Available online freely at www.isisn.org

Bioscience Research

Print ISSN: 1811-9506 Online ISSN: 2218-3973

Journal by Innovative Scientific Information & Services Network

RESEARCH ARTICLE BIOSCIENCE RESEARCH, 2018 15(3): 2283-2392. **OPEN ACCESS**

Evaluation of regeneration, active ingredients and antioxidant activities in jojoba tissue cultures as affected by carbon nanotubes

Alaa A. Gaafar[1], Rania A. Taha*[2,3], Nesreen H. Abou-Baker[4], Esam A. Shaaban[5], Zeinab A. Salama[1]

[1]Department of Plant Biochemistry, National Research Centre (NRC), Dokki, Giza, **Egypt**
[2]Biotechnology and Micropropagation Lab., Pomology Department, NRC, Dokki, Giza, **Egypt**
[3]Tissue Culture Technique Lab., Central Laboratories Building, NRC, Dokki, Giza, **Egypt**
[4]Soils and Water Use Department, NRC, Dokki, Giza, **Egypt**
[5]Pomology Department, NRC, Dokki, Giza, **Egypt**

***Correspondence:** rania_abdelghaffar@yahoo.com **Accepted:** 05 July 2018 **Published online:** 29 Sep. 2018

Tissue culture technique could be the most suitable method for studying the impact of nanotechnology on the plant. Recently, carbon nanotubes (CNTs) application on plant attracts a great attention. This study was to demonstrate the effect of CNTs on *in vitro* culture of jojoba shoots. Carbon in two sizes (activated carbon and carbon nanotubes) was used. Generally, CNTs at 0.002, 0.02 and 0.2 g/L enhanced multiplication rate; as shoot and leaf number as well as callus degree. It also increased growth vigor, at 0.002 g/L, as plantlet length, leaf number and stem thickness in rooting stage, compared with the control and activated charcoal (AC) at 2.0 g/L. Decreasing concentration of CNTs to 0.002 g/L led to higher antioxidant activity, phenolic, flavonoid and tannin contents compared with its other concentrations used. Methanol extract (80%) of jojoba treated with AC had the highest content of total phenolic, flavonoid and tannin and showed the highest antioxidant activity. It is assumed that the AC used is able to exhibit an antioxidant effect because of its radical capacity due to its adsorption ability, increasing phenols which may have a scavenging effect or increase the production of antioxidant enzymes in plant tissues. As our knowledge, this investigation on CNTs and activated charcoal is the first in jojoba *in vitro* cultures

Keywords: activated charcoal, *in vitro*, nanoparticles, nutrients, metal chelating, *Simmondsia chinensis*.

INTRODUCTION

Nanotechnology field is expected to use in a huge selection of applications in biology and medicine (Bruches et al., 1998; Ma et al., 2003), electronics (Wang et al., 2009) and agriculture (Bouwmeester et al., 2009; Nair et al., 2010; Taha, 2016a).

Recently, carbon nanotubes (CNTs) applications showed very promising results in agriculture (Taha, 2016b). It shows an impressive role due to its unique mechanical, electrical, thermal and chemical properties (Siddiqui et al., 2015). Some researchers indicated that carbon nanotubes treatment encouraged growth, shooting and other aspects of plant growth parameters**.** It enhanced germination of date palm embryo culture (Taha et al., 2016)**,** induced germination and growth of tomato seeds (Khodakovskaya et al., 2012) and can penetrate the coat of plant seeds (Khodakovskaya et al.,

2009). Otherwise, multi-walled CNTs did not show any positive effect on seed germination in some plants (Lin and Xing, 2007; Husen and Siddiqi, 2014). Moreover, toxicity of multi-walled CNTs was occurred in some other studies (Tan and Fugetsu, 2007; Stampoulis and Sinha, 2009) and that is explained as the higher concentrations used might be the reason, including plant sensitivity in those investigations (Taha et al., 2016). These properties and response surely differ with normal bulk of carbon; like activated charcoal (AC). Incorporating activated charcoal to both liquid and semi-solid plant tissue culture media may influence plant growth and development due to its adsorption of inhibitory substances inside the culture medium, going throw promoting plant growth (Johansson et al., 1990).

Jojoba (*Simmondsia chinensis* (Link) Schneider) is related to the family *Simmondsiaceae*. It grows in arid and semi-arid zones. Its originality belongs to Arizona, California and northern Mexico. The seed contains 50-60% liquid wax. Jojoba is an intensively drought, hot and salinity resistant plant and is gaining worldwide credit, for its benefits in cosmetics, pharmaceuticals and lard industries (Ali et al., 2013; Hussein et al., 2017). According to FAO (2017) data harvested area of jojoba seed and its production was reduced from 490 ha, producing 351 tonnes, in 1996 to 302 ha, resulting 147 tonnes, in 2014. It surly needs more studies for improving its propagation, planting and production. Tissue culture is a method to micropropagate plants and became a traditional tool to produce needed plants with huge numbers.

This research aimed to study the effect of carbon nanotubes on *in vitro* jojoba cultures and investigate their impact on some plant active ingredients and nutrient contents.

MATERIALS AND METHODS

This study was carried out in Biotechnology and Micropropagation Lab., Pomology Dep. and Tissue Culture Technique Lab., Central Labs Building; for *in vitro* culture treatments, Dep. of Plant Biochemistry; for biochemical analysis and Soil and Water Use Dep.; for nutrients analysis, all at Agricultural and Biological Research Division, National Research Centre, Dokki, Giza, Egypt, from 2016 to 2017.

Plant material

Jojoba nodal segments (1-2 cm) were cut from a unique shrub, taken to the lab and thoroughly washed with running water for 30 minutes, sterilized with 50% Clorox (commercial bleach) with two drops of tween 20 for 20 minutes then, washed in sterilized distilled water three times each for five minutes. The prepared explants were cultured on MS medium (Murashige and Skoog, 1962) solidified with 0.6% agar and supplemented with 1.0 mg/L 6-benzylaminopurine (BAP) (Fayek et al., 2007). The pH of the medium was adjusted to 5.7 and autoclaved at 121°C and 15 Ib/ inch^2 for 15 minutes. The cultured explants were incubated under 16 hours of fluorescent light and 8 hours of dark with average temperature at 25±2°C. Subculturing was done regularly at six weeks intervals. *In vitro* shoot clusters similar in size and length were selected for this study. In addition, rooted plantlets introduced by the method of Taha and Hassan (2016) were also used for this investigation (plantlets produced from rooting medium containing silver nitrate and IBA at 3 and 7 mg/L, respectively) .

Carbon nanotubes treatment

Multiwalled carbon nanotubes (CNTs), ALDRICH®, diam. = 110– 170 nm, length = 5–9 µm, 90+ %; were firstly characterized using transmission electron microscope (TEM) to analytically confirmed. CNTs were added to the media before autoclaving. CNTs were investigated at 0.0, 0.2, 0.02 and 0.002 g/L and compared with 2.0 g/L activated charcoal. Two *in vitro* stages were investigated; multiplication and rooting stages. Shoot number, shoot length, leaf number and callus degree were determined in multiplication stage. In addition, plantlet lengths, leaf number and stem thickness of rooted plantlets were assessed.

Biochemical analysis

Chemicals

Butyl hydroxy toluene (BHT); potassium ferricyanide; · 2, 2-diphenyl-1-picrylhydrazyl (DPPH); (3- (2 - pyridyl) - 5, 6- bis- (4-phenylsulfonic acid)-1, 2, 4-triazine (ferrozine); Folin-Ciocalteau reagents; gallic acid; quercetin; were from Sigma Chemical Co., St. Louis, MO, USA.

Preparation of plant extract

Jojoba shoot tissues were dried. Briefly, 10 g of the dried powder were soaked in 100 ml of 80% methanol and shaken at room temperature for 48 h. The extract was filtered using filter paper (Whatman No.1). Methanolic extracts were used

for further phytochemical and antioxidant analysis. The absorbance of the extract for each treatment was determined using spectrophotometer (Unicom UV e300). All samples were analyzed in triplicates.

Total phenolic content (TPC)

The total phenolic content was determined by Folin Ciocalteu reagent assay as described in Singleton and Rossi (1965). A suitable aliquot (1 ml) of jojoba extracts was added to 25 ml volumetric flask, containing 9 ml of distilled water. One milliliter of Folin Ciocalteu phenol reagent was added to the mixture and shaken. Five minutes later, 10 ml of Na_2CO_3 (7 % solution) were added to the mixture. Dilution to 25 ml was performed with distilled water, then the solution was mixed. The absorbance was determined at 750 nm, after incubation for 90 min at room temperature, against prepared reagent as blank. A total phenolic content in samples was expressed as mg gallic acid equivalents (GAE)/g dry weight.

Total flavonoid content (TFC)

The aluminum chloride method was used for the determination of total flavonoid content (Zhishen et al., 1999). One ml of jojoba extracts was added to 10 ml volumetric flask, containing 4 ml of distilled water. After that, 0.3 ml of 5 % $NaNO_2$ was added to the flask and after 5 min, 0.3 ml of 10 % $AlCl_3$ was added. At the sixth minute, 2 ml of 1M NaOH were added and the total volume was made up to 10 ml with distilled water. The solution was mixed well and the absorbance was measured against prepared reagent blank at 510 nm. Total flavonoid in sample was expressed as mg quercetin equivalents (QE)/ g dry weight.

Total tannins content (TTC)

Total tannins of different jojoba extracts were measured using the Folin-Ciocalteu reagent assay according to Polshettiwar et al. (2007). One ml of jojoba extracts or standard solution of (tannic 20-120 mg/l) was added to 7.5 ml distilled water (dH_2O). After that, addition of 0.5 ml of Folin reagent and 1 ml of 35% sodium carbonate solution was performed. The volume was made up to 10 ml with distilled water. Absorbance was measured against prepared reagent blank at 775 nm. Total tannins in sample were expressed as mg tannic acid equivalent (TE)/g dry weight.

Antioxidant activity

DPPH• radical scavenging assay

The method described by Chu et al. (2000) was used to assess the DPPH• (2, 2-diphenyl-1-picryl hydrazyl) radical scavenging activity of jojoba extracts. 0.1 mM of DPPH• in methyl alcohol was prepared and 0.5 ml of this solution was added to 1 ml of jojoba extracts at different concentrations (25, 50, 75, 100 µg/ml). The mixture was shaken vigorously and allowed to stand at room temperature in the dark for 30 min. Butyl Hydroxytoluene (BHT, Sigma) was used as positive control; and negative control contained the entire reaction reagent except the extract. Then the absorbance was measured at 515 nm against blank. The capacity to scavenge the DPPH• radical was calculated using the following equation:

DPPH• scavenging effect (Inhibition %) = $[(A_c - A_s / A_c) \times 100]$

Where: A_c was the absorbance of the control reaction and A_s the absorbance in the presence of the plant extracts. The results were expressed as IC_{50} (the concentration (µg/ml) of plant extract that scavenge 50% of DPPH• radical).

Ferrous ion chelating activity

Metal chelating effects of ferrous ions was carried out as described by Su et al. (2003). One ml of jojoba extracts, or EDTA solution as a positive control at different concentrations (25, 50, 75, 100 µg/ml) was spiked with 0.1 ml of 2 mM $FeCl_2$-$4H_2O$ and 0.2 ml of 5 mM ferrozine solution and 3.7 ml methanol were mixed in a test tube and reacted for 10 min, at room temperature, then the absorbance was measured at 562 nm. Mixture without extract was used as the control. A lower absorbance indicates a higher ferrous ion chelating capacity. The percentage of ferrous ion chelating ability was calculated using the following equation:

Iron chelating activity (Inhibition %) = $[(A_c - A_s / A_c) \times 100]$

Where: A_c was the absorbance of the control reaction

A_s the absorbance in the presence of the plant extracts.

Reducing power

The reducing power was assayed as described in (Kuda et al., 2005). One ml of jojoba extracts at different concentrations (25, 50, 75, 100 µg/ml) was mixed with 2.5 ml of phosphate buffer (50 mM, pH 7.0) and 2.5 ml of 1% potassium

ferricyanide. The mixture was then incubated at 50 ˚C for 20 min. After, 2.5 ml of trichloroacetic acid (10 %) was added to the mixture, centrifuged at 3000 rpm for 10 min. Finally, 1.25 ml from the supernatant was mixed with 1.25 ml of distilled water and 0.25 ml $FeCl_3$ solution (0.1%, w/v). The absorbance was measured at 700 nm. BHT was used as positive control. The assays were carried out in triplicate and the results were expressed as EC_{50} (the concentration (µg/ml) of plant extract that provide the reading 0.5 absorbance at 700 nm).

Nutrient content

Jojoba clusters were cleaned, dried in electric oven at 65°C until the weight was fixed and digested using sulphuric and perchloric acids. Water content of plant tissue (%) was calculated as described by Romero-Aranda et al. (2006). Nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg) and sodium (Na) concentrations were determined as mentioned by Cottenie et al. (1982).

Transmission electron microscope image

Leaves samples of CNTs treated- jojoba- shoots were prepared and examined with a High Resolution Transmission Electron Microscope (JEOL- 2100 TEM) to detect the penetration of CNTs inside the tissues (Fig. 1 A).

Statistical analysis

In this investigation, each treatment had three replicates and each replicate had three clusters for shoot multiplication. At rooting stage, each treatment had three replicate and each replicate had three plantlets. Each experiment was repeated at least three times. Chemical analysis assumed on jojoba *in vitro* shoots after four subcultures. Analysis of variance was conducted using one-way ANOVA test, Costat statistical package (Anonymous, 1989) and means were compared by Duncan's test at the 0.05 level of confidence.

RESULTS AND DISCUSSION

Effect of carbon nanotubes or activated charcoal on *in vitro* multiplication of jojoba shoots

CNTs, in this investigation, found to be entered jojoba tissues as they were detected using TEM image (Fig.1 A). Our data revealed that CNTs application enhanced multiplication rate of *in vitro* jojoba shoots significantly (as shoot number, shoot length, leaf number and callus production) compared with the control and activated charcoal treatment (Table 1 and Fig.1 B to F). The lowest concentration of carbon nanotubes (0.002 g/L) gave the highest average shoot number (16.0) followed by the higher concentrations as the more increase in concentration the less shoot number occurred. Concerning leaf number response, it is clear that the high concentrations used to raise average leaf number significantly compared with the control and activated charcoal treatment. Similarly, the highest concentration of CNTs increased callus production presented as degrees. Meanwhile, the increase in shoot length due to CNTs was insignificant. Similar results were achieved with Taha et al. (2016) who indicated that CNTs enhanced growth and regeneration of date palm *in vitro* cultures. However, this response differed from stage to other. Low concentrations of CNTs were found to promote callus fresh weight, increase number of germinated embryos as well as shoot length and leaf number. They can penetrate date palm tissues and enter its cells. CNTs also had a positive effect on the growth of broccoli seedlings and helped plants tolerating saline conditions (Martinez‐Ballesta et al., 2016). Otherwise, activated charcoal gave the lowest multiplication rate as shoot number. This might be as a response to its cytokinins adsorption (Yam et al., 1990).

Effect of carbon nanotubes or activated charcoal on regenerated plantlets of jojoba shoots

Data in Table 2 indicated that carbon nanotubes concentrations generally enhanced plantlet length, leaf number and stem thickness. The lowest concentration of CNTs gave the highest average plantlet length, leaf number and stem thickness followed by other concentrations used compared with the control and activated charcoal treatment. Similar results were achieved with Taha et al. (2016) who indicated that CNTs enhanced plantlet length. CNT could be considered as a regulator for seed germination and growth. It was found to increase cell divisions, cell wall formation and water transport indicating that it might organize the marker genes to enhance cell culture growth (Khodakovskaya et al., 2012). In our study, it is worth mentioned that neither AC nor CNTs affected rooting percentage of jojoba shoots (unpublished data).

Changes in total phenolic, total flavonoids and total tannins content of jojoba in vitro shoots

Data shows that total phenolic, flavonoid and tannins content of jojoba plants significantly increased with activated charcoal treatment; these increments were phenolic (23.17 mg GAE/g DW), flavonoids (20.66 mg QE/g DW) and tannins (6.35 mg TE/ g DW), respectively as compared to control and CNTs treated plants (Table 3) .

Table 1. Effect of carbon nanotubes or activated charcoal on *in vitro* multiplication of jojoba shoots

Treatment	Shoot number	Shoot length (cm)	Leaf number	Callus (degree)
Control	9.23d	1.0a	3.22c	1.24b
AC (2.0g/L)	6.5e	1.15a	3.85b	1.0b
CNTs (0.002g/L)	16.0a	1.36a	3.31c	1.23b
CNTs (0.02 g/L)	14.5b	1.36a	4.0ab	1.38b
CNTs (0.2 g/L)	13.5c	1.27a	4.19a	2.25a

Means with different letters within each column were significantly different at the 5 % level.

Table 2. Effect of carbon nanotubes or activated charcoal on regenerated jojoba plantlets

Treatment	Plantlet length (cm)	Leaf number	Stem thickness (mm)
Control	2.0d	4.0e	1.5d
AC at 2.0 g/L	2.8c	4.4d	1.7cd
CNTs at 0.002 g/L	4.3a	10.2a	2.6a
CNTs at 0.02 g/L	3.2b	8.6b	2.1b
CNTs at 0.2 g/L	3.17b	6.17c	2.0bc

Means with different letters within each column were significantly different at the 5 % level.

Table 3. Changes in total phenolic, total flavonoids and total tannins content of jojoba tissues in carbon nanotubes or activated charcoal presence

Treatments	TPC mg GAE /g DW	TFC mg QE /g DW	TTC mg TE /g DW
Control	22.27b	15.63b	3.46b
AC at 2.0 g/L	23.17a	20.66a	6.35a
CNTs at 0.002 g/L	17.98c	10.35c	2.39c
CNTs at 0.02 g/L	16.46d	10.12c	1.89d
CNTs at 0.2 g/L	15.71e	10.13c	1.98d

Means with different letters within each column were significantly different at the 5 % level. GAE, gallic acid equivalents. QE, quercetin equivalents. TE, Tannic equivalents.

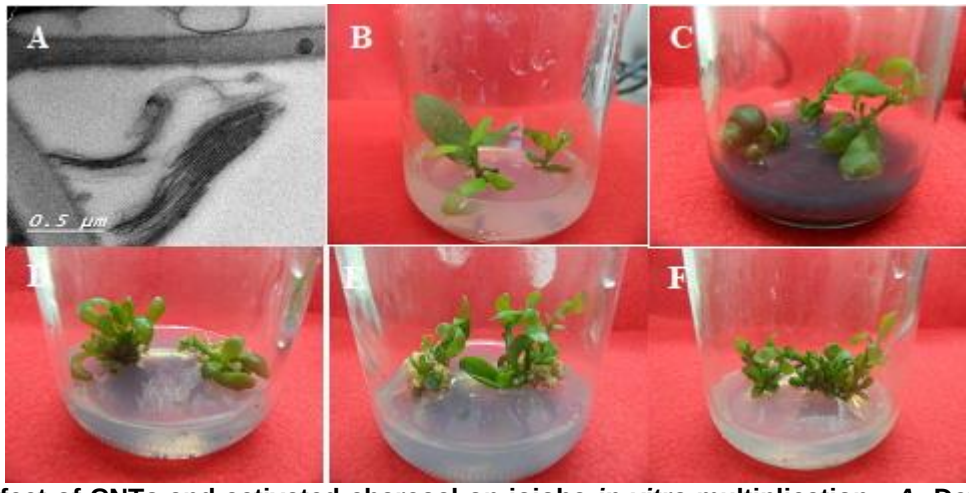

Figure 1. The effect of CNTs and activated charcoal on jojoba *in vitro* multiplication: *A*- Detected CNTs in jojoba tissues using TEM; *B*- Control, *C*- AC at 2.0 g/L, *D*- CNTs at 0.2 g/L, *E*- CNTs at 0.02 g/L, *F*- CNTs at 0.002 g/L

However, treatment of the lowest concentration of CNTs (0.002 g/L) led to the highest increment of phenolic (17.98 mg GAE/g DW), flavonoids (10.35 mg QE/g DW) and tannins (2.35 mg TE/ g DW) compared with its other concentrations used. Results were in agreement with Wang et al., (2009) stating that lower concentrations of CNTs resulted in the accumulation of phenolic, flavonoids and tannins. In addition, Ghorbanpour and Hadian (2015) assured that effect of CNTs on plant phenols and flavonoids content was concentration dependant. The process of producing secondary metabolites such as phenolic, flavonoids and tannins treated with CNTs and AC is required to have further studies at molecular level.

However, the safety of CNTs is still not clear and their use in soil must be associated with safety for the ecosystem and the environment.

Changes in antioxidant activities of jojoba *in vitro* shoots

As containing complicated chemicals in plant tissues, different assays are required to assess their antioxidant activity. Number of methods is used to determine antioxidant activities based on both the free radical scavenging and the redox mechanisms. In this study, the antioxidant activities in presence of activated charcoal and CNTs treatments of jojoba shoots were examined using $DPPH^{\cdot}$, Fe^{2+}- chelating and reducing power assays (Table 4).

The $DPPH^{\cdot}$ assay was based on the reduction of the stable radical $DPPH^{\cdot}$ to yellow colored in the presence of a hydrogen donor . The IC_{50} of $DPPH^{\cdot}$ scavenging capacities of treated jojoba was in the range of 66.03 - 116.63 µg/ml. As shown in Table 4 generally, activated charcoal treatment showed the highest $DPPH^{\cdot}$ radical scavenging activity, Fe^{2+}- chelating and reducing power compared with the control and CNTs treatments.

Considering CNTs treatments, $DPPH^{\cdot}$ radical scavenging activity of jojoba shoots treated with the lowest concentration of CNTs (0.002 g/L) recorded the highest $DPPH^{\cdot}$ radical scavenging activity (94.92 µg/ml) compared with other concentration tested. Ghorbanpour and Hadian (2015) conducted a maximum oxidative stress index (H_2O_2) with the highest concentration tested (0.5 g/L) from CNTs. The lowest IC_{50} means the highest antioxidant capacity. It was mentioned that, the increased level of antioxidant activity might be a self-defensive response against the effects of oxidative stress (Smirnoff, 1995). The tissue exhibiting high antioxidant activities could be more resistant to oxidative stress than tissue with lower antioxidant potential due to capability of antioxidants to scavenge reactive oxygen species (Lester, 2008).

Metal ions can initiate lipid peroxidation and start a chain reaction that leads to the deterioration of food (Gordon, 1990). Ferrous ions are the most effective pro-oxidants, (Yamaguchi et al. 1998). In the present study AC exhibited higher chelating ability (98.30 µg/ml) comparable to control and CNTs used. All the treatments of CNTs demonstrated considerable ability to chelate metal ions (Fe^{+2}). Oyaizu (1986) assured that presence of reluctant in sample would result in the reduction of the ferric (Fe^{3+}) to ferrous ion (Fe^{2+}) through the donation of an electron and the creation of the Perl's Prussian blue complex. This complex was estimated by measuring absorbance at 700 nm. We can observe that lower concentrations of CNTs showed higher chelating ability than the highest concentration. Whereas, Gordon et al. (2012) claimed that no consistent trend was derivable for the antioxidant activity at different concentrations of nanoparticles.

Table 4 also describes the reducing power of jojoba shoots treated with AC or several concentrations of CNTs. As can be seen, all jojoba shoots extracts showed reducing power ability either by application of AC, CNTs or control extracts. Jojoba treated with AC had the superiority in increasing reducing power ability as EC_{50} (the concentration (µg/ml) of plant extract that provide the reading 0.5 absorbance at 700 nm) as EC_{50} = 50.55µg/ml, compared to control. While, CNTs at 0.2 concentration exhibits the minimum antioxidant capacity (EC_{50}= 141.07µg/ml).

From the previous data, it can be clearly observed that there was a relationship between total phenolic content and antioxidant activity in jojoba tissues. Phenolic compounds might contribute with antioxidant activity in jojoba shoots. This is in agreement with Bendini et al. (2006) and Wojdylo et al. (2007) who assured that phenols are very important compounds for plants due to their radicals scavenging ability concerning their hydroxyl groups. They expected that phenolic content of plants might be correlated directly to their antioxidant action, phenylpropanoid and flavonoid biosynthesis. In addition, jojoba extract is seemed to have a radical scavenging effect due to its phenolic content (Aksoy et al., 2013).

Table 4. Changes in scavenging activities by DPPH$^{\cdot}$, Fe^{2+}-chelating and reducing power activities of jojoba tissues in carbon nanotubes or activated charcoal presence

Treatments	DPPH$^{\cdot}$	Fe^{2+}- chelating	Reducing power
	IC_{50} µg/ml	IC_{50} µg/ml	EC_{50} µg/ml
Control	80.54d	105.74c	63.87d
AC at 2.0 g/L	66.03e	98.30d	50.55e
CNTs at 0.002 g/L	94.92 c	116.38b	89.11c
CNTs at 0.02 g/L	104.42b	116.24b	121.41b
CNTs at 0.2 g/L	116.63a	135.53a	141.07a
EDTA	--------	23.12e	---------
BHT	29.64f	--------	11.01f

Means with different letters within each column were significantly different at the 5 % level.

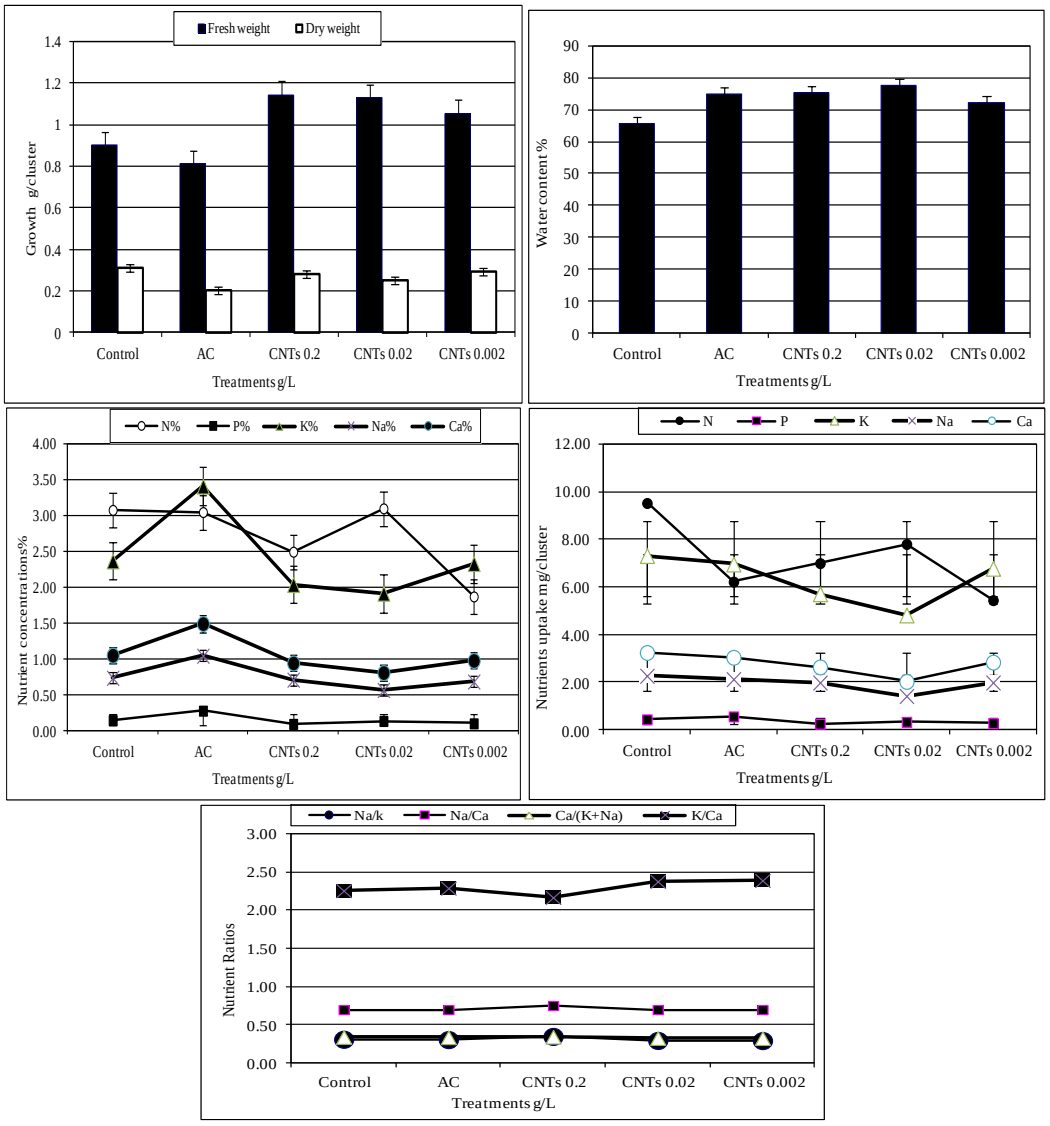

Figure 2. Fresh and dry weight of jojoba (g/cluster) and water content (%), nutrient concentrations (%) and content (mg/cluster), elements ratios in jojoba *in vitro* clusters as affected by activated charcoal and carbon nanotubes rates

Our findings show a new interpretation of the effect of activated charcoal on plant tissues. The presence of activated charcoal in the culture medium lead to an increase in phenols inside the plant, which corporate with a clear antioxidant activity indicating that either phenols have a scavenging effect on the radicals or cause an increase in the production of antioxidant enzymes with its increase.

CNTs might act as chelator agent for nutrients such as iron and zinc which are involved in the biosynthesis of enzymes leading to increase their activities that regulate the synthesis of phenolic compound (Salama et al., 2015; Barz and Koster, 1981; Cochrane, 1994) and enhanced plant growth. In addition, an increase in production of phenolic compounds has been associated with a decrease in growth (Arnaldos et al., 2001) which appeared with our control and AC treatment.

Nutrient status and growth of jojoba *in vitro* shoots

Although application of activated charcoal to growth media decreased dry and fresh weight of jojoba shoots compared to untreated one, all rates of CNTs led to increase both of fresh and dry weight compared to control and activated charcoal (Fig. 2). The highest growth was produced by CNTs at 0.2 and 0.02 g/L without significant difference between them. The water content of treated jojoba shoots was higher than untreated plants. The highest water content percentage was given by the second rate of CNTs.

These results are in agreement with those obtained by Martínez‐Ballesta et al. (2016) where carbon nanotube increased broccoli growth by 1) increased aquaporin transduction occurred which improved water uptake and transport 2) enhanced net assimilation of CO_2 3) induced changes in lipid composition 4) improving of rigidity and permeability of the root plasma membranes. The highest concentrations and contents of measured elements (N, P, K, Na and Ca) were observed in control and AC treatment followed by CNTs which gave the highest growth (Fig. 2). This may be attributed to the dilution effect of high growth, especially with stable concentrations of these elements in the media. This claim is conformity with obtained by Abou-Baker et al., (2011) where the nutrient concentration trend take the opposite line of growth values and they refer that to the dilution effect of high growth. All measured elements are essential macro nutrients except for Na which consider harmful element. The lowest concentration and content of Na was observed by addition of CNTs at 0.02 g/L. In this concern, Taha et al., (2016) reported that the Na concentration in date palm shoots was decreased with increasing the concentration of carbon nanotubes.

CONCLUSION

Carbon nanotubes generally enhanced multiplication rate and growth vigor of jojoba *in vitro* cultures. It improved some properties needed for adaptation process of tissue culture plants such as plant length and stem thickness. Addition of CNTs affected secondary metabolites, DPPH radical scavenging activity, Fe^{+2}-chelating activity and reducing power ability. More studies are needed to understand the role of carbon nanotubes in plants.

CONFLICT OF INTEREST

The authors declare that present study was performed in absence of any conflict of interest.

ACKNOWLEGEMENT

The authors wish to thank the National Research Centre, 33 El Bohouth st. –Dokki – Giza – Egypt, for funding this research as in-house project (11090108).

AUTHOR CONTRIBUTIONS

All the authors contributed to the conception or design of the work; the acquisition, analysis, or interpretation of data for the work. They contributed in drafting the work or revising it. They gave final approval of the version to be published and agreed to be accountable for all aspects of the work.

REFERENCES

Abou-Baker NH, Abd-Eladl M, Abbas MM, 2011. Use of silicate and different cultivation practices in alleviating salt stress effect on bean plants. Austral. J. Basic Appl. Sci. 5:

769-781.

Aksoy L, Kolay E, Ağılönü Y, Aslan Z, Kargıoğlu M, 2013. Free radical scavenging activity, total phenolic content, total antioxidant status, and total oxidant status of endemic *Thermopsis turcica*. Saudi J. Biol. Sci. 20: 235-239.

Ali EF, Bazaid S, Hassan FAS, 2013. Salt effects on growth and leaf chemical constituents of *Simmondsia chinensis* (Link) Schneider. J Med. Plants Stud. 1: 22-4.

Anonymous A, 1989. Cohort Software Crop. Costat user manual version 3.03, Berkeley CA, USA.

Arnaldos TL, Munoz R, Ferr MA, Calderon AA, 2001. Changes in phenol content during strawberry callus cultures. Physiol. Plant. 113: 315-322.

Barz W, Koster J, 1981. Turnover and degradation of secondary (natural) products. -In EE Conn, ed. The Biochemistry of Plants. Vol. 7. Academic Press, New York, USA, pp 35-82

Bendini A, Cerretani L, Pizzolante L, Gollina-Toschi T, Guzzo F, Andereetta F, Levi M, 2006. Phenol content related to antioxidant and antimicrobial activity of *Passiflora spp*. Extracts. Eur. Food. Res. Technol. 233: 102-109.

Bouwmeester H, Dekkers S, Noordam MY, Hagens WI, Bulder AS, De Heer C, Ten Voorde SE, Wijnhoven WP, Marvin HJP, Sips AJ, 2009. Review of health safety aspects of nanotechnologies in food production. Regulatory Toxicol. Pharmacol. 53: 52-62.

Bruchez M, Moronne M, Gin P, Weiss S, Alivisatos AP, 1998. Semiconductor Nano crystals as fluorescent biological labels. Science 281: 2013-2016.

Chu YH, Chang CL, Hsu HF, 2000. Flavonoids content of several vegetables and their antioxidant activity. J. Sci. Food Agri. 80: 561–566.

Cochrane MP, 1994. Observation on the germ aleurone of Barley: Phenol oxidase and peroxidase activity. Annal. Botany 73: 121-128.

Cottenie A, Verloo M, Kiekens L, Velghe G, Camerlynck R, 1982. Chemical Analysis of plants and soils. Laboratory of Analytical and Agrochemistry State Univ., Ghent-Belgium.

FAO, 2017. Production/yield quantities of jojoba seed in world. http://www.fao.org/faostat

Fayek MA, Shaaban EA, El-Obeidy AA, Taha RA, 2007. *In vitro* propagation of three female jojoba clones (*Simmondsia chinensis* (Link) Schneider). Egypt. J. Appl. Sci. 22: 652-665.

Ghorbanpour M, Hadian J, 2015. Multi-walled carbon nanotubes stimulate callus induction, secondary metabolites biosynthesis and antioxidant capacity in medicinal plant *Satureja khuzestanica* grown *in vitro.* Carbon 94: 749-759.

Gordon A, Cruz APG, Cabral LMC, De Freitas SC, Taxi CMAD, Donangelo CM, Mattietto RA, Friedrich M, Da Matta VM, Marx F, 2012. Chemical characterization and evaluation of antioxidant properties of Açaí fruits (*Euterpe oleraceae* Mart.) during ripening. Food Chem. 133: 256–263.

Gordon MH, 1990. The mechanism of antioxidant action *in vitro*. In: BJF Hudson, ed. Food antioxidants, Elsevier Applied Science, New York, USA.

Husen A, Siddiqi KS, 2014. Carbon and fullerene nanomaterials in plant system. -J Nanotechnol. 12: 1-10.

Hussein MM, El-Faham SY, Abd El-Moti EZ, Abou-Baker NH, 2017. Jojoba irrigated with diluted seawater as affected by ascorbic acid application. Inter. J. Agri. Res. 12: 1-9.

Johansson LB, Calleberg E, Gedin A, 1990. Correlations between activated charcoal, Fe-EDTA and other organic media ingredients in cultured anthers of Anemone canadensis. Physiol. Plant. 80: 243–9.

Khodakovskaya MV, De Silva K, Biris AS, Dervishi E, Villagarcia H, 2012. Carbon nanotubes induce growth enhancement of tobacco cells. ACS Nano 6: 2128-2135.

Khodakovskaya MV, Dervishi E, Mahmood M, Yang X, Li Z, et al., 2009. Carbon nanotubes are able to penetrate plant seed coat and dramatically affect seed germination and plant growth. ACS Nano 3: 3221-3227.

Kuda T, Tsunekawa M, Goto H, Araki Y, 2005. Antioxidant properties of four edible algae harvested in the Noto Peninsula, Jpn. J. Food Compos. Analysis 18: 625–633.

Lester GE, 2008. Antioxidant, sugar, mineral, and phytonutrient concentrations across edible fruit tissues of orange-fleshed Honeydew melon (*Cucumismelo* L.). J. Agri. Food Chem. 56: 3694-3698.

Lin D, Xing B, 2007. Phytotoxicity of nanoparticles: Inhibition of seed germination and root growth. Environmental Pollution 150: 243-250.

Ma J, Wong H, Kong LB, Peng KW, 2003.

Biomimetic processing of nanocrystallite bioactive apatite coating on titanium. Nanotechnol. 14: 619-623.

Martinez‑Ballesta C, Zapata L, Chalbi N, Carvajal M, 2016. Multiwalled carbon nanotubes enter broccoli cells enhancing growth and water uptake of plants exposed to salinity. J. Nanobiotech. 14: 42.

Murashige T, Skoog F, 1962. A revised medium for rapid growth and bioassay with tobacco tissue culture. Physiol. Plant. 15: 473-497.

Nair R, Varghese SH, Nair BG, Maekawa T, Yoshida Y, Kumar DS, 2010. Nanoparticulate material delivery to plants. Plant Science 179: 154-163.

Oyaizu M, 1986. Studies on products of browning reactions: Antioxidative activities of products of browning reaction prepared from glucosamine. Jpn. J. Nutr. 44: 307–315.

Polshettiwar SA, Ganjiwale RO, Wadher SJ, Yeole PG, 2007. Spectrophotometric estimation of total tannins in some ayurvedic eye drops. Indian J. Pharmaceut. Sci. 69: 574-576.

Romero-Aranda MR, Jurado O, Cuartero J, 2006. Silicon alleviates the deleterious salt effect on tomato plant growth by improving plant water status. J. Plant Physiol. 163: 847-855.

Salama ZA, EL Fouly MM, Gaafar AA, 2015. Role of chelated zinc (Zn-HEDTA) foliar spray in regulation salt. Analele Universităţii din Oradea, Fascicula Biologie, Tom. XXII: 7-12.

Siddiqui MH, Al-Whaibi MH, Firoz M, AlKhaishany MY, 2015. Role of Nanoparticles in Plants. In MH Siddiqui, et al., eds., Nanotechnology and Plant Sciences, Springer International Publishing, Switzerland.

Singleton VL, Rossi JA, 1965. Colorimetric of total phenolics with phosphomolibdic – phosphor tungstic acid reagents. Amer. J. Enol. Viticul. 16: 144–158.

Smirnoff N, 1995. Antioxidant systems and plant response to the environment. In N Smirnoff, ed, Environment and Plant Metabolism: Flexibility and Acclimation, Oxford: Bios Scientific, p 217-243.

Stampoulis D, Sinha SK, White JC, 2009. Assay-dependent phytotoxicity of nanoparticles to plants. Environ. Sci. Technol. 43: 9473-9479.

Su C, Chen W, Weng Y, Tseng C, 2003. Chemical composition, physical properties, and antioxidant activities of yam flours as affected by different drying methods. Food Chem. 83: 85–92.

Taha RA, 2016a. Nanotechnology and its application in agriculture. Adv. Plants Agric. Res. 3, 00089. Doi: 10.15406/apar.2016.03.00089.

Taha RA, 2016b. Nano carbon applications for plant. Adv. Plants Agric. Res. 5, 00172: 00172. DOI: 10.15406/apar.2016.05.00172.

Taha RA, Hassan SAM, 2016. Studies on silver nitrate impact on jojoba *in vitro* culture. Inter. J. PharmTech Res. 9: 77-83.

Taha RA, Hassan MM, Ibrahim EA, Baker NHA, Shaaban EA, 2016. Carbon nanotubes impact on date palm *in vitro* cultures. PCTOC 127: 525-534. DOI 10.1007/s11240-016-1058-6.

Tan X-m, Fugetsu B, 2007. Multi-walled carbon-nanotubes interact with cultured rice cells: evidence of a self-defense response. J Biomed Nanotechnol 3, 285-288.

Wang Y, Mirkin CA, Park SJ, 2009. Nanofabrication beyond electronics. ACS Nano 26, 1049–1056.

Wojdylo A, Oszmianski J, Czmerys R, 2007. Antioxidant activity and phenolic compounds in 32 selected herbs. Food Chem. 105: 940-949.

Yam TV, Ernst R, Arditti J, Nair H, Weatherhead MA, 1990. Charcoal in orchid seed germination and tissue culture media: A review. Lindleyana 5: 256-265.

Yamaguchi T, Takamura H, Matoba T, Tero J, 1998. HPLC method for evaluation of the free radical-scavenging activity of foods by using 1, 1-dicrylhydrazyl. Biosc. Biotechnol. Biochem. 62: 1201-1204.

Zhishen J, Mengcheng T, Jianming W, 1999. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chem. 64: 555–559.