 de	10.0 c	17.633a	11.378 A	1.917 ef	3.667 c	9.9 a	5.161 A
Ag80mg/l	2.5 d	6.0 c	10.0 b	6.167 B	5.0 ef	8.0 d	12.3 b	8.433 B	1.417 fg	2.5 de	6.9 b	3.606 B
Zn40mg/l	1.250 d	2.0 d	1.9 d	1.717 C	3.25 fg	3.6 fg	3.2 fg	3.35 CD	0.75 gh	0.833 gh	2.633 d	1.406 C
Zn80mg/l	1.0 d	1.75 d	2.5 d	1.75 C	2.0 g	2.967 fg	2.167 g	2.378 D	0.583 h	0.467	1.333 fg	0.794 D
Means	1.85 C	4.117 B	7.18 A		4.0 C	5.613 B	7.86 A		1.083 C	1.673 B	4.573 A	

Means followed by different letters are significantly different at $p \leq 0.05$

4. Discussion

Recently, nanomaterials are starting to be used in a variety of scientific fields, including plant tissue culture and other agricultural fields. The widespread application of different NPs in industries like biotechnology, electronics, medicine, energy, cosmetics, and pharmaceuticals has also shown beneficial effects in the removal of microorganisms. Numerous studies have looked for non-toxic ways to reduce microbial contamination of plant tissue cultures because successful micropropagation depends on doing so. Because of its powerful ability to release small particles of silver, nanoparticles not only has the ability to diminish or eliminate bacterial and fungal infections, but it can also destroy viruses [39], [40].

In this study, results from *in vitro* microbial contamination trails, demonstrated that it was possible to use the nanoparticles as antibacterial agents in the tissue culture media. The inhibitory capacity of nanoparticle agents of *in vitro* microorganisms has been confirmed by previous studies on the antibacterial activity of the tested nanoparticles [5], [41], [42]. Here, with the lowest contamination percentage, AgNPs have a great capacity to remove microbial contamination from culture media, which is statistically comparable to sterilization by autoclaving. These results were in confirmation with [2], [12], [43], [44]. The strong antibacterial properties of AgNPs' can be attributed to the severe toxicity of silver ions against a wide range of microorganisms. Furthermore, interactions and binding of silver ions with cell membrane proteins, which results in bacterial cell death, depend on the small particle size (5–15 nm) of the silver nanoparticles [45].

The fact that ZnONPs can interact with the cell surface and/or nucleus during cell penetration may account for the antibacterial action of ZnONPs seen in this investigation at varied concentrations. Although, the exact mechanism of zinc oxide nanoparticles' toxicity is still not fully understood, it is thought to be due to the direct contact of ZnONPs with bacterial cell walls, which ruptures membranes and releases antimicrobial ions (mainly Zn^{2+}) and reactive oxygen species [19], [23], [46].

Nanoparticles application in culture media had a beneficial impact on both establishment and multiplication stage. Results cleared that adding AgNPs to the establishment medium significantly increased growth efficiency compared with ZnONPs treatment. The results go in line with those founds by [6] which cleared that, olive shoots grown *in vitro* was significantly influenced by AgNPs concentrations.Furthermore, [7] found that, adding silver Nanoparticles (AgNPs) to the olive medium recorded the highest percentage of bud sprouting of Picual olive explants. [3]reported that, Argovit™ silver nanoparticles effectively reduce contaminant levels during the establishment of woody plant cultures. Additionally, incorporating nanoparticles into the culture medium positively impacted olive explants compared to the control. The NPs significantly decreased necrosis in the three olive cultivars and enhanced growth vigor and development of the cultured olive explants [47].

These data also agree with [48] who stated that, adding AgNPs directly to MS medium increased the mean number of fresh shoots/explant of *Tecomella undulata*. Hence, the addition of AgNPs directly to the culture medium significantly reduced both internal and external contaminations compared to the control group. Using nanoparticles in the culture medium proved to be

more effective in reducing fungal and bacterial contamination than immersing in the hybrid G × N15 (a hybrid of almond × peach) [39].

Moreover, the obtained results demonstrated that using AgNPs at 40 mg/l had a positive effect on the growth parameters of avocado cultivars *i.e*. Hass, Fuerte and Red. The results are consistent with the previous evidence of positive effects of using silver nanoparticles with low concentrations. Hassanin et al., [6] cleared that, culture medium supplemented with 20 and 30 mg/l AgNPs significantly influenced the shoot growth of *in vitro* olive resulted in the highest shoots number, shoot length, and leaf count for the Picual cultivar compared to the media without nanoparticles. However, higher concentrations of AgNPs negatively affected the growth parameters of the Dolce cultivar, which showed decreased values compared to the lower concentration of 10 mg/l. Darwesh et al., [49] noticed that AgNPs at 5 or 10 mg/l resulted in greater values for shoot number, shoot length, leaf number, and multiplication rate compared to treatments with chitosan and selenium nanoparticles. Similar results were observed by [13], who found that AgNPs at 12 mg/l led to approximately a threefold increase in growth parameters such as the number of shoots per explant, shoot length, and leaf surface area compared to the control treatment. Also,[50] reported that, the addition of AgNPs at 100 mg/l had a significant effect improving productivity of apricot micropropagation. [7], demonstrated that, the maximum percentage of bud sprouting, shoot length, number of shoots/explant, and number of leaves/shoot of olive were recorded by adding 5 mg/l of AgNPs. It was discovered that adding 5 mg/l of AgNPs to the culture medium increased the growth vigor and development of olive explants and greatly decreased necrosis in the three olive cultivars when compared to the control [47].

The beneficial effects of AgNPs on plant growth and development have been explained by a number of theories [14]. Their effect on plant hormones provides one logical explanation. For example, AgNPs inhibit ethylene action, which makes them useful in plant tissue culture [51]. Additionally, AgNPs have been shown to raise plant tissues' cytokinin levels. [52], highlighting their role in enhancing growth responses. Furthermore, research has demonstrated that AgNPs can raise plant levels of vital nutrients such as iron (Fe), magnesium (Mg), and nitrogen (N) [15], [16]. It is thought to be that increased chlorophyll production is connected to this nutritional increase, and that higher photosynthetic activity can result in improved plant development.

However, the impact of AgNPs on higher plants depends on several factors, including plant species, plant age, particle size, and concentration. Optimization of nanoparticle application is crucial to maximize plant growth while avoiding toxicity from higher doses. Excessive concentrations of NPs can be harmful to plant growth, regeneration, biomass production, leaf area, shoot growth, and cell viability [53], [54]. Toxicity from NPs caused morphological changes and abnormalities in cell growth in *Spirodela Polyrhiza*[55]. In rice seedlings, higher concentrations of AgNPs have been found to significantly reduce plant biomass, inhibit shoot growth, and decrease root elongation [56]. [57] noted that while lower concentrations of AgNPs (20 and 40 ppm) can stimulate the growth of *Phaseolus vulgaris* and maize, whilst inhibitory effects were observed under higher concentrations (100 ppm).

5. Conclusion

The distinct chemical, physical, and biological characteristics of nanomaterials in contrast to their bulk counterparts offer numerous advantages to agricultural science. In this work, various bacterial and fungal strains were used to examine the impact of AgNPs and ZnONPs in removing contamination. Additionally, the study explored their ability to improve *in vitro* growing explants under two stages of establishment and multiplication using the avocado as a model for the first time, utilizing three different accessions, "cv." Hass, Red, and Furete. Results demonstrated a significant variation in the inhibitory potential of the studied NPs on *in vitro* microbial contamination between the various types and concentrations of NPs. Results stated that, the addition of 40 mg/l AgNPs to establishment media significantly enhanced growth of three avocado cultivars *in vitro*. Also during the multiplication stage, the same concentration of AgNPs under successive subcultures, resulted in increasing of number of shoots/explant, number of leaves/explant and shoot length/explant in all studied avocado accessions. Since the AgNPs improve the growth of the avocado plants and decreased bacterial and fungal contamination during cultivation, this work is regarded as a novel contribution to the tissue culture of avocado plants.

6. Conflicts of interest

The authors declare that they have no competing interests.

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