Enhancing *In Vitro* Multiplication of some Olive Cultivars Using Silver, Selenium And Chitosan Nanoparticles

Osama M. Darwesh (darweshosama@yahoo.com)
National Research Centre https://orcid.org/0000-0002-7742-4982
Sayed A.M. Hassan
National Research Centre
Abdou M. Abdallatif
Faculty of Agriculture, Cairo University

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Abstract

Olive trees are commercially propagated by cuttings or grafting on clonal or seedling rootstocks; recently, olive micropropagation has emerged as a powerful technique for mass production of true to type and pathogen free plants. The current study was carried out to evaluate the effect of silver, chitosan and selenium nanoparticles as microbial decontamination agents, as well as evaluate its possible effects on *in vitro* shoot growth and multiplication of three olive cultivars namely Koroneiki, Picual and Manzanillo. Validation and characterization of the biosynthesized nanoparticles was carried out by Transmission Electron Microscopy. The produced nanoparticles were added to the olive culture medium, and the growth parameters were determined. The tested nanoparticles showed varied degree of antimicrobial activity, silver nanoparticles were highly effective to inhibit *in vitro* microbial contamination. The effect of genotypes on shoot growth and multiplication was significant; Koroneiki and Picual cultivars showed better growth measurements compared with Manzanillo. The addition of nanoparticles to the culture medium had a significant outcome on growth and multiplication rate of *in vitro* growing olive shoots. Silver nanoparticles produced higher values of number of shoots, shoots length, leaf number and multiplication rate compared with the other treatments. In conclusion; the current results showed that nanoparticles were efficient as *in vitro* disinfectant agent and the nanoparticles addition to culture medium especially silver NPs had a positive effect on growth and multiplication rate of *in vitro* growing olive shoots.

Introduction

Olive (*Olea europaea*) is one of the traditionally cultivated fruit crops in the Mediterranean region and one of the most important fruit crops in Egypt (**Baldoni and Belaj 2009**). Olive trees are generally adapted to the semi-arid environment and traditionally grown under arid desert conditions (**Dag et al. 2008; Guerfel et al. 2009**). Olive fruits, oil and leaves are rich sources of valuable nutrients and bio-active pharmaceutical materials (**Ghanbari et al. 2012**). The benefits of olive products to human health have been widely recognized (**Visioli et al. 2002**). Olive trees are usually propagated by leafy stem cuttings under mist conditions; the hard to root cultivars are propagated by grafting onto seedlings or clonal rootstocks (**Fabbri et al. 2004**). Olive micropropagation has been reported as a powerful technique for mass production of pathogen-free and true to type cultivars, and offers a valuable tool for genetic improvement and germplasm conservation (**Zuccherelli and Zuccherelli 2002; Zacchini and De Agazio 2004**). *In vitro* propagation of olive is affected by several factors including plant genotype, growth medium, cytokinin type and concentration (**Grigoriadou et al. 2002; Zuccherelli and Zuccherelli 2002; Peixe et al. 2007**). Generally, microbial contamination, oxidation of phenolic compounds and slow shoot growth are the major problems of olive micropropagation (**Lambardi et al. 2012**). Moreover, olive shoots are characterized by a strong apical dominance and the formation of axillary shoots is very limited (**Fabbri et al. 2004**). Microbial contamination is a serious problem of *in vitro* propagation; eliminate the microbial contamination is one of the basic requirements for successful initiation, growth and development of cultured plant tissues (**Abdi et al. 2008; Msogoya et al. 2012**). To eliminate microbial contamination, plant

materials must be surface sterilized; disinfection procedures usually involve using of sodium hypochlorite, ethyl alcohol, hydrogen peroxide, mercury chloride and antibiotics; nevertheless, these substances are frequently toxic to plant tissues and have shown inhibitory effects on explant growth and development (**Teixeira da Silva et al. 2003; Abdi et al. 2008**). Therefore, there is a need for more studies to improve olive micropropagation efficiency with inhibition of pathogens growth. Recently, nanotechnology have received much attention, it expected to support a wide range of applications in agriculture fields, e.g. enhancing seed germination, plant growth, yield, nutritional value and production of secondary metabolites, moreover nanotechnology aims to develop a new delivery system of mineral nutrients and pesticide, thereby, reducing environmental pollution (**Tarafdar et al. 2013; Nuruzzaman et al. 2016; Wang et al. 2016; Ismail et al. 2017; Javed et al. 2017**). The unique properties of nanoparticles provide excellent chemical, mechanical, and electrical activity, which are generally depending on material type, particle size and their uptake and translocation in plant tissues (**Franklin et al. 2007; Jiang et al. 2009; Salehi et al. 2018; Hussein et al. 2019a,b**). A wide range of NPs including, silver, titanium dioxide and zinc oxide have antimicrobial activities against different types of microorganisms (**Allahverdiyev et al. 2011; Applerot et al. 2012**). In plant tissue culture, several reports have proved the positive effects of NPs on seed germination, callus induction, shoot multiplication and plant growth (**Kumar et al. 2009; Aghdaei et al. 2012; Wang et al. 2016; Spinoso-Castillo et al. 2017**). However, the effect of nanoparticles on plants performance is highly depending on plant genotype and nanoparticles type, size and concentration (**Asgari-Targhi et al. 2018**). AgNPs treatments stimulate growth and delayed explants senescence of *in vitro* growing *Tecomella undulate* (**Sarmast et al. 2015**) and *Brassica juncea* (**Sharma et al. 2012**). The anti-microbial activity of chitosan nanoparticles against different plant pathogens was reported previously (**Qi et al. 2004; Du et al. 2009; Ali et al. 2011**). Chitosan application improves plant growth, photosynthesis and increase plant resistance to abiotic stress condition (**Guan et al. 2009; Zong et al. 2017**). Chitosan NPs has the advantages of biocompatibility, and biodegradability, and safety (**Manikandan and Sathiyabama 2016**). Selenium NPs have a wide range of applications in medical and agricultural fields (**Shoeibi et al. 2017**). It was reported that selenium nanoparticles stimulate callus induction and improve morphogenesis of tobacco and increased shoot length and shoot number of *in vitro* growing artichoke (**Abdalla et al. 2021; Domokos-Szabolcsy et al. 2012**). However more studies are required to clarify the possible effect of selenium and chitosan NPs on *in vitro* plant tissues culture. Thus, the current study aimed to investigate the effect of silver, chitosan and selenium nanoparticles on microbial contamination in tissue culture medium as well as evaluate its possible effects on *in vitro* propagation of three dominant olive cultivars under Egyptian conditions (Koroneiki, Picual and Manzanillo).

Material And Methods

Chemicals

All chemicals used in this study were reagent-grade; absolute ethanol (Chem Lab- Zedelgem, Belgium); silver nitrate, sodium hydroxide and acetic acid (Merck-Darmstadt, Germany); chitosan (Oxford Lab Fine Chem-Navghar, India); tri-sodium polyphosphate (Dop Organik Kimya- Ankara, Torkiya); sodium selenite

(SD Fine-Chem Limited- Mumbai, India); ascorbic acid (Lab-Scan Analytical Sciences- Sowinskiego, Poland). All solutions were prepared with deionized water.

Preparation of silver, selenium and chitosan nanoparticles

Silver nanoparticles (AgNPs) were prepared using the supernatant free cells of *Fusarium oxysporum* (**Elshahawy et al. 2018**). A 250 ml flasks containing 100 ml sterilized potato dextrose broth (PDB) medium was inoculated with *F. oxysporum* and incubated in a rotary shaker at 28˚C for 3 days, then the cell free supernatant was obtained by filtration (Whatman No.1). Ten ml of supernatant was added to 10 ml of 1M silver nitrate solution in bottles and incubated under static conditions at room temperature for 24 h. The prepared AgNPs were obtained by centrifugation (10000 rpm for 10 min) and washed three times by deionized water and then washed by absolute ethanol and oven dried at 50 °C (**Marrez et al. 2019**). In case of selenium nanoparticles preparation, stock solutions of 100 mM sodium selenite and 50 mM ascorbic acid were prepared in deionized water. The ascorbic acid was mixed drop wise with the sodium selenite solution under magnetic stirring (600 rpm) at room temperature till achieve a final ratio of 1:4 sodium selenite to ascorbic acid. The mixtures were allowed to react till the color changed from colorless to light orange (**Hussein et al. 2019a**). Chitosan nanoparticles were prepared by ionic gelation technique using tri-sodium polyphosphate (TPP) (**Darwesh et al. 2018**). One gram of medium molecular weight chitosan (deacetylation degree> 80%), were dissolved in 100 ml of 1% (v/v) acetic acid solution by stirring at room temperature overnight, the final pH of chitosan solutions was adjusted to 5.9 with 1M of NaOH (**Tsai and Su 1999**). Chitosan nanoparticles were obtained by drop wise addition of 14 ml of 0.1 %TPP solution to 35 ml of chitosan solution under continuous stirring (550 rpm) at room temperature. Chitosan nanoparticles were precipitated by centrifugation at 10000 rpm for 10 min. The pellet was washed with distilled water followed by absolute ethanol then air dried.

Characterization of the synthesized nanoparticles

Dimension and shape of the manufactured nanoparticles have been analyzed using transmission electron microscopy (JEOL, JEM 1400, USA) at an accelerating voltage of 80 kv. Samples for TEM analysis have been prepared by drop-coating nanoparticles solution onto carbon-coated copper grids. The films on the TEM grids have been allowed to stand for 2 min, then extra solution was removed and the grid was allowed to dry prior to measurement.

Effect of synthesized nanoparticles on *in vitro* microbial contamination

In order to validate the efficiency of the studied nanoparticles on microbial contamination, silver at 5 and 10 mg l^{-1}, selenium at 2.5 and 5 mg l^{-1} and chitosan at 40 and 60 mg l^{-1}, were added to the non-sterilized Rugini olive medium (**Rugini 1984**) supplemented with 30g l^{-1} mannitol and 6 g l^{-1} agar; the experiment including comparative negative control treatment (non-sterilized free nanoparticles medium) and positive control treatment (autoclave sterilized free nanoparticles medium). The prepared media were dispensed in glass petri dishes and incubated at 25°C with 16 h photoperiod for 48h; fungus or bacteria colony

formation was monitored by visual examining of culture plates, and the visible colonies were counted and the contamination percentage was calculated in relation to the negative control treatment.

Plant materials and explants preparation

Active growing shoots of 30 to 40 cm were collected during June and July from mature own rooted olive trees of 'Koroneiki', 'Picual' and 'Manzanillo' cultivars, grown at experimental olive orchard (experimental orchard location is situated at 031°12'65"E longitude, 30°00'48"N latitude, Giza, Egypt). The shoots were collected from each cultivar and immediately transferred to the laboratory; shoots were stripped of leaves, divided into nodal cuttings and washed several times with running tap water. The nodal segments were surface sterilized by 20% commercial bleach (5.5% NaOCl) for 10 min followed by 1000 mg l^{-1} mercuric chloride ($HgCl_2$) for 5min and then rinsed 3 times with distilled and sterilized water for 5 min.

Effect of the synthesized nanoparticles on in vitro performance of olive explants

Sterilized nodal segments of the selected cultivars were cultured on Rugini olive medium (**Rugini 1984**), supplemented with 2.5 mg l^{-1} zeatin, 30 g l^{-1} mannitol and 6 g l^{-1} agar. The pH was adjusted to 5.8 and the media were sterilized by autoclaving at 121°C for 15 min. The cultures were incubated into growth chamber at 25±2ºC under 4000 lux light intensity for 16h/8h light/dark photoperiods supplied by white cool fluorescent lamp. Four weeks later, the sprouted axillary shoots were transferred to fresh Rugini olive medium supplemented with one type of the above mentioned nanoparticles; silver NPs at 5 and 10mg l^{-1}, selenium NPs at 2.5 and 5mg l^{-1} and chitosan NPs at 40 and 60mg l^{-1}, in addition to the control free nanoparticles medium. The nanoparticles were added to the proliferation medium then the media were autoclaved at 121°C for 15 min. Four explants were transferred to 200ml jar containing 50ml semi-solid medium (each treatment consisted of 20) and incubated at 25±2ºC under 4000 lux light intensity for 16h/8h light/dark photoperiods supplied by white cool fluorescent lamp, the sub-culture was performed every four weeks. At the 3rd subculture, number of shoot per explant, shoots length, multiplication rate and number of leaves per shoot was determined. Multiplication rate calculated as the total number of shoots per explant multiplied by number of potentially nodal cuttings per shoot (**Lambardi et al. 2012**).

Experimental design and statistical analysis

The experiment was carried out in a completely randomized design, the assumptions of normality were tested using Shapiro–Wilk's test (**Shapiro and Wilk 1965**). Normally distributed data was subjected to two-way analysis of variance to investigate the effect of olive genotype, nanoparticles treatments and their interaction (**Snedecor and Cochran 1967**). Analysis of variance was performed using the SAS software (version 9.0; SAS Institute, Cary, NC, USA). The mean and standard error (SE) were calculated from three replicates per treatment and the significant differences within and between treatments were assessed by means of multiple Duncan range test at significance level of 0.01 (**Duncan 1955**).

Results And Discussion

Characterization of the synthesized nanoparticles

The produced silver, chitosan and selenium nanoparticles were characterized by TEM instrument. The diameter and shape of the tested nanoparticles were illustrated in **Fig. (1)**. TEM micrograph demonstrates the formation of spherical shape with a narrow range of particle size distribution, the spherical nanoparticles were produced with size of 5-15 nm (Silver NPs), 15-35 nm (Selenium NPs) and 20-50 nm (Chitosan NPs). Analysis of TEM micrograph demonstrated that, the mean size of the obtained nanoparticles are comparable to the particle size that has been reported in previous studies for silver (**Hmmam et al. 2021**), selenium (**Srivastava and Mukhopadhyay 2015**), and chitosan nanoparticles (**Asgari-Targhi et al. 2018**). Therefore, the prepared nanoparticle in our study represented typical nanoparticle in term of shape and size.

Effect of synthesized nanoparticles on *in vitro* microbial contamination

Regarding the variation in inhibition potential of the tested nanoparticles on *in vitro* microbial contamination (**Fig. 2**), the obtained results showed that, there was a significant difference between the different nanoparticles types and concentrations on microbial contamination. Silver nanoparticles at concentration of 10 mg l^{-1} recorded the lowest value of microbial contamination in tissue culture medium compared with the negative control plates. Chitosan nanoparticles were ranked in the second order, while selenium nanoparticles recorded the lowest value. The obtained results indicated the feasibility for applying the nanoparticles in the tissue culture medium as antimicrobial agents without sterilization. The inhibition potential of nanoparticles agents in vitro growth of microorganisms confirm our previous studies about the antimicrobial activity of the tested nanoparticles (**Darwesh et al. 2018; Elshahawy et al. 2018; El-Shanshoury et al. 2020**). Silver NPs has a high potential for eliminates microbial contamination in culture medium; AgNPs recorded the lowest contamination percentage, which is statistically similar to media sterilization by autoclaving (**Fig. 2**). The high antimicrobial activity of AgNPs may be attributed to the strong toxicity of silver ions to a wide range of microorganism (**Abdi et al. 2008; Jo et al. 2009; Sobha et al. 2010; Hwan et al. 2017; Ismail et al. 2017**). Moreover, the small particle size (5-15 nm) of the obtained silver nanoparticles are important for interactions and binding of silver ions with cell membrane proteins, which resulting cell death (**Sondi and Salopek-Sondi 2004**).

Chitosan nanoparticles showed relatively higher anti-microbial activity compared with selenium nanoparticles and control treatment; the anti-microbial activity of chitosan nanoparticles against different plant pathogens was reported previously (**Qi et al. 2004; Du et al. 2009; Ali et al. 2011**). Chitosan NP affecting cell membrane permeability and inhibiting DNA replication (**Chandrasekaran et al. 2020**). Our results show that SeNPs have low anti-microbial activity compared with AgNPs and ChNPs; the low toxicity of Se NPs may be due to the negative charge of Se NPs which results in relatively repulsive with bacterial membrane (**Nguyen et al. 2017**). Also, higher concentrations of SeNPs may be needed to show significant effect on microbial contamination. A previous study showed that, 50 mg l^{-1} is the minimum dose for inhibition of *E. coli* or *S. aureus* (**Guisbiers et al. 2016**). According to **Nguyen et al. (2017)**, SeNPs

exhibited dose-dependent antimicrobial properties; 10 mg l^{-1} inhibited *in vitro* growth of *S. aureus* but have a no effect on *E. coli*, *Salmonella*, and *L. monocytogenes*.

Effect of the synthesized nanoparticles on *in vitro* performance of olive explants

According to the data illustrated in **Tables (1)**, *in vitro* growth of olive shoots was significantly affected with both of plant genotype and nanoparticles treatments at the proliferation stage. Picual recorded higher number of shoots/explant compared with Koroneiki and Manzanillo. The highest number of shoots /explant was recorded for AgNPs at 5 and 10 mg l^{-1} (1.77±0.43 and 2.28±0.56, respectively), while SeNPs treatments had a negative effect on shoot growth and recorded the lowest value of number of shoots/explant, there was a non-significant differences between ChNPs and control treatment.

Table 1 The effect of nanoparticles type and concentration on shoot number of different olive cultivars

Treatments	Concentration (mg L^{-1})	Manzanillo	Picual	Koroneiki	Mean
Selenium NP	2.5	1.00±0.00 g	1.50±0.05 e	1.30±0.05 f	1.27±0.07D
	5	1.00±0.00 g	1.00±0.00 g	1.00±0.00 g	1.00±0.00E
Silver NP	5	1.20±0.05f	2.00±0.00bc	2.12±0.02 b	1.77±0.14B
	10	1.83±0.05 cd	2.00±0.11bc	3.00±0.05 a	2.28±0.18A
Chitosan NP	40	1.00±0.00 g	1.80±0.20 d	1.30±0.00 f	1.36±0.11C
	60	1.00±0.00 g	1.00±0.00 g	1.00±0.00g	1.00±0.00CD
Control	------	1.00±0.00 g	2.00±0.00bc	1.00±0.00 g	1.33±0.16CD
Mean		1.14±0.06 B	1.61± 0.09 A	1.53±0.15 A	

Values of interaction (treatments x cultivars) followed by different lowercase letters are significantly different ($p < 0.01$). Mean values of treatment or cultivar followed by different uppercase letters are significantly different ($p < 0.01$); each value represent mean of three replicate± standard error (SE).

As shown in **Table (2)**, Koroneiki cv. recorded the highest shoot length compared with Picual and Manzanillo cv. It is evident that the addition of nanoparticles to culture medium affected growth of *in vitro* cultured olive shoots compared with the control. The shoot length varied between the different nanoparticles treatments, AgNPs at 10 mg L^{-1} recorded the highest value, while SeNPs at 5 mg L^{-1} recorded the lowest value. Due to the strong apical dominance of olive the formation of secondary axillary shoots is very limited; olive shoot multiplication is achieved by segmentation of elongated shoots at each subculture (**Fabbri et al. 2004; Lambardi et al. 2012**). The highest multiplication rate of the cultivated olive cultivars was recorded for Picual cv. (**Table 3**) compared with the other cultivars. There

was a significant difference between the nanoparticles treatments regarding multiplication rate; silver NPs at both concentration improved multiplication rate of the studied olive cultivars compared with the other treatments while selenium NPs at 5 mg L^{-1} and chitosan NPs at 60 mg L^{-1} produced the lowest value.

Table 2 The effect of nanoparticles type and concentration on shoot length (cm) of different olive cultivars

Treatments	Concentration (mg L^{-1})	Manzanillo	Picual	Koroneiki	Mean
Selenium NP	2.5	6.0±0.57c	6.0±0.57c	7.0±0.57bc	6.33±0.33B
	5	3.0±0.00d	4.0±0.00d	3.0±0.00d	3.33±0.16D
Silver NP	5	6.0±0.57c	6.0±0.00c	8.0±0.57b	6.67±0.41B
	10	7.0±0.00bc	8.0±0.57 b	10.0±0.00 a	8.33±0.47A
Chitosan NP	40	7.0±0.57bc	6.0±0.00 c	6.5±0.28bc	6.50±0.24 B
	60	4.0±0.00d	5.5±0.00c	6.0±0.57c	5.17±0.34C
Control	——	7.0±0.00bc	6.0±0.00 c	7.0±0.57bc	6.67±0.24B
Mean		5.71± 0.35 B	5.92± 0.26 B	6.78± 0.46 A	

Values of interaction (treatments x cultivars) followed by different lowercase letters are significantly different ($p < 0.01$). Mean values of treatment or cultivar followed by different uppercase letters are significantly different ($p < 0.01$); each value represent mean of three replicate± standard error (SE).

Table 3 The effect of nanoparticles type and concentration on multiplication rate of different olive cultivars

Treatments	Concentration (mg L^{-1})	Manzanillo	Picual	Koroneiki	Mean
Selenium NP	2.5	3.0±0.12d	3.2±0.05d	2.2±0.12e	2.80±0.16C
	5	1.2±0.00fg	1.3±0.00f	1.2±0.00fg	1.23±0.07E
Silver NP	5	3.2±0.00d	4.3±0.05b	4.8±0.12 a	4.10±0.24B
	10	4.3±0.17b	4.0±0.00c	5.0±0.00 a	4.43±0.16A
Chitosan NP	40	2.0±0.00 e	2.0±0.00e	1.3±0.12f	1.77±0.12D
	60	1.0±0.00 g	1.2±0.00fg	1.1±0.00fg	1.10±0.03E
Control	———	2.0±0.00e	2.0±0.00e	1.1±0.00fg	1.70±0.15D
Mean		2.38± 0.24 B	2.57±0.26 B	2.38±0.36 A	

Values of interaction (treatments x cultivars) followed by different lowercase letters are significantly different ($p < 0.01$). Mean values of treatment or cultivar followed by different uppercase letters are significantly different ($p < 0.01$); each value represent mean of three replicate± standard error (SE).

Data presented in **Table (4)** showed that the highest leaf number was recorded for Picual cv. compared with the other cultivars, there was a statistically significant difference between the nanoparticles treatments regarding the leaf number, silver NPs at 10 mg L^{-1} results in the highest leaf number followed by silver NPs at 5 mg L^{-1} while selenium NPs at 5 mg L^{-1} and chitosan NP at 60 mg L^{-1} showed the lowest leaf number. The nanoparticles application may lead to stimulate or inhibit effects on plant growth and development; the impact of nanoparticles on plant growth depending on particle size, shape, concentrations, plant genotype and age (**Khan et al. 2019; Goswami et al. 2019; Kranjc and Drobne 2019**). The obtained results indicated the positive effect of nanoparticles, especially AgNPs in improving the efficiency of olive micropropagation. As reported previously, silver ion (Ag^{+}) has a positive effect on plant tissue culture e.g. increased survival and delayed explants senescence (**Sarmast et al. 2015**), improve somatic embryogenesis (**Al-Khayriet al. 2001**), organogenesis (**Ricci et al. 2020**), increase shoot multiplication rate and plant growth (**Kotsias and Roussos 2001**). Shoot growth and number of shoots per explant were increased in *Brassica juncea*, *Tecomella undulate* Roxb. and *Vanilla planifolia* cultured on medium supplemented with AgNPs (**Kumar et al. 2009; Aghdaei et al. 2012; Sharma et al. 2012; Spinoso-Castillo et al. 2017**), which was attributed to the effect of Ag^{+} as an ethylene blockage agent (**Songstad et al. 1988**). Selenium is not an essential nutrient for higher plants and their effect differs depending on the concentration. Selenium at low concentration can stimulate plant growth, whereas, higher doses had an inhibitory effect (**Hawrylak et al. 2015**). It was reported that selenium nanoparticles stimulate callus induction and improve morphogenesis of tobacco and artichoke (**Domokos-Szabolcsy et al. 2012; Abdalla et al. 2021**). Recent studies showed that selenium stimulate shoot growth and increased fresh and dry weight of in vitro growing olive shoots (**Regni et al. 2021**).

Table 4 The effect of nanoparticles type and concentration on leaf number of different olive cultivars

Treatments	Concentration ($mg\ L^{-1}$)	Manzanillo	Picual	Koroneiki	Mean
Selenium NP	2.5	9.0±0.57cd	10.0±0.57c	6.0±0.57fg	8.33±0.66C
	5	4.0±0.00hi	5.0±0.00gh	4.0±0.00 hi	4.33±0.17E
Silver NP	5	8.0±0.00de	10.0±0.57c	12.0±0.57b	10.00±0.62B
	10	12.0±0.57b	10.0±0.57c	14.0±0.57a	12.00±0.65A
Chitosan NP	40	6.0±0.00fg	7.0±0.00ef	6.7±0.33efg	6.55±0.18D
	60	3.0±0.00 i	6.0±0.00fg	6.7±0.66efg	5.22±0.60E
Control	———	7.0±0.57ef	7.0±0.00ef	6.0±0.00fg	6.67±0.24D
Mean		7.00± 0.64 B	7.85± 0.45 A	7.90± 0.76 A	

Values of interaction (treatments x cultivars) followed by different lowercase letters are significantly different ($p < 0.01$). Mean values of treatment or cultivar followed by different uppercase letters are significantly different ($p < 0.01$); each value represent mean of three replicate± standard error (SE).

In contrast, our results showed that SeNPs had a negative effect on growth of olive shoots, which may be due to the higher absorption and mobility of selenium nanoparticles in plant tissues. Also, there are limited studies on the *in vitro* applications of chitosan NPs on the plant growth, supplementations of culture medium with chitosan NPs promote plant growth, however the higher doses of chitosan NPs dramatically caused reduction of plant growth and development, the toxicity of chitosan NP was higher than the chitosan bulk type, which may be due to the physicochemical properties of chitosan NP (**Asgari-Targhi et al. 2018**). Despite the fantastic effects of nanoparticles, the application of nanoparticles in culture media should be optimized to avoid the toxic effect of high doses; according to our results, the higher concentration of selenium and chitosan NPs had a negative effect on olive shoot growth. Several studies have shown that, higher concentrations of NPs had adverse effects on cell viability, organogenesis, shoot growth and plant regeneration (**Chichiriccò and Poma 2015; Da Costa et al. 2016; Salehi et al. 2018**). NPs affect the mitotic activity and change the DNA structure and gene expression in different plant species (**Ewais et al. 2015; Tripathi et al. 2017; Hassan et al. 2019**). According to **Nakasato et al. (2017)**, high concentration of chitosan NP severely inhibited germination, and negatively affected growth of *Zea mays*, *Brassica rapa* and *Pisum sativum*. Se toxicity may be due to the replacement of sulfur atoms by selenium in sulfur containing amino acids; which result in changes in proteins structure and function, simultaneously selenium can cause oxidative and stresses, cellular damage and disrupts plant metabolism and reduce plant growth (**Terry et al. 2000; Hasanuzzaman et al.**

2011). Therefore, the effects of different types and concentration of NPs on plant tissue should be optimized in order to determine the optimum dose with minimal phytotoxicity (**Kim et al. 2017**).

Conclusion

According to the obtained results, the tested nanoparticles showed varied degree of antimicrobial activity; AgNPs were highly effective to inhibit *in vitro* microbial contamination, ChNPs and SeNPs showed low anti-microbial activity. The addition of nanoparticles to the culture medium had a significant effect on growth and multiplication rate of *in vitro* growing olive shoots. AgNPs had a positive effect on growth and multiplication rate of *in vitro* growing olive shoots while the higher concentration of chitosan and selenium nanoparticles had a negative effect on shoot growth under in vitro conditions.

Declarations

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Figures

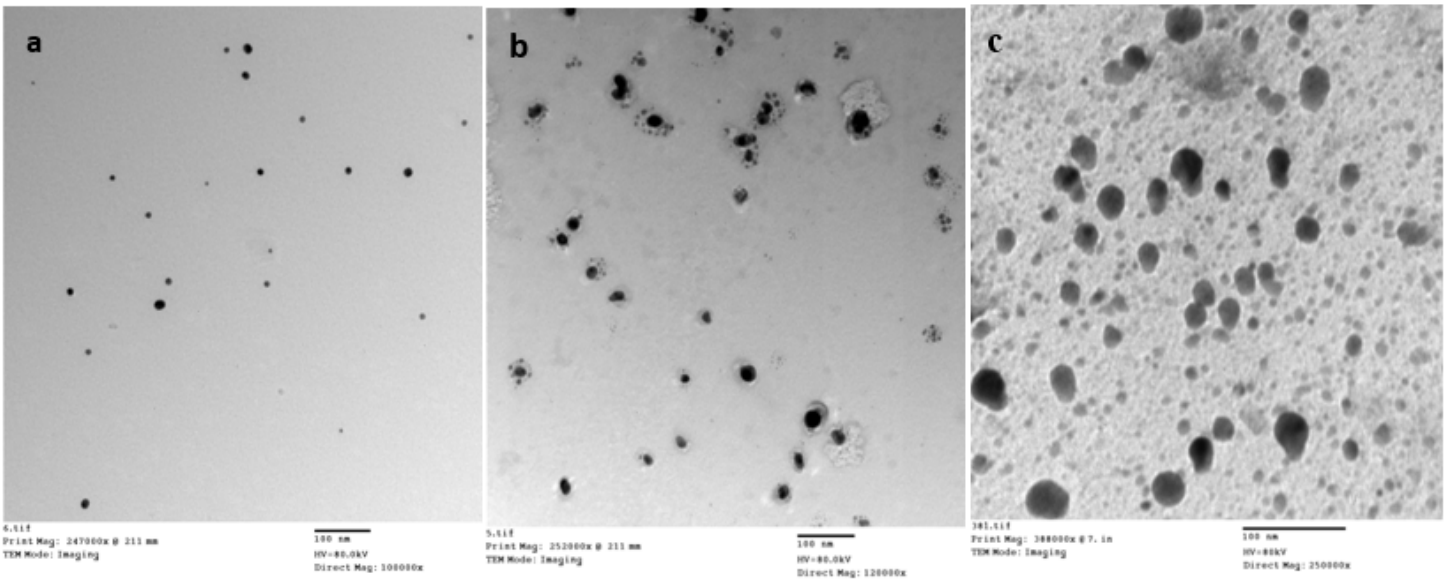

Figure 1

the transmission electron microscope (TEM) micrographs of silver (A), selenium (B) and chitosan (C) nanoparticles

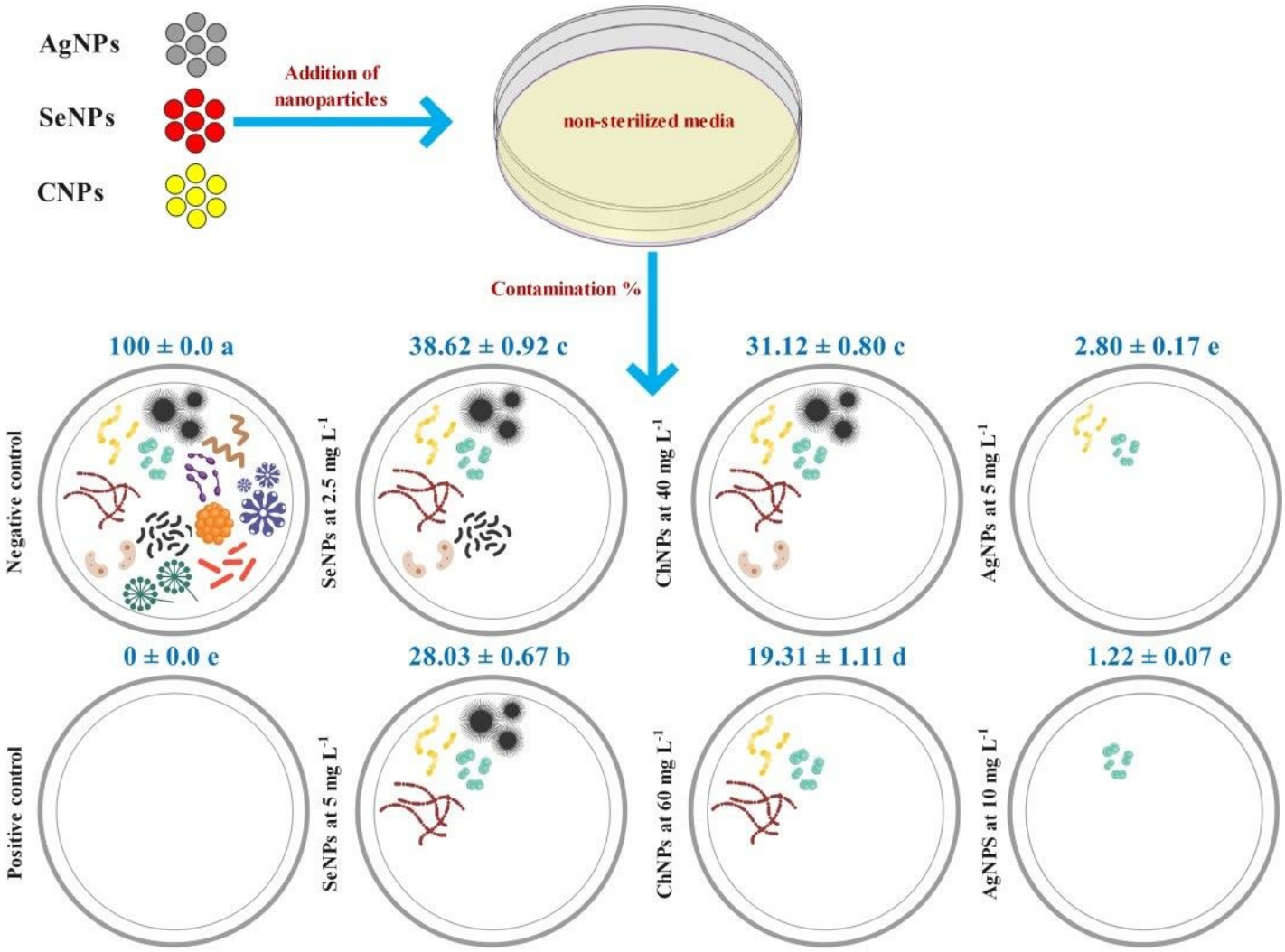

Figure 2

the effect of silver, selenium and chitosan nanoparticles on in vitro microbial contamination; AgNPs, silver nanoparticles; SeNPs, selenium; ChNPs, chitosan nanoparticles; negative control (non-sterilized medium); positive control (autoclaved medium).