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Effects of in vitro addition of silver and zinc oxide nanoparticles on contamination and growth parameters of three olive genotypes

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Abstract

Field of nanotechnology is an innovative science, which can develop a numerous of agriculture fields, as plant growth enhancing and crop incomes, nanoparticles (NPs) can be applied to plant tissue culture but with definite protocol of NPs concentration to encourage explants germination and decrease contamination. The present study aims to investigate the effects of two nanoparticles, zinc oxide and silver, on micropropagation of three olive genotypes, Koroneiki, Dolcie, and Kalamata. Additionally, a strategy to reduce contamination and promote the sprouting of olive explants in tissue culture lab is being determined. Three nanoparticle concentrations 312.5, 156.25, and 78.125 mg/l were employed; based on the antimicrobial experiment's minimum inhibition concentration (MIC). Olive media (OM) was used as starting medium, and standard Morishige and Skoog (MS) medium was utilized as the multiplication medium. Occasionally, OM was modified media by adding 4 mg/l of zeatin instead of 2 mg/l to encourage the germination of dolcie and kalamata genotypes explant buds. After assessing all of the outcomes, study focused upon analyzing the first set of data, results revealed that, in general, the Koroneiki genotype was the most successful at sprouting through tissue culture methods; however, dolcie and kalamata proved to be quite challenging to micropropagate. Koroneiki olive was chosen for the rest of the study and dilute the NPs concentration to new ones 160, 80 and 40 mg/L. At the end of the study data revealed that contamination percent decreased as NPs concentration increase, while other growth parameters (bud germination), shoot length mean, number of leaves/ shoot and number of shoots/explant) were improved with using NPs. Additionally, zinc oxide NPs outperformed silver nanoparticles in terms of effectiveness, and when utilizing the Koroneiki cultivar as explants, the concentration of 40 mg/l was the most significant in growth parameters outcomes. Using NPs in olive micropropagation depends on genotype and dose, whereas, koroneiki cultivar is more reliable to micropropagate protocols than dolcie and kalamata cultivars. The usage of NPs in the micropropagation culture of plants enables the possibility to learn about many features of plant growth and development in controlled environmental conditions.

Keyword: Olive, Genotypes, Silver & Zinc NPs, Micropropagation, Contamination, Nanotechnology.

Introduction

Overview along with the large-scale breeding of endangered species **(1),** plant micropropagation has made a substantial contribution to the research of their growth and improvement as well as the impact of environmental conditions on them (**2**). However, there are a number of issues with woody plant species micropropagation, such as shoot tip necrosis **(3),** challenging shootlet rooting **(4),** hyperhydricity (5), phenolization **(6),** and a low morphogenetic capacity of the *in vitro* shoots **(7).**

There are numerous methods for micropropagating olive trees, including suckering, cuttings, and seeds **(8).** Stem cutting is the primary method of propagating olives to encourage the emergence of roots, although it has a very poor effectiveness **(9)** and is not appropriate for commercial production in order to prevent pests and illnesses. Many investigations, including **(8, 10, 11, 12),** were fearless in their attempts to cultivate various olive cultivars in vitro. Olives (*Olea europaea* L.), a Mediterranean tree, are distributed around the world but are most commonly utilized in Mediterranean nations. They have been used as traditional agricultural trees since antiquity **(12, 13, 14,15).**

The main obstacle preventing woody plant propagation is bacterial and fungal contamination **(16).** According to **Dabai et al. (17),** there are numerous ways to reduce *in vitro* contamination, including the use of antibiotics. However, employing antibiotics lead to the possibility to release bacterial resistance and/or impact the growth and responsiveness of the explants. Mercuric chloride was previously employed to reduce internal contaminations in the micropropagation of olive and certain woody plants **(18). Martino et al. (19)** discovered that mercuric chloride is extremely harmful to olive explants and can harm completely explants. Thus, researchers have discovered more safe methods for managing contamination in olive tissue culture laboratories.

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Recently , nanotechnology permeates every aspect of our lives, including clothing, cosmetics, tools, applications, food, and the surroundings.Whether we like it or not, nanomaterials are present in and around us **(20).** The foundation of plant biology is plant micropropagation, which is essential for plant conservation, genetic modification, large-scale propagation, the synthesis of bioactive compounds, and plant improvement. Applications of nanoparticles have successfully reduced the amount of microbiological pollutants, demonstrated a progressive improvement of the callus induction, genetic transformation, organogenesis, somaclonal variation, somatic embryogenesis, and secondary metabolite production. this may led to develop germination of seeds, enhancement plant growth and harvest, facilitate the plant genetic variation and succeed in plant safety **(20, 21).**

Rostami and Shahasavar (8) discovered that sliver nanoparticles have non-toxic and strong antimicrobial properties against bacteria ,viruses and fungi. About 600 microorganisms have been shown to benefit from nano-silver's beneficial properties **(22).** This is due to the release of tiny silver particles, which have the power to eradicate viruses as well as bacteria and fungi **(23).** When nanoparticles are added to tissue culture media, It can increase the morphological potential of cultured samples and eliminate bacterial contamination. It also leads to somaclonal variation **(20).**

In latest eras Zn oxide NPs have been further destructive to bacteria and scarcer sensitive to human cells when compared to other metal oxides **(24, 25).** Since ZnO NPs have a tremendous antibacterial mechanism based on the potentiality to produce the reactive oxygen species (ROS) which destroy bacterial cells **(26, 27)**. The information about Zn particles toxicity and its mechanism is not revealed yet, generally these NPs revealed negative effects on the tested organisms used **(28, 29)** also, have a dose dependent response **(30).** Zinc oxide improved the content of flavonoids, anthocyanins, phenolic compounds glycyrrhizin and tannins **(31)**.

In recent decades, the consumption of metal NPs has extremely enlarged. In agriculture, NPs are utilized as fertilizers like Ag, Se, Au, Mg, Si, however, others are used as pesticides or pesticide carriers like Fe3O4, S, CuO, chitosan, or Ca-alginate-chitosan NPs, **(32, 33).** Moreover, currently several industries included depend on NPs usage. Widespread usage of NPs can have a harmful influence on the environment and could be known as a contaminant product **(34).** However NPs such as Ag, Zn, iron and CuO, could be showed plant growth promotion, on the other hand they have also been associated to phytotoxicity **(35, 36).**

This research study the effect(s) of the addition of three different concentrations of two nanoparticles types namely silver and zinc oxide on contamination percentage and growth parameters for explants of three olive genotypes Koroneiki, dolcie and Kalamata.

2. **Materials and methods**:

2.1. Nanoparticles:

Two types of readymade nanoparticles were used:

a-Silver nanoparticles characterized by Size: 5 - 28 nm, Shape: spherical and Methods: chemical synthesis (Alex biotechnology lab. product).

b-Zinc oxide nanoparticles, characterized by Size: 23-222 nm, Shape: spherical and Methods: chemical synthesis (Alex biotechnology lab. product).

2.2. Characterization of nanoparticles:

Characterization of nanoparticles was done with transmission electron microscope (TEM).

Transmission Electron Microscope (TEM):

The images of transmission electron microscopy were used to determine of the particle size, size distribution and shape of the nanoparticles. Imaging direct gives a fast automated image to analysis solution.

2.3. Plant materials:

Small, green and fresh branches of three genotypes cultivars of olive (*Olea europaea*), (Kalamata, Dolcie and Koroneiki) were used in this experiment with the same media and culture conditions. Four branch cuts were used in each jar as explants. Branches of koroneiki cultivar were only used in the rest of the study.

2.4. Culture media:

Starting medium : Olive medium + 2 mg/l or 4 mg/l zeatin + 0.1 mg/l IBA (Indole Butyric Acid) + 1mg/l BA + 30 gm sucrose+ 1.0 gm/l PVP (polyvinyl pyrolidone) + 0.3 GA3 +1ml/l capanthocyanide + 7 gm agar, pH5.7.

Modified olive medium : The components of the previous olive medium modified with 4 mg/l zeatin instead of 2mg/l to promote dolce and kalamata varieties.

Multiplication medium: MS medium + 0.1 IBA+ 1mg/l BA+ 30 gm sucrose+ 1.5 gm PVP (polyvinyl pyrolidone) + 1ml/l Ca-panthocyanide + 7 gm agar, ph 5.7.

2.5. Nanoparticles addition:

Firstly, three concentrations from the readymade powder silver and zinc oxide nanoparticles were added to media (78.125 = C, 156.25 = B and 312.5 = A mg/L), previous concentrations concluded after determination of MIC (Minimum Inhibition Concentration) by antimicrobial activity experiment for each nanoparticles used, besides jars without any nanoparticles as control with dolcie, kalamata and koroneiki genotypes as explants. Then, after evaluating the first data we were diluting the NPs concentrations were dilutes into new ones 160, 80 and 40 mg/L.

2.6. Sterilization procedure:

Branches of the olive plant were cut into pieces with 3cm in length and leaves were removed. Plant pieces kept in water with Clorox for 30 minutes, then washed with running tap water for one hour, after that plant pieces were soaked in mixture of citric acid (150 mg/L) and ascorbic acid (100 mg/ L) for 1 hour. Finally, they sprayed with 70% ethyl alcohol and transferred to culture cabinet.

Inside cabinet plant pieces were soaked in 20% Naocl2 for 15 minutes, after that they were kept in 0.1 $HgCl_2$ for 2 minutes and washed with sterilized distilled water for 3 times, in last step they were immersed in 9.9% H_2O_2 for 1 minute then washed once.

2.7. Antimicrobial activity:

***In vitro* antibacterial and antifungal assay;**

Antimicrobial activity was measured by an agar diffusion method **(37, 38).** The compounds were tested against reference strains, (*Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* ATCC 6633, *Escherichia coli* ATCC 25922 and *Salmonella enterica* ATCC 25566), yeast (*Candida albicans* ATCC10321, *Candida tropicalis* ATCC750), fungi (*Fusarium oxisporium and A. niger)*, Bacteria and yeast strains were obtained from the American Type Culture Collection Thiophenicol (Thiamphenicol, Sanofiaventis, France) and Treflucan (Fluconazole, Egyptian International Pharmaceutical Industries Company EIPICO) were used as antibacterial and antifungal positive controls, respectively, in a concentration of 100 µg/disk (diagram 1).

Different concentrations of silver and zinc oxide nanoparticles (1000, 800, 600 µg/5 µl) (table 1), were dissolved in dimethyl sulfoxide (DMSO) and spotted on paper disks prepared from blotting paper (5 mm diameter) with a concentration of DMSO/disk for isolated compounds. The disks were applied on inoculated agar plates and incubated for 24 h at 30 °C for bacteria and 72 h at 28 °C for fungi strains. The zone of inhibition was recorded and compared with the control treatments.

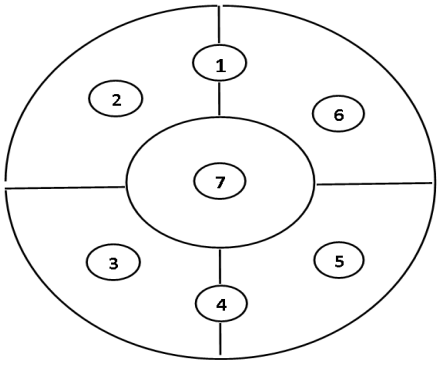

Diagram (1): Illustrate the microorganisms distribution used in antimicrobial activity experiment.

Table (1): Nanoparticles types used and its concentrations used in antimicrobial activity experiment..

Type of nanoparticles	Concentration	Disk No.
Silver	1000 ug	1
Silver	800 ug	2
Silver	600 ug	3
Zinc oxide	1000 ug	4
Zinc oxide	800 ug	5
Zinc oxide	600 ug	6
Control	Positive Control	7

2.8. Data Recorded:

Four weeks later after cultured on starting medium, the bud germination percentage, average no. of shoot/explant, no. of leaves/explant, shoot length (cm) and contamination percentage were recorded. After two successive subcultures the same data were recorded. Subculture was done every 30 days.

2.9. 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 ± SE (standard error) was assessed using Duncan's multiple range test at $p < 0.05$, using Genstat (21st Edition) software.

3. Results and discussion:

Olive micropropagation is affected by numerous factors including growth medium, cytokinin type, plant genotype, contamination, phenolic compounds oxidation, imperfect proliferation rates and concentration **(39, 40)**.

Nowadays, application of NPs has enormously increased in agriculture, NPs of Ag, Si, Au, Mg and Se are employed as fertilizers, whereas Ca-alginate-chitosan or Fe3O4, S, CuO and chitosan NPs, are used as pesticides and/or In the same time, many studies exclude that NPs such as Ag, Zn, CuO, and iron, in addition to some other studies, have been found to stimulate development of plant, nevertheless they have also been associated toxicity to plants **(35, 36, 41).**

3.1. Characterization of nanoparticles:

TEM: Determination the NPs particle size, distribution and shape by transmission electron microscopy (figure 1). As shown in figure (1a) silver nanoparticles were round shape with different sizes ranged from 5 nm to 28 nm as well as figure (1b) zinc oxide nanoparticles were different shapes as oval and rounded with different sizes ranged from 23 nm to 222 nm.

Results of transmission electron microscope showed that silver nanoparticles have different sizes ranged from 5 to 28 nm (figure 1a). Moreover, the small particle size (5-28 nm) of Ag NPs have ability to interactions and binding of Ag with proteins of cell membrane, which leads to cell death **(14, 23).** Numerous studies approved the inhibition potential of nanoparticles on microorganisms growth in plant micropropagation media (**42, 43).** Whereas, Ag nanoparticles have high ability for removing infectious contamination from culture medium; Ag nanoparticles verified the lowest contamination percent. Also, their antimicrobial action may be related to the strong toxicity of these ions to a widespread range of microbes **(14, 44, 45).**

Zinc oxide nanoparticles size found ranged from 23-222 nm (figure 1b) which is a wide range of sizes. Recently zinc oxide nanoparticles have gained much interest because of their distinctive properties compared to their bulky complements, in which their use in biological applications increased **(46).** Since ZnO NPs have an unique antibacterial activity established by the formation of ROS (reactive oxygen species) that can destroys bacteria (**26, 27**). Zinc oxide NPs have been more antibacterial activity and less reactive to human cells when compared to other metal oxides **(24, 25).**

3.2. Antimicrobial activity:

MIC measurement: The two nanoparticles were assessed for their minimal inhibitory concentration MIC. Zinc oxide and silver NPs were estimated at the final concentrations; 1000, 800 and 600 µg (table 1). The lowermost concentration display inhibition zone around the disk was taken as the MIC as shown in table (2) and figure (2), MIC was recorded 312.5 mg/L. Olive branches were used as explants, and three concentrations of silver nanoparticles and zinc oxide nanoparticles were used as the following; 78.125 (C)(25%), 156.25 (B)(50%), and 312.5 (A) (100%), mg/L (figure 2), the concentrations were determined according to antimicrobial activity test, which determined by MIC value.

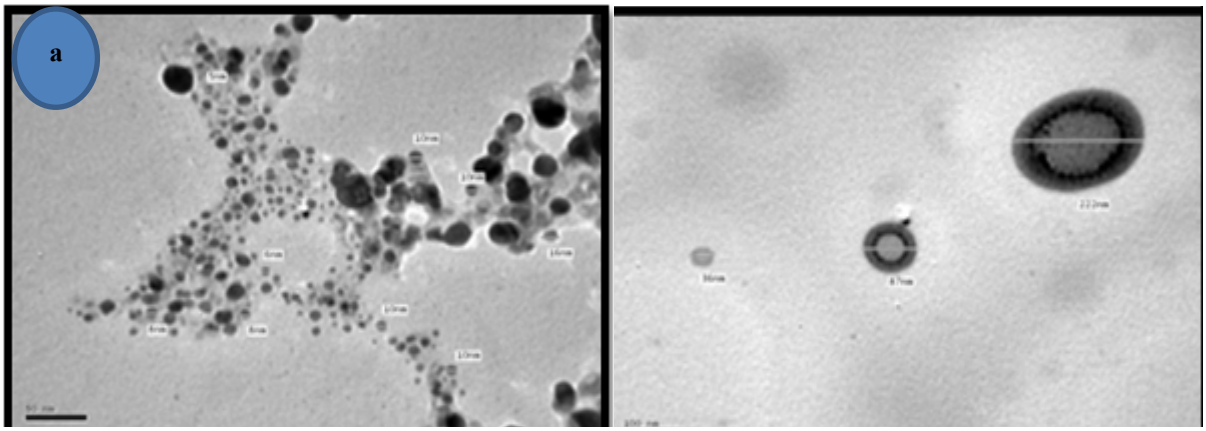

Figure 1: Transmission electron microscopy for a) Silver and b) Zinc oxide NPs.

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

	Inhibition zone diameter (mm)						
Microorganism	Gram positive bacteria		Gram negative bacteria	Fungi			
Nanoparticles	*B. subtilis*	*S. aureus*	*E. coli*	*C. tropicalis*	*C. albicans*	*F. oxysporium*	*A. niger*
Silver (1000ug)	0.9	0.7	0.9	N.A	N.A	0.7	N.A
Silver (800ug)	0.9	0.7	0.8	N.A	N.A	N.A	N.A
Silver (600ug)	0.8	0.7	0.8	N.A	N.A	N.A	N.A
Zinc oxide (1000ug)	1.0	0.8	0.9	0.7	N.A	1.1	0.9
Zinc oxide (800ug)	0.9	0.8	0.9	N.A	N.A	1.1	0.8
Zinc oxide (600ug)	0.8	0.7	0.8	N.A	N.A	1.1	0.8
Positive control	2.7	2.7	1.6	0.7	N.A	1.7	0.7

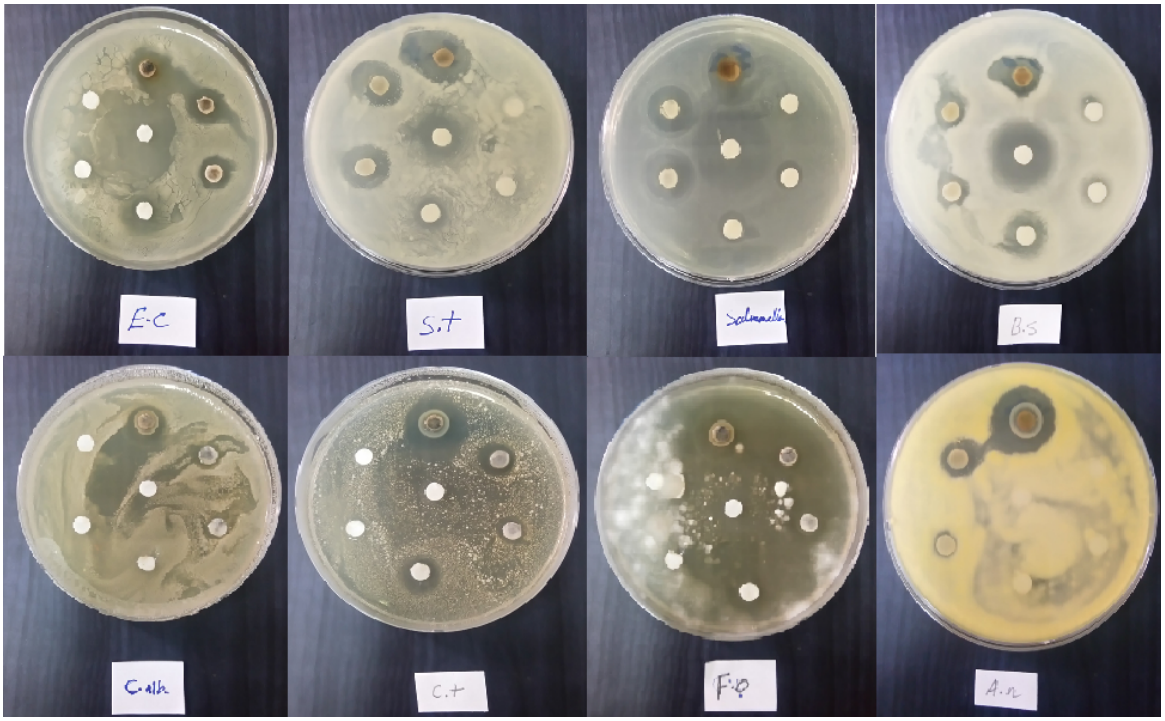

Figure 2: Agar Diffusion Technique and the Inhibition Zone Diameter with the examined microorganisms treated with different concentrations (1000, 800, 600 µg/5 µl) of nanoparticles to determine MIC.

3.3. Guidance experiment:

Three olive genotypes and two NPs with three concentrations beside control were used as shown in tables (3&4), dolcie and kalamata explant branches never respond to show germination in any medium with or without NPs, as well as not germinate in medium contains 2 mg/l zeatin or 4 mg/l zeatin. Only Koroneiki genotype was respond in medium contains 2 mg/l zeatin with NPs and control (figure 3), and so study was completed by using Koroneiki genotype as explant for the rest of the research

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Table (3): Percentage of buds germination and contamination were recorded after using zinc oxide NPs for three olive cultivars Koroneiki, dolcie and kalamata. (guidance experiment)

Zinc oxide NPs								
Concentration	Control		A (312.5 mg/L)		B (156.25 mg/L)		C (78.125 mg/L)	
Olive cultivar	% germination	% contamination	% germination	% contamination	% germination	% contamination	% germination	% contamination
Koroneiki (2 mg/l zeatin)	28	49	8	12	31	15	39	18
Dolcie (2 mg/l zeatin)	0	0	0	0	0	0	0	0
Dolcie (4 mg/l zeatin)	0	0	0	0	0	0	0	0
Kalamata (2 mg/l zeatin)	0	0	0	0	0	0	0	0
Kalamata(4 mg/l zeatin)	0	0	0	0	0	0	0	0

Table (4): Buds germination percentage and contamination percentage were recorded after using silver NPs for three olive cultivars from Koroneiki, dolcie and kalamata. (guidance experiment)

Silver NPs								
Concentration	Control		A (312.5 mg/L)		B (156.25 mg/L)		C (78.125 mg/L)	
Olive cultivar	% germination	% contamination	% germination	% contamination	% germination	% contamination	% germination	% contamination
Koroneiki (2 mg/l zeatin)	28	49	5	9	18	10	22	15
Dolcie (2 mg/l zeatin)	0	0	0	0	0	0	0	0
Dolcie (4 mg/l zeatin)	0	0	0	0	0	0	0	0
Kalamata (2 mg/l zeatin)	0	0	0	0	0	0	0	0
Kalamata (4 mg/l zeatin)	0	0	0	0	0	0	0	0

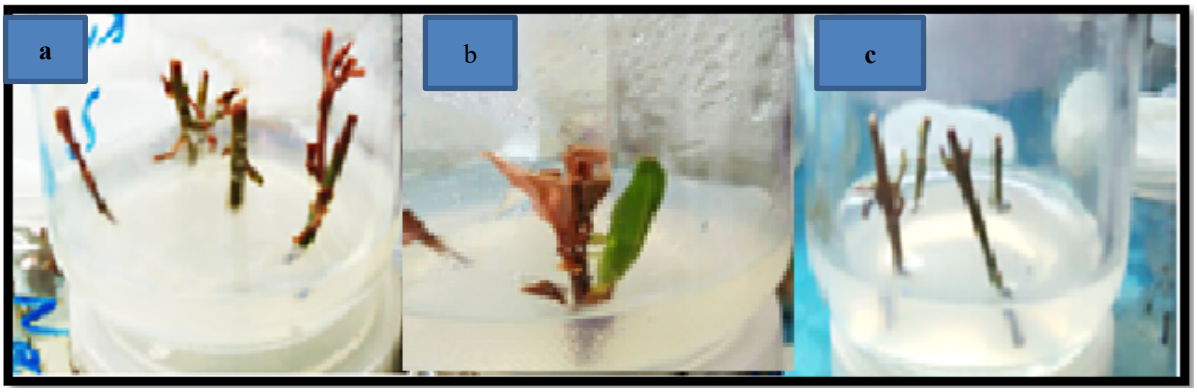

Figure 3: The response of three olive cultivars explanted on control medium during guidance experiment, a) Dolcie, b) Koroneiki and c) kalamata.

3.4. Koroneiki genotype (second experiment):

In this experiment NPs concentrations were diluted, as shown in (table 5 & figures 6,7) when branches of koroneiki olive genotype used as an explant, and addition of two types of nanoparticles zinc oxide and silver (each one found in three concentrations) to OM media where. The results of contamination percentages were significantly decreased after all NPs addition when compared with control, the lowest contamination percent was found with nano-silver 160 mg/ (fig. 4).

In the same time the buds germination percentage of the explant branches were recorded the maximum and significant increase after addition of nano-zinc (40 mg/L) but the lowermost was recorded after addition of nano-silver (160 mg/L). In case of number of shoots/explant the resulted differences were between results was found not significant (table 5 & fig. 4). The best outcomes of all NPs treatments almost noted after the addition of nano-zinc (40 mg/L).

Compared with bulk material Ag nanoparticles have an extensive applications in numerous fields because of their superior biological, physical and chemical characteristic (**47**). Ag NPs have vital properties which depend on the particle's shapes and size (48**)**. Furthermore, the effect of Ag nanoparticles is vastly dependent on plant genotype, the little quantity of Ag nanoparticles can decrease shoot length in *Hordeum vulgare*, but 100mg/L of Ag nanoparticles has no significant influence on *Lactuca sativa* and *Cucumis sativus* **(49).** Additionally, Ag nanoparticles at 10–20 mg/L stimulate *Eruca sativa* growing whereas higher concentration (100 mg/L of Ag nanoparticles) recorded lower growing values **(50).**

Data of this study revealed that nanoparticles using *in vitro* growth of different olive cultivars could be depends on genotypes of the plants used where koroneiki genotype respond to addition of zinc oxide and silver NPs but dolcie and kalamata genotypes have not any response, also NPs using is dose dependent where, addition of 40 mg/L from zinc oxide NPs is best dose for koroneiki growth parameters. Whereas, **Hasanin et al., (13)** found that effect of NPs could be depend on genotypes where their experiment data when they used picual and dolcie cultivars as explants. Their results disclosed that the good influence of Ag nanoparticles on shoot growth of *in vitro* olive shoots is high dependent on olive cultivar genotype and NPs concentration. Where, picual cv. recorded higher values of shoot growing parameters compared with dolcie cv. Ag NPs addition to the culture media affected significantly the development of *in vitro* olive shoots. But the higher concentration of

Ag nanoparticles damaged the growth parameters of dolcie genotype and recorded lower growth values. Ag nanoparticles effect significantly on morphology and anatomical leaf structure.

Table (5): The effect of different types of silver and zinc oxide NPs of different concentrations, Contamination percentage, buds germination and vegetative growth parameters (shoot length by cm, number of shoots/explant and number of leaves/shoot) of Koroneiki olive cultivar as affected by using NPs during second experiment. Means followed by different letters are significantly different at $p \leq 0.05$

Treatment (starting)	Contamination %	Bud germination (%)	Mean of Shoot length (cm)	No. of shoots/explant	No. of leaves/ shoot
Control	46.00 a	42.00 e	2.10 b	1.27 a	8.01 b
Nano-Zinc 160	11.00 d	17.00 b	2.17 bc	1.09 a	5.08 f
Nano-Zinc 80	12.00 cd	43.00 e	2.45 d	1.32 a	7.00 c
Nano-Zinc 40	15.00 c	57.00 f	2.79 e	1.48 a	9.77 a
Nano-silver 160	13.00 cd	13.00 a	1.99 a	1.13 a	4.91 g
Nano-silver 80	10.00 d	25.00 c	2.15 bc	1.22 a	6.52 e
Nano-silver 40	18.00 b	29.00 d	2.21 c	1.29 a	6.94 d

3.5. Multiplication experiment:

After 4 weeks the germinated explants from koroneiki genotype were sub-cultured twice into MS medium, the results of the experiment were shown in table (5& fig. 8). Contamination percent was improved and decreased after almost all treatments when compared with starting experiment, as well as contamination decreased significantly after treated with all NPs addition when compared with control (table 6 & fig. 5). Data of shoot length mean (by cm), number of leaves/shoot and number of shoots/explant were found all measurements changed significantly when compared to control. As well as starting experiment, the best outcomes of all NPs treatments almost noted after the addition of nano-zinc C (40 mg/L).

Nanoparticles have distinctive property enable them extremely applicable in many sciences such as chemistry, physics, biology and environment **(51).** Many studies have demonstrated the optimistic effects of nanoparticles in plant micropropagation, as shoot multiplication, callus induction and plant development **(52).**

The plant tissue phytotoxicity of higher nanoparticles concentrations demonstrated by many researches **(53).** Most nanoparticles can change the physiological, biochemical, genetic structures, morphological and anatomical of the treated plant tissue **(54).** Plants treated with nanoparticles this can be affect the genetic structures through the mitotic cycle and change the protein and DNA profile **(55).** Phytotoxicity of Ag nanoparticles may be produced by the creation of ROS (Reactive Oxygen Species), this lead to proteins destruction, lipid peroxidation and DNA damage. Silver nanoparticles may be induce variation in morphology and anatomy of callus and enlarged somaclonal variation in calli and regenerated shoots **(56, 57)**.

Table (6): The effect of different types of silver and zinc oxide NPs of different concentrations, contamination percentage buds germination and vegetative growth parameters (shoot length by cm, number of shoots/explant and number of leaves/shoot) for Koroneiki olive cultivar as affected by NPs during second experiment, a after 8 weeks of culturing in multiplication media during second experiment. Means followed by different letters are significantly different at $p \leq 0.05$

Treatment (multiplication)	Contamination %	Mean of Shoot length (cm)	No. of shoots/explant	No. of leaves/ shoot
Control	41.00 a	4.10 b	1.53 a	15.81 b
Nano-Zinc 160	9.00 d	3.83 d	1.12 e	8.41 d
Nano-Zinc 80	11.00 cd	3.95 c	1.36 b	6.58 e
Nano-Zinc 40	14.00 c	4.27 a	1.55 a	17.09 a
Nano-silver 160	13.00 cd	2.13 g	1.16 de	5.35 f
Nano-silver 80	11.00 cd	2.45 f	1.25 cd	7.36 e
Nano-silver 40	15.00 b	3.57 e	1.31 bc	11.39 c

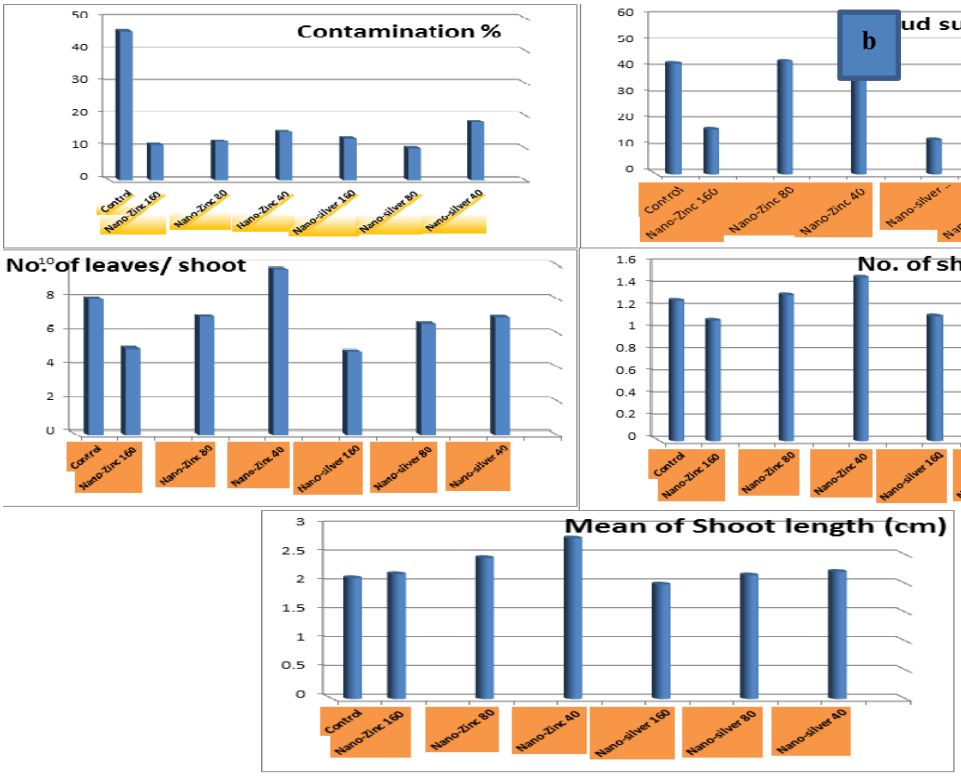

Figure 4: The effect of different types of nanoparticles (zinc oxide and silver) on contamination, bud germination percentage and vegetative growth parameters (shoot length by cm, number of shoots/explant and number of leaves/ shoot) during starting experiment for Koroneiki olive cultivar.

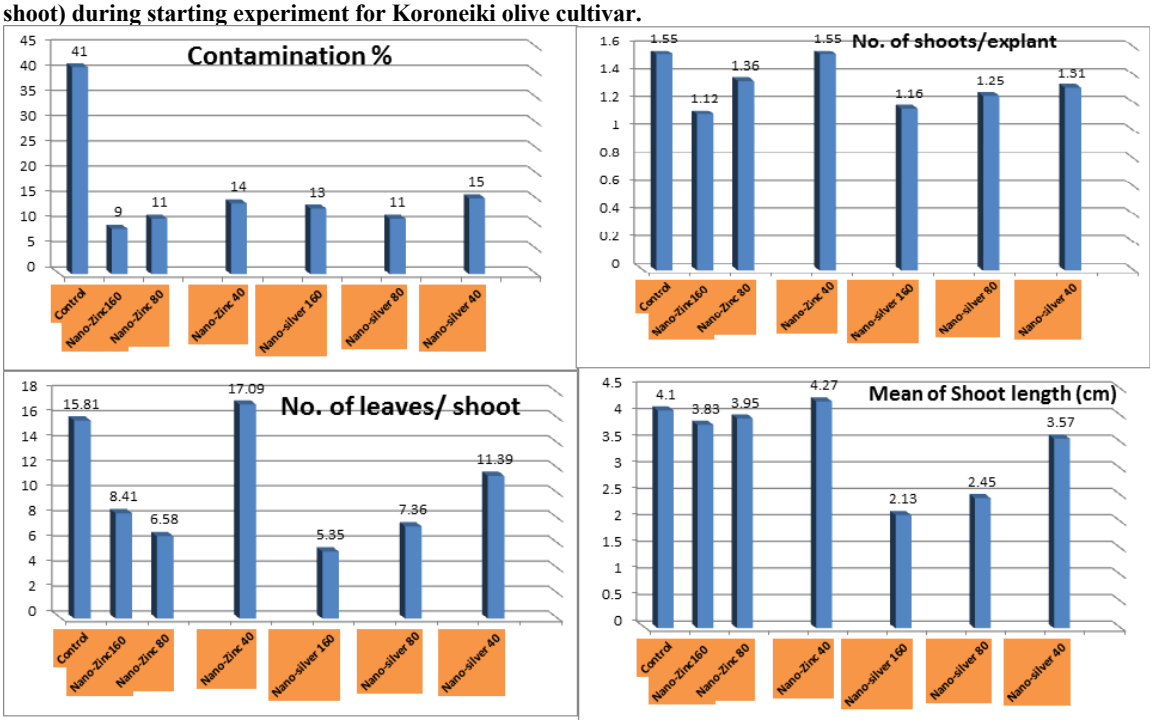

Figure 5: The effect of different types of nanoparticles (zinc oxide and silver) on contamination, bud germination percentage and vegetative growth parameters (shoot length by cm, number of shoots/explant and number of leaves/ shoot) during multiplication experiment for Koroneiki olive cultivar.

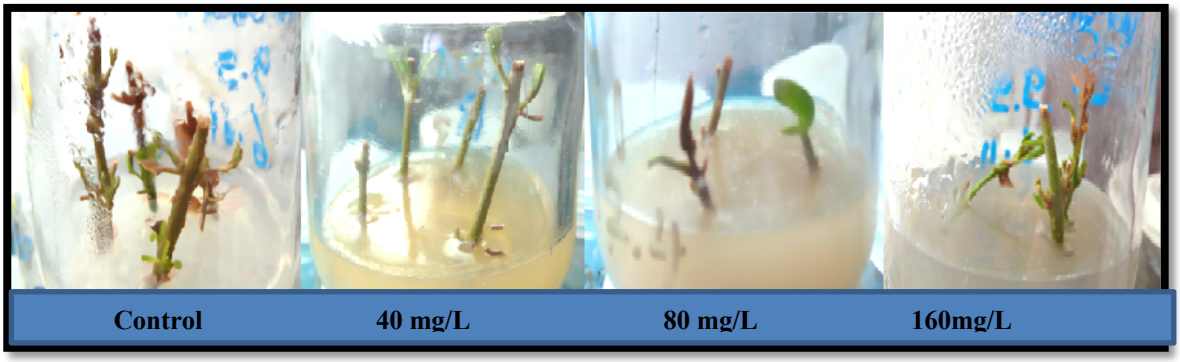

Figure 6: The response of koroneiki olive cultivar as affected by three different concentrations of silver NPs cultivated on OM media after 4 weeks of culture.

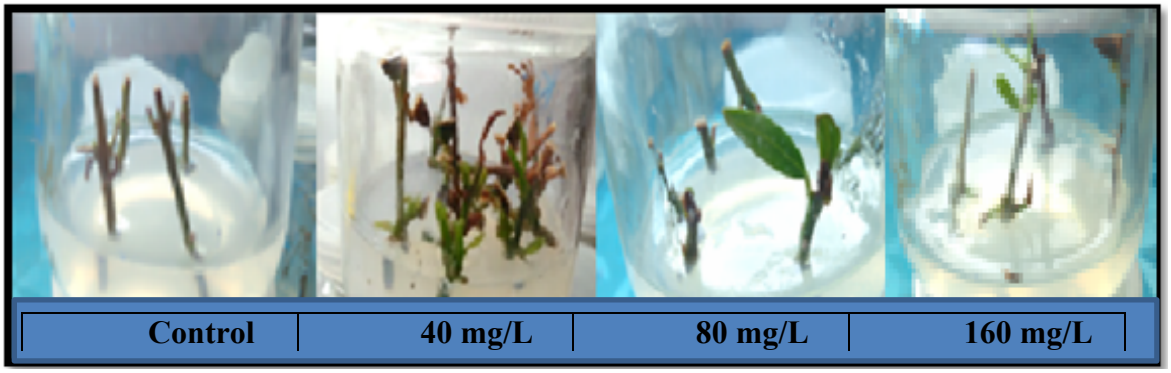

Figure 7: The response of koroneiki olive cultivar as affected by three different concentrations of zinc oxide NPs cultivated on OM media after 4 weeks of culture.

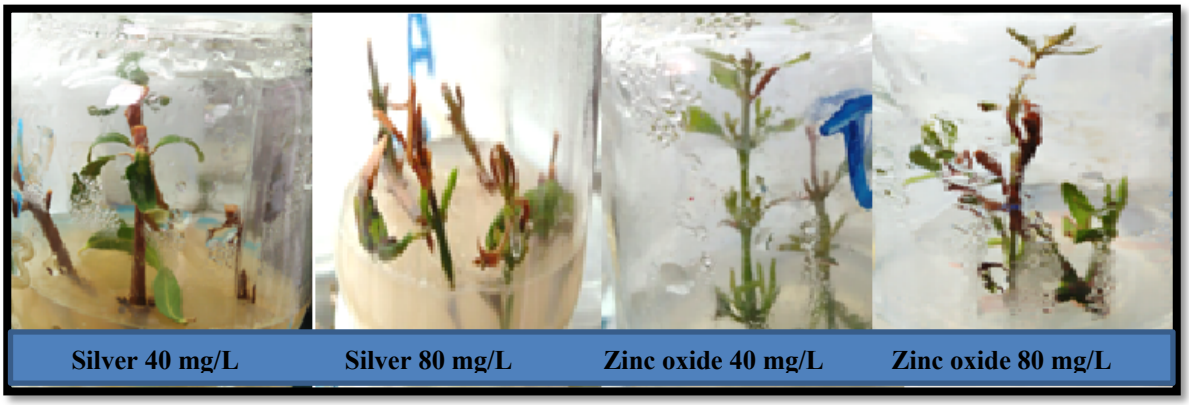

Figure 8: The response of koroneiki olive cultivar as affected by two different concentrations of silver and zinc oxide NPs cultured on OM media after two subcultures.

Results of these study support that silver nanoparticles can lead to an enhancement in plant growth and decrease contamination, nevertheless olive koroneiki genotype growth parameters not improved with any concentration used from silver nanoparticles, but contamination percentage improved. Data showed that Ag nanoparticles had stimulate the shoot regeneration of micropropagation culture the shoots of koroneiki cultivar and the impact of NPs on shoot growth was high dependent on nanoparticles concentration as many previous studies **(13, 14).** It is clearly that Ag NPs most decreased and the efficiency of nanoparticles is dose dependent, which is reliable with many studies **(12, 13, 14).**

Koroneiki genotype showed improvement in some growth parameters in this study. Ag ions have positive effects on micropropagation of growing shoots, for example, improved explants survival and delayed their senescence **(58),** as well as shoot multiplication rate increased, shoot growth and organogenesis improved **(59).** Many other researchers concluded that shoot growth and number of shoots/explant were improved in *Vanilla planifolia, Brassica juncea* and *Tecomella undulate Roxb.* cultured on media supplemented with silver nanoparticles **(60)** which was related to the effect of silver ion as an ethylene blockage factor; as the addition of cytokinin to culture medium is known to enable ethylene production; Ag+ would produce blocking of ethylene action and encourage shoot regeneration and delay explant senescence. Moreover, **Syu et al.**

(61) indicated that silver nanoparticles inhibited ethylene action through reduced the expression of *ACC* synthase *7* and *ACC* oxidase *2*.

Many studies applied in different plants support our finding, as *Vigna radiate* was recorded increase in the dry weight when treated with 500, 1000, 2000, and 4000 ppm of zinc oxide nanoparticles **(62).** wheat was recording an increase in the growth, seed germination and chlorophyll content after seed priming with zinc oxide nanoparticles **(63, 64).**

In this research contamination decreased as NPs concentration increase, this is evidence to the presence of antimicrobial activity recorded in this study after using zinc oxide and silver NPs. The outcome results recommended a considerable difference between the various nanoparticle types and concentrations on explant growth and microbial contamination whereas, zinc oxide NPs found to be more effective in explant growth than silver NPs, where the addition of 40 mg/L from zinc oxide NPs is the most effective concentration, but both were effective against microbial contamination. The inhibition possibility of NPs agents micropropagation of microorganisms confirmed by numerous studies about the antimicrobial action of the tested nanoparticles **(14, 43, 44, 48, 65).**

In the present study, three concentrations of ZnO NPs were used to evaluate their outcome on the growth and contamination of olive koroneiki explants. The optimistic results here from the addition of 40 mg/L to micropropagation of koroneiki which recorded the best data for explant growth may attributed to that Zn is essential trace element with low toxicity. Zn is a fundamental portion of several enzymes and cofactors that are involved in photosynthesis **(66).** Also, is considered as a micronutrient, essential by plants in a minor amount for their proper growth. It is involved in DNA/RNA metabolism, is part of the structure of transcription factors, and confirms the stability and normal functioning of chromatin **(67).** It also e affects the production of plant growth hormones, as it is required for the biosynthesis of tryptophan, a precursor of indole-3-acetic acid **(68).**

The present outcomes of our present study obviously indicate that the higher levels of zinc oxide nanoparticles leads to a negative effect on explants, and so, it was concluded to be dose dependent. **Alsuwayyid et al., (69)** found that the plant Zn necessitates up to 0.05 ppm for typical growing, and unnecessary zinc may lead to plant toxicity **(69)**. The present results are reliable with previous studies carried on ryegrass, lettuce, maize, cucumber and radish **(70, 71)**, peanut **(72),** wheat **(69),** in addition to, soybean seedlings cytotoxicity at 4000 ppm of zinc oxide nanoparticles **(73). Regni et al., (74),** study the effect of ZnO NPs on olive micropropagation and highlighted that zinc oxide nanoparticles exerted beneficial effects on the olive explants in vitro, improving the efficiency of the micropropagation procedure.

The broad usage of NPs can lead to a negative effects on the environment and may be considered a pollutant **(34).** Furthermore, if they move to the food chain they may source for many hazardous effects **(75).** Consequently, it is essential to estimate the effect of these NPs on plants and other living beings. Many scientists have described both the positive and negative effects of different NPs on living organisms. But, extensive investigations are required in this field since there is a shortage of definite information about the impact of NPs on all living beings specially plants **(69).**

4. Conclusion:

Established on the data achieved in the current study, nanoparticles can be applied to plant tissue culture but with definite protocol of NPs concentration to encourage explants germination and decrease contamination. Also, it can be concluded that adding zinc oxide nanoparticles is more effective than silver nanoparticles (Ag NPs) which were both added to Olive media at low concentrations of 40 mg L-1 where improved the bud survival percentage and shoot growth and decreases contamination percentage. Zeatin at 4mg /L cannot improve the proliferation rate and growth of olive dolcie and kalamata cultivars during starting stage. Thus, these results will be helpful in the refinement of protocol for olive *in vitro* propagation. Finally, using NPs in olive micropropagation depend on genotype, also is dose dependent, whereas, koroneiki cultivar is more reliable to micropropagate protocols than dolcie and kalamata cultivars. The usage of NPs in the micropropagation culture of plants enables it possible to learn about many features of plant growth and development in controlled environmental conditions.

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References:

[1] Ibrahim FH, Alhenzab MT, Elbadawi AO (2018) Establishment of an efficient in vitro regeneration protocol for mass propagation via organogenesis of endangered plant Rhanterium epapposum in Qatar. In: Qatar foundation annual research conference proceedings, vol 1. HBKU Press Qatar, pp EEPD85.

[2] Espinosa CA, Puente CA, Garcia S (2018) In vitro plant tissue culture: means for production of biological active compounds. Planta 348:1–18.

[3] Surakshitha NC, Soorianathasundaram K, Ganga M, Raveendran M (2019) Alleviating shoot tip necrosis during in vitro propagation of grape cv. Red Globe Sci Hortic 248:118–125.

[4] Benmahioul B, Dorion N, Kaid-Harche M, Daguin F (2012) Micropropagation and ex vitro rooting of pistachio (Pistacia vera L.). Plant Cell Tissue Org 108(2):353–358.

[5] Cuenca B, Shnchez C, Aldrey A, Bogo B, Blanco B, Correa B, Vidal N (2017) Micropropagation of axillary shoots of hybrid chestnut (Castanea sativa × C. crenata) in liquid medium in a continuous immersion system. Plant Cell Tissue Org 131(2):307–320.
[6] Restrepo C, Gmez FA, Gil A, Torres JM, Urrea AI (2018) In vitro propagation of avocado (Persea americana Mill.) cv. Hass through morphogenesis. Acta Agron 67(1):160–167.
[7] Bello-Bello JJ, Iglesias L, Snchez L, Casas J, Santana N (2012) In vitro regeneration of Pinus brutia Ten. var. eldarica (Medw.) through organogenesis. Afr J Biotechnol 11(93):15982–15987.
[8] Rostami AA, Shahsavar A (2009) Olive "Mission" explants. Asian J Plant Sci 8(7):505–509.
[9] Rugini E., Gutiérrez Pesce P. (2006). Genetic improvement of olive. Pomol Croati, 12:43-72.
[10] Bartolini, G.. A.R. Leva and A. Benelli. l990. Adrances in in vitro cultures of olive: Propagation of cv. Maurino. Acta Houic., 286: 41-44.
[11] Cozza, R., D. Turco, C.B. Bati and M,B. Bitoni, 1997. Influenoe of growth medium on mineral composition and leaf histology in micropropagaied plantlets. Plant Cell. Tissue Organ C ult., 5l: 215-223.
[12] Hegazi E. S., Aml R. M. Yousef, Abdallatif A. M., Thanaa Sh. M. Mahmoud and Mona K.M. Mostafa. Effect of Silver Nanoparticles, Medium Composition and Growth Regulators on in vitro propagation of Picual Olive Cultivar. Egyptian Journal of Chemistry. Vol. 64, No. 12 pp. 6961 - 6969 (2021).
[13] Hasanin M.S., S.A.M. Hassan, A.M. AbdAllatif and O.M. Darwesh, 2024. Unveiling the silver lining: examining the effects of biogenic silver nanoparticles on the growth dynamics of in vitro olive shoots. Microbial Cell Factories, 23:79.
[14] Darwesh O. M., S. A.M. Hassan, and A. M. Abdallatif (2023). Improve In vitro Multiplication of Olive Shoots Using Environmental-Safe Chitosan, Selenium, and Silver Nanostructures. Biointerface research in applied chemistry, Volume 13, Issue 5, 2023, 419 p 1-12.
[15]Fraga, H.; Moriondo, M.; Leolini, L. and João A. Santos (2021). Mediterranean olive orchards under climate change: A review of future impacts and adaptation strategies. Agronomy, 56 (11):1-15.
[16] Houng, J,, H,N, Murty and K.Y. Peak, 200I , High frequency adventitious shoot induction and plant regeneration from leaves of siatice. Plant Cell Tissue Organ Cult., 65:123-128.
[17] Dabai y.u.,Arzai A.H.,Muhammad S.,2007.In vitro antibacterialactivity of single and combined antibiotics on bone bacterial isolate. Pak.J.Bio.sci.6:1-3.
[18] Leifert C. and weedward S., 1997.Laboratory contamination Management ; The requirement for microbial contamination management in Micropropagation. Cassells A.C.(ed) .Kluwer Academic publisher ,Dordrecht ,pp: 237-244.
[19] Martino, L., L. Cuozzn and A. Bninori, 1999. Establishment of meristem lip culture from field- grown olire (Olea eumpaea L.). Moraiolo. Agric. Med., 129: 193-198.
[20] Kim DH, Gopal J, Sivanesan I (2017) Nanomaterials in plant tissue culture: the disclosed and undisclosed. RSC Adv 7(58):36492–36505.
[21] Wang P, Lombi E, Zhao F-J, Kopittke PM. Nanotechnology: a new opportunity in plant sciences. Trends Plant Sci. 2016;21(8):699–712.
[22] Abdi, G.; Salehi, H. and Khosh-Khui, M. (2008). Nano silver: A novel nanomaterial for removal of bacterial contaminants in valerian (Valeriana officinalis L.) tissue culture. ActaPhysiol Plant 30: 709-714.
[23]Sondi I, Salopek-Sondi B (2004) Silver nanoparticles as antimicrobial agent: a case study on E. coli as a model for Gram-negative bacteria. J Colloid Interface Sci 275(1): 177-182.
[24]Mir, S.A,. V. Shrotriya, T.I. Al-Muhimeed, M.A. Hossain, M.B. Zaman, Metal and metal oxide nanostructures applied as alternatives of antibiotics, Inorg. Chem. Commun. (2023) 110503.
[25] Riahi S., Moussa N. B., Lajnef M., Jebari N., Dabbek A., Chtourou R., Guibiers G., Vimont S., Herth E.. Bactericidal activity of ZnO nanoparticles against multidrug-resistant bacteria, J. Mol. Liq. 387 (2023) 122596.
[26] Espitia P.J.P., N. d. FF Soares, JS d. R. Coimbra, NJ de Andrade, RS Cruz and EAA Medeiros, Food Bioprocess Technol 5 (2012) 1447–1464.
[27] Dwivedi S., R. Wahab, F. Khan, Y.K. Mishra, J. Musarrat, A.A. Al-Khedhairy, Reactive oxygen species mediated bacterial biofilm inhibition via zinc oxide nanoparticles and their statistical determination, PloS One 9 (11) (2014) e111289.
[28] Wang BWY, Feng WY, Wang C, Jia M, Wang C, Shi W, Zhang F, Zhao L, Chai F (2006). Acute toxicity of nano-and micro scale zinc powder on healthy adult mice. Toxicol Lett. 161:115-123.
[29]Yang L, Watts DJ (2005). Particle surface characteristics may play an important role in phytotoxicity of alumina nano-particles. Toxicol Leu. 158:122-132.
[30]USPEA (U.S. Environmental Protection Agency) (2005). Nanotechnology white paper external review.
[31] Oloumi, H.; R. Soltaninejad and A. Baghizadeh, (2015). The comparative effects nano and bulk size of CuO and ZnO on glycyrrhizin and phenolic compounds contents in Glycyrrhiza glabra L. seedling. Indian J. Plant Physiol., 20, 157–161.
[32] Singh, R.P., Handa, R., Manchanda, G., 2021. Nanoparticles in sustainable agriculture: an emerging opportunity. J. Control. Release. 329. https://doi.org/ 10.1016/j.jconrel.2020.10.051.
[33] Ale, S., Himanshu, S.K., Mauget, S.A., Hudson, D., Goebel, T.S., Liu, B., Baumhardt, R.L.,Bordovsky, J.P., Brauer, D.K., Lascano, R.J., Gitz, D.C., 2021. Simulated dryland cotton yield response to selected scenario factors associated with soil health. Front. Sustain. Food Syst. 4.
[34] Rajput, V., Minkina, T., Sushkova, S., Behal, A., Maksimov, A., Blicharska, E., Ghazaryan, K., Movsesyan, H., Barsova, N., 2020. ZnO and CuO nanoparticles: a threat to soil organisms, plants, and human health. Environ. Geochem. Health 42.

[35] Akbarnejad-Samani, Z., Shamili, M., Samari, F., 2020. The toxicity potential of Ag nanoparticles synthesized from Cordia myxa L. Adv. Hortic. Sci. 34.
[36] Fedorenko, A.G., Minkina, T.M., Chernikova, N.P., Fedorenko, G.M., Mandzhieva, S.S., Rajput, V.D., Burachevskaya, M.V., Chaplygin, V.A., Bauer, T.V., Sushkova, S.N., Soldatov, A.V., 2021. The toxic effect of CuO of different dispersion degrees on the structure and ultrastructure of spring barley cells (Hordeum sativum distichum). Environ. Geochem. Health 43.
[37] Abd El-All, A.S.; S.A. Osman, H.M.F. Roaiah, M.M. Abdalla, A.A. Abd El Aty, and W.H. AbdEl- Hady,(2015). Potent anticancer and antimicrobial activities of pyrazole, oxazole and pyridine derivatives containing 1,2,4 t-triazine moiety. Med. Chem. Res. 24, 4093.
[38] El-serwy, W.S.; N.A. Mohamed, W.S. El-serwy, E.M.M. Kassem, and A.A. Abd El Aty, (2015). Synthesis of new benzofuran derivatives and evaluation of their antimicrobial activities. Biol. Chem. Sci. 6, 213.
[39] Peixe A, Raposo A, Lourenço R, Cardoso H, Macedo E. Coconut water and BAP successfully replaced zeatin in olive (Olea europaea L.) micropropagation. Sci Hort. 2007;113(1):1–7.
[40] Lambardi M, Ozudogru EA, Roncasaglia R. In vitro propagation of olive (Olea europaea L.) by nodal segmentation of elongated shoots, Protocols for Micropropagation of Selected Economically-Important Horticultural Plants, Springer2012, pp. 33–44.
[41] Goswami P, Mathur J (2019) Positive and negative effects of nanoparticles on plants and their applications in agriculture. Plant Sci Today 6(2): 232-242.
[42] El-Shanshoury A, Darwesh O, Sabae S, Awadallah O, Hassan S (2020) Bio-manufacturing of selenium nanoparticles by Bacillus subtilis isolated from Qarun Lake and evaluation their activity for water remediation. Biointerf Res Appl Chem 10(4): 5834–5842.
[43] Darwesh OM, Sultan YY, Seif MM, Marrez DA (2018) Bio-evaluation of crustacean and fungal nanochitosan for applying as food ingredient. Toxicol Rep 5: 348-356.
[44] Ismail M, Prasad R, Ibrahim AIM, Ahmed ISA (2017) Modern prospects of nanotechnology in plant pathology. In: Prasad R, Kumar M, Kumar V. (Eds) Nanotechnology. Springer Nature Singapore Pte Ltd., Singapore, pp 305–317.
[45] Hwan KD, Gopal J, Sivanesan I (2017) Nanomaterials in plant tissue culture: the disclosed and undisclosed. RSC Adv 7: 36492–36505.
[46] Al-darwesh M.Y., S. S. Ibrahim, and M. A. Mohammed, 2024. A review on plant extract mediated green synthesis of zinc oxide nanoparticles and their biomedical applications. Results in Chemistry 7 (2024) 101368.
[47] Syafiuddin A, Salim MR, Beng Hong Kueh A, Hadibarata T, Nur H. A review of silver nanoparticles: research trends, global consumption, synthesis, proper¬ties, and future challenges. J Chin Chem Soc. 2017;64(7):732–56.
[48] Elsayed H, Hasanin M, Rehan M. Enhancement of multifunctional properties of leather surface decorated with silver nanoparticles (Ag NPs). J Mol Struct. 2021;1234:130130.
[49] El-Temsah YS, Joner EJ. Ecotoxicological effects on earthworms of fresh and aged nano-sized zero-valent iron (nZVI) in soil. Chemosphere. 2012;89(1):76–82.
[50] Ray S, Maunsell JH. Different origins of gamma rhythm and high-gamma activity in macaque visual cortex. PLoS Biol. 2011;9(4):e1000610.
[51] Mourad R, Darwesh O, Abdel-Hakim A. Enhancing physico-mechanical and antibacterial properties of natural rubber using synthesized Ag-SiO2 nanoparticles. Int J Biol Macromol. 2020;164:3243–9.
[52] Spinoso-Castillo J, Chavez-Santoscoy R, Bogdanchikova N, Pérez-Sato J, Morales-Ramos V, Bello-Bello J. Antimicrobial and hormetic effects of silver nanoparticles on in vitro regeneration of vanilla (Vanilla planifolia jacks. Ex Andrews) using a temporary immersion system. Plant Cell Tissue Organ Cult (PCTOC). 2017;129(2):195–207.
[53] Musante C, White JC. Toxicity of silver and copper to Cucurbita pepo: differential effects of nano and bulk-size particles. Environ Toxicol. 2012;27(9):510–7.
[54] Tripathi DK, Singh S, Singh VP, Prasad SM, Chauhan DK, Dubey NK. Silicon nanoparticles more efficiently alleviate arsenate toxicity than silicon in maize cultivar and hybrid differing in arsenate tolerance. Front Environ Sci. 2016;4:46.
[55] Landa P, Prerostova S, Petrova S, Knirsch V, Vankova R, Vanek T. The tran¬scriptomic response of Arabidopsis thaliana to zinc oxide: a comparison of the impact of nanoparticle, bulk, and ionic zinc. Environ Sci Technol. 2015;49(24):14537–45.
[56] Ewais EA, Desouky SA, Elshazly EH. Evaluation of callus responses of Solanum nigrum L. exposed to biologically synthesized silver nanoparticles. Nanosci Nanotechnol. 2015;5(3):45–56.
[57] Kokina I, Mickeviča I, Jermaļonoka M, Bankovska L, Gerbreders V, Ogurcovs A, Jahundoviča I. Case study of somaclonal variation in resistance genes Mlo and Pme3 in flaxseed (Linum usitatissimum L.) induced by nanoparticles. Int J Genomics 2017 (2017).
[58] Zhao Q, Liu Y, Wang C (2005) Development and evaluation of electroless Ag-PTFE composite coatings with anti-microbial and anti-corrosion properties. Appl Surf Sci 252(5):1620–1627.
[59] Betts AJ, Dowling DP, McConnell ML, Pope C (2005) The influence of platinum on the performance of silver–platinum antibacterial coatings. Mater Des 26(3):217–222.
[60] Pulit-Prociak J, Banach M (2016) Silver nanoparticles—a material of the future…? Open Chem 14:76–91. https ://doi.org/10.1515/ chem-2016-0005.
[61] Syu Y-y, Hung J-H, Chen J-C. -w. Chuang, impacts of size and shape of silver nanoparticles on Arabidopsis plant growth and gene expression. Plant Physiol Biochem. 2014;83:57–64.

[62] Mahajan, P., Dhoke, S.K., Khanna, A.S., 2011. Effect of nano-ZnO particle suspension on growth of mung (Vigna radiata) and gram (Cicer arietinum) seedlings using plant agar method. J. Nanotechnol.
[63] Munir, T., Rizwan, M., Kashif, M., Shahzad, A., Ali, S., Amin, N., Zahid, R., Alam, M.F.E., Imran, M., 2018. Effect of zinc oxide nanoparticles on the growth and Zn uptake in wheat (Triticum aestivum L.) by seed priming method. Dig. J. Nanomater. Biostructures 13.
[64] Rai-Kalal, P., Jajoo, A., 2021. Priming with zinc oxide nanoparticles improve germination and photosynthetic performance in wheat. Plant Physiol. Biochem. 160.
[65] El-Banna, A.N.; El-Mahrouk, M.E.; Dewir, Y.H.; Farid, M.A.; Abou Elyazid, D.M.; Schumacher, H.M. Endophytic Bacteria in Banana In vitro Cultures: Molecular Identification, Antibiotic Susceptibility, and Plant Survival. Horticulture 2021, 7, 526,.
[66] Hu, H., Sparks, D., 2019. Zinc deficiency inhibits chlorophyll synthesis and gas exchange in 'stuart' pecan. HortScience 26.
[67] Ramesh, A.; Sharma, S.K.; Sharma, M.P.; Yadav, M.; Joshi, O.P. Inoculation of zinc solubilizing Bacillus aryabhattai strains for improved growth, mobilization and biofortification of zinc in soybean and wheat
[68] Puzina, T.I. Effect of zinc sulfate and boric acid on the hormonal status of potato plants in relation to tuberization. Russ. J. Plant Physiol. 2004, 51, 209–215. cultivated in Vertisols of central India. Appl. Soil Ecol. 2014, 73, 87–96.
[69] Alsuwayyid A. A., A. S. Alslimah, K. Perveen, N. A. Bukhari, and L. A. Al-Humaid, 2022. Effect of zinc oxide nanoparticles on Triticum aestivum L. and bioaccumulation assessment using ICP-MS and SEM analysis. Journal of King Saud University – Science 34 (2022) 101944.
[70] Lin, D., Xing, B., 2007. Phytotoxicity of nanoparticles: Inhibition of seed germination and root growth. Environ. Pollut. 150, 243–250.
[71] Lin, D., Xing, B., 2008. Root uptake and phytotoxicity of ZnO nanoparticles. Environ. Sci. Technol. 42.
[72] Prasad, T.N.V.K.V., Sudhakar, P., Sreenivasulu, Y., Latha, P., Munaswamy, V., Reddy, K.R., Sreeprasad, T.S., Sajanlal, P.R., Pradeep, T., 2012. Effect of nanoscale zinc oxide particles on the germination, growth and yield of peanut. J. Plant Nutr. 35.
[73] López-Moreno, M.L., de la Rosa, G., Hernández-Viezcas, J.Á., Castillo-Michel, H., Botez, C.E., Peralta-Videa, J.R., Gardea-Torresdey, J.L., 2010. Evidence of the differential biotransformation and genotoxicity of ZnO and CeO2 nanoparticles on soybean (Glycine max) plants. Environ. Sci. Technol. 44.
[74] Regni L., D. Del Buono, M. Micheli, S. L. Facchin, C. Tolisano and P. Proietti. Effects of Biogenic ZnO Nanoparticles on Growth, Physiological, Biochemical Traits and Antioxidants on Olive Tree In Vitro. Horticulturae 2022, 8, 161.
[75] Hawthorne, J., De la Torre Roche, R., Xing, B., Newman, L.A., Ma, X., Majumdar, S., Gardea-Torresdey, J., White, J.C., 2014. Particle-size dependent accumulation and trophic transfer of cerium oxide through a terrestrial food chain. Environ. Sci. Technol. 48.