In vitro Assessment of ZnO Nanoparticles on *Phoenix dactylifera* L. Micropropagation

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ABSTRACT

Microbial contaminants are the major challenges in plant's in vitro propagation during different stages of culture processes, ZnO-NPs exhibit attractive antibacterial properties due to increased specific surface area as the reduced particle size leading to enhanced particle surface reactivity. The aim of present study was to evaluate the effect of ZnO NPs on microbial contamination; vitrification and growth promoting of date palm cultured tissues. Several concentrations of ZnO NPs were selected to assess their activity. The percentage of shoot formation, the number of proliferated shoot and the percentage of contamination of cultures were investigated, as well as several biochemical characteristics. Twofold of increase multiplication rate of proliferated shoot was observed in ZnO NPs at 150 mg L^{-1} compared to control treatment. The multiplication rate was 46.6% at control and increased significantly to 86.67% and 93.34% in ZnO NPs before and after sterilisation, respectively. No microbial contamination and vitrification were observed at all ZnO NP treatments compared with control treatment. Biochemical analysis showed that ZnO NPs had no toxic effects at all examined concentrations on date palm cultured tissues. A positive effect was observed in carbohydrates, protein and amino acid accumulations at high ZnO concentration (150 mg l^{-1}). The results provide basis for the application of ZnO NPs in media date palm tissue cultures at 150 mg l^{-1}.

Key Words: Micropropagaion, Nano biotechnology, Phytotoxicity, ZnO nanoparticles.

Abbreviations: BSA: Bovine serum albumin, cv: Cultivar, FW: Fresh weight, MDA: Malondialdehyde, MS: Murashige and Skoog, NAA: Naphthalene acetic acid, NPs: Nanoparticles, RPM: Revolution per minute, RT: Room temperature, ZnO: Zinc oxide.

INTRODUCTION

Nanotechnology has attracted considerable research attention in modern applications, such as antimicrobials, agriculture, and therapeutics, as well as high-sensitivity biomolecular detection and diagnostics (Monica and Cremonini, 2009; Safavi *et al.*, 2011). Nanoparticles (nano-scale particles NSPs) could be defined as particles (atomic or molecular aggregates) having at least one dimension between 1 to 100 nm, as a diameter (Roco, 2003; Auffan *et al.*, 2009); NPs have considerably distinct physio-chemical properties compared to the bulk material (Nel *et al.,* 2006). The interactions between nanoparticles and different plants have been studied on morphological and physiological levels, as well as in genetic stability (Wang *et al.*, 2016; Ruttkay-Nedecky *et al.*, 2017). The influence of nanoparticles on plant tissues is mainly dependent on several variables, such as the chemical composition, size, reactivity, surface covering and concentration (Ma *et al.*, 2010; Khodakovskaya *et al.*, 2012). In recent years, various types of metal oxide nanoparticles have been used in biological application, such as antibiotics, DNA detection, and food safety (Wang *et al.*, 2017; Veerachandra *et al.*, 2017; Liu *et al.*, 2018). Zinc oxide (ZnO) nanoparticles are widely used for physical, biomedical, and other application because of their excellent properties. ZnO is non-toxic, stable under high temperature, easy to prepare, and cost-effective (Hassan *et al.*, 2013; Mirzaeia and Darroudi, 2017; Asuncion *et al.*, 2018).The positive effect of ZnO NPs on the growth of plants and their development has been demonstrated in different plants, including peanut (*Arachis hypogaea*), soybean (*Glycine max*), wheat (*Triticum aestivum*), onion (*Allium cepa*), banana (*Musa paradisiaca*) and sesame (*Sesamum indicum*) (Prasad *et al.*, 2012; Ramesh *et al.*, 2014; Raskar and

Laware, 2014; Helaly *et al.*, 2014; Narendhran *et al.*, 2017). Plant tissue culture technique is designed to maintain and multiply the growth of plant cells or different parts on specific cultural medium supplemented with different nutrients and hormones taking the advantage of totipotency (Abass, 2016a). This technique has been employed for various applications, including large-scale production, germplasm conservation, pathogen-free plant production, genetic transformation, genetic toxicity, metabolite production, embryogenesis, morphogenesis, and nutrition (Thorpe, 2007; Abass *et al*., 2017; Kim *et al*., 2017). The supplementation of plant tissue culture medium [mainly Murashige and Skoog (MS) media] with different nanoparticles has been reported in a wide range of plants for several purposes. For example, Ag NPs have been applied as antimicrobial agent by Safavi *et al.* (2011) at 50 mg L^{-1} into MS medium. Ag and TiO_2 NPs showed a significant microbial growth inhibition when used with MS medium in potato and tobacco plants (Safavi, 2012; 2014) The addition of Ag NPs at concentrations of 100–200 mg l^{-1} to MS medium with both *Rose hybrid* and *Bacopa monnieri* plants significantly reduces bacterial contamination (Shokri *et al.*, 2014; Kalsaitkar *et al.*, 2014). The addition of ZnO NPs at 100 mg L^{-1}in MS medium decreases the bacterial contamination of banana cultures (Helaly *et al.*, 2014). ZnO NPs have been used in tomato tissue cultures on MS medium to induce callus production and plant regeneration, as well as mitigate the deleterious effect of NaCl (Alharby *et al.*, 2016). Moreover, ZnO NPs (1–20 mg L^{-1}) are also employed in MS medium to induce root formation of *Brassica nigra* plants. ZnO NPs (0.1–1000 mg L^{-1}) could enhance shoot formation in *Stevia rebaudiana*(Zafar *et al.*, 2016; Javed *et al.*, 2017). The effect of ZnO NP nanoparticles on date palm tissue cultures at different stages of *in vitro* propagation, such as callus induction, organogenesis, shoot multiplication, shoot elongation, and rooting as well as their phytotoxicity on date palm cultures is not well documented. The present study aimed to evaluate the efficiency of different concentrations of NPs and date palm tissue micropropagation.

MATERIALS AND METHODS

ZnO nanoparticles preparation for characterization.

ZnO nanoparticles with a diameter of 20 nm were purchased from mkNANO (Mississauga, Canada). These particles were successfully used as a seed layer to grow zinc oxide Nanowires on Silicon (Al-Asadi *et al.*, 2016) and aligned Carbone nanotube (Al-Asadi *et al*. 2017).

A 0.2 g of ZnO nanoparticles was put in 10 ml of deionized water and shacked well and then drops of the solution were deployed onto silicon substrate and left to dry naturally for FESEM images that was analyzed by scanning electron microscopy (SEM; Zeiss Supra 55VP). For optical absorbance, same ZnO NPs solution was measured using UV-V in spectrophotometer type Shimadzhu1800.

Tissue cultures experiments

Experiments were conducted at the Tissue Culture Laboratory, Date Palm Research Centre; Basra University/ Iraq to maximize the numbers and growth of the *in vitro* proliferated shoots of date palm. Briefly, bud clusters were cut into two to three segments and sub cultured in MS medium (Murashige and Skoog, 1962) containing NAA at 1 mg l^{-1}, BA at 0.5 mg l^{-1} and 0.5 mg l^{-1} of kinetin (K) for multiplication.

ZnO nanoparticles (NPs) was examined for its potential in *in vitro* shoot multiplication of date palm cv. Barhee, for this purpose, various concentrations (0.0 control, 50, 100 and 150) mg l^{-1} of ZnO (NPs) were added to MS media before and after sterilization of the media by autoclave. The pH of the medium was changed to 5.7 using NaOH (0.1 N).

Autoclaving of medium was conducted at 121° C and 1.04 kg/cm^2 for 20 minutes. All cultures were incubated in a culture room under 8/16 h dark/light period provided by daylight fluorescent lamps at 272°± C. Subcultures on the same medium and growth

conditions were performed every four weeks. The percentage of response of cultures on shoot formation was calculated after 8 weeks. The number of proliferated shoots and the percentage of contamination and vitrification of cultures were recorded after 10 weeks of culturing in different media. Additionally, a biochemical analysis was followed to evaluate the shoot responses to ZnO addition including:

Total soluble Carbohydrates

The total carbohydrates of proliferated date palm shoots have been measured according to Watanabe *et al*. (2000). The plant tissues (0.5 g as fresh weight) were homogenized in 80% ethanol and centrifuged for 10 minutes at 5k rpm. The supernatant (1 ml) was transferred into new test tube containing an anthrone reagent, which was previously prepared (50 ml of H_2SO_4 95%+ 50 mg reagent), the mixture left in water bath 100° C up to 10 min, then ice cooled. The measuring of total carbohydrates was performed spectrophotometrically at 620 nm with glucose as standard curve.

Total soluble protein.

The procedure of Bavei *et al.* (2011) was followed to determine the total soluble proteins. 300 mg of date palm shoots tissues were ground in liquid nitrogen, a 3 ml of Tris-HCl buffer (0.1 M, pH 7.5) containing 1 mM phenylmethane sulfonylfluoride (PMSF) was used to homogenize the powder at 4º C. A centrifugation for 13 krpm for 30 minutes was followed. The Bradford reagent was prepared prior to use (100 mg Coomassie Brilliant Blue R-250 in 50 ml 95% ethanol, 100 ml 85% (w/v) phosphoric acid) and used to determine protein content with crystalline bovine albumin as standards curve in the concentrations of 5-100 µg at 595 nm.

Free amino acid content.

Ninhydrine reagent was used to determine the free amino acid content in treated and untreated date palm tissues according to Lee and Takahashi (1966). Briefly, 500 mg of fresh tissues was incubated overnight in ethanol (70%) at room temperature. A mixture of glycerol (55%) and ninhydrine solution (0.5 ml) was added, and mixed well then boiled at 100° C for 20 minutes. Double distilled water was used to make up the final volume of 6 ml. Finally, the wavelength of 570 was used to measure free amino acid content.

Proline content

The protocol of Bates *et al.* (1973) was used to measure proline content in date palm tissues. Ten ml of 3% aqueous sulfosalicylic acid was used to homogenize 500 mg of shoot tissues, followed by centrifugation at 6k rpm. After centrifugation, 2 ml of supernatant was transferred to new test tube containing acid ninhydrine (2 ml) and mixed well, heated at 100º C for one hour and cooled on ice to stop reaction. Toluene (4 ml) was added to the mixture and stirred for 30 seconds. The proline content was measured spectrophotometrically at 520 nm against toluene blank.

Malondialdehyde (MDA) content.

To measure MDA content in date palm shoot tissues, 500 mg of tissues was used and homogenized in 5 ml Trichloroacetic acid (TCA: 0.1%, w/v), followed by a centrifugation at 10k rpm for 5 minutes. One ml of supernatant was transferred into new test tube containing 4 ml of Thiobaributric acid (TBA; 0.5% w/v). The mixture kept in water bath until boiling and then ice cooled to stop reaction, followed by a centrifugation at 10k rpm for 15 minutes. The absorbance then measured at 532 and 600 nm wavelength and the MDA was determined as extinction coefficient of 155 (Heath and Packer, 1968).

Statistical analysis

SPSS-21 statistical software (SPSS In., Chicago, and IL., USA) was used to conduct one-way analysis of variance (ANOVA). The differences between means were compared using LSD test at $P \leq 0.05$ level. Bars on diagrams represent standard deviations. All treatments were triplicates.

RESULTS

ZnO nanoparticles characterization:

Figure (1) shows the FESEM image of ZnO nanoparticles put onto Si wafer. Results showed that Nanoparticles have very high surface to volume ratio which means that examined particles have unsaturated surface bonds. Therefore, ZnO nanoparticles were aggregated together as shown in Figure (2).

Figure (2) shows the optical absorbance spectrum of ZnO NPs measured in the wavelength range of 300-1000 nm. The absorbance peak is located at a wavelength of 375.2 nm which is equivalent to energy band gap (Eg) of 3.30 eV. However, compared by the value of energy band gap of ZnO (3.37eV), the obtained Eg is expanded and this confirms the nature of nanoparticles (Hassan *et al*., 2013).

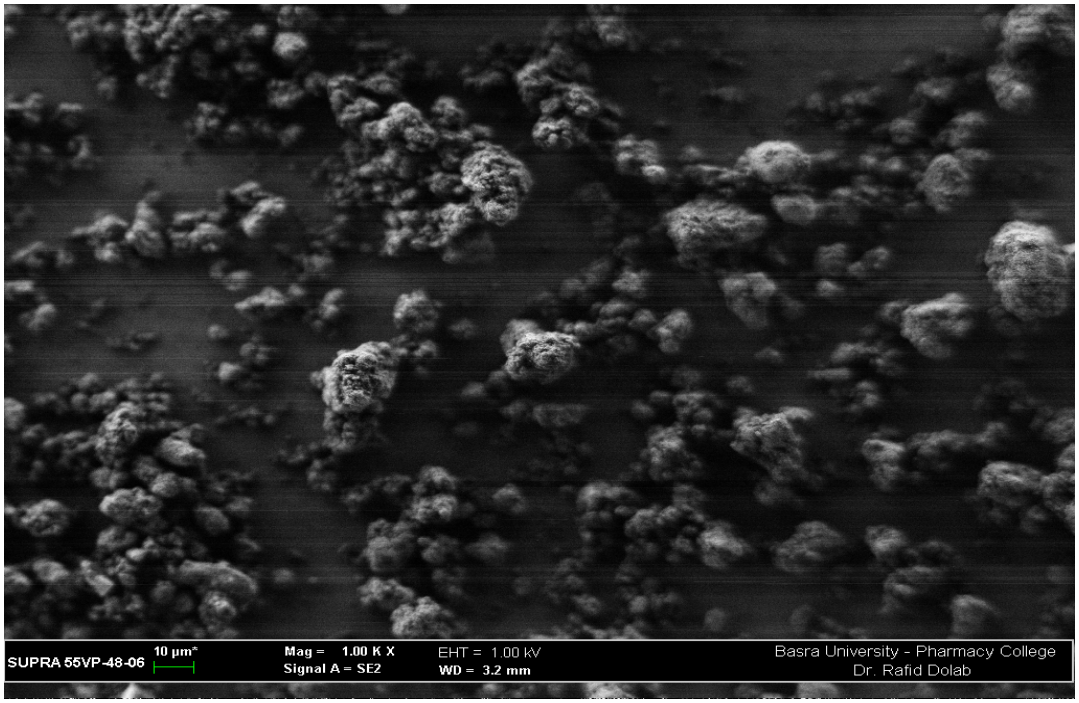

Fig. (1) FESEM image of used ZnO NPs in date palm micropropagation.

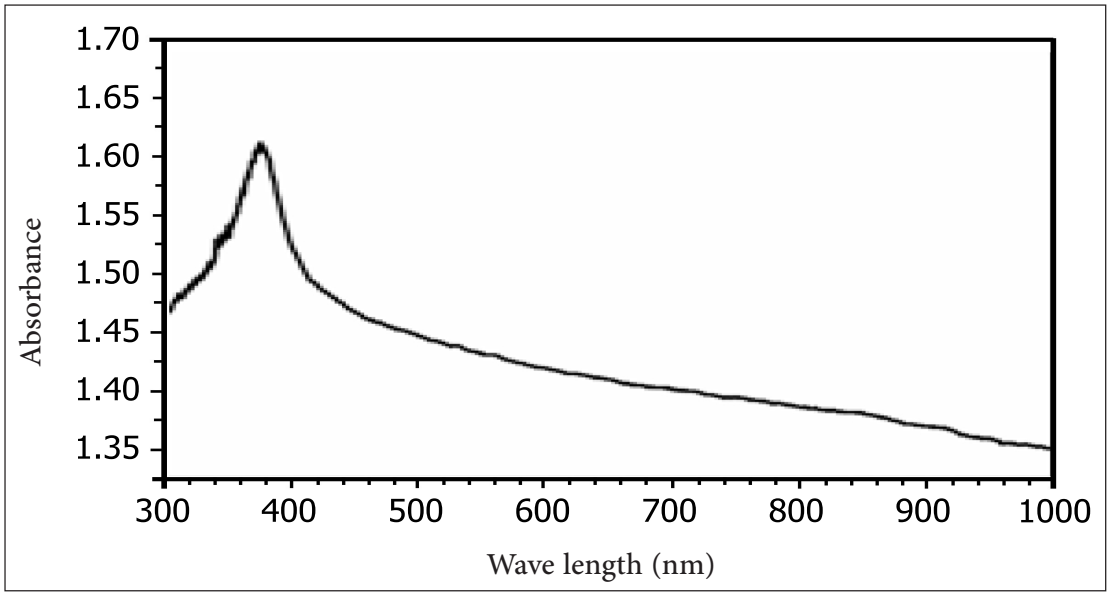

Fig. (2) Optical absorption spectrum of used ZnO NPs in date palm micropropagation.

Effect of ZnO (NPs) on date palm multiplication.

The formation of shoots in cultured buds showed significant variations ($p<0.05$) undergo the influence of ZnO NPs treatments. The best response percentages of explants producing shoots with the highest numbers of shoots per jar were examined on MS medium supplemented with 150 mg l^{-1} of ZnONPs both before and after sterilization as a percent of 93.34% and 86.67%, respectively (Fig. 3A and 3B).

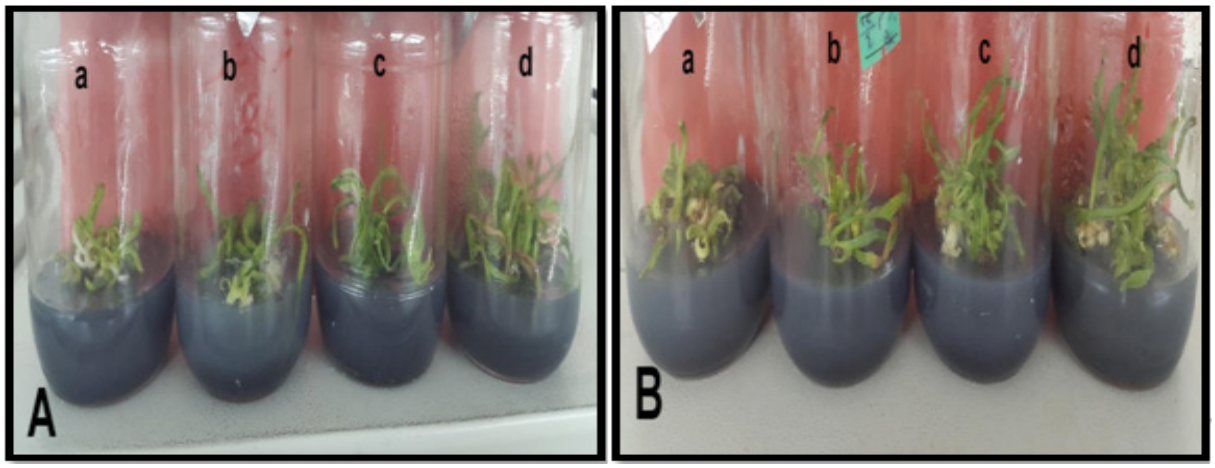

Fig.(3) The effect of ZnO (NPs) at different concentrations in the date palm multiplication before (B) and after (A) sterilization media.

This increase was accompanied with an increase in the average of proliferated shoots and reached 16.2 and 15.8 shoots per jar after 10 week respectively (Table 1), with significant differences than other treatments, followed by 100 mg $l^{-1}$of ZnO (NPs) (Fig.3A and 3B). However, the lowest response percentage of explants producing shoots and number of shoots were recorded in control treatment without addition ZnO NPs which were 46.67% and 6.8 shoots/ jar, respectively.

Table 1: Effect of ZnO NPs on cultures responding percentage and number of shoots for date palm cv. Barhee.

ZnO NPs treatment (mg l^{-1})		Percentage of culture responding (after 8 weeks)	Average number of shoots / jar
Control	0.0	46.67* ± 5.93 e	6.80 ± 0.55 d**
50	B	60.00 ± 6.01 cd	9.20 ± 0.43 c
	A	53.34 ± 7.29 de	8.80 ± 0.50 c
100	B	66.67 ± 4.81 bc	11.60 ± 0.70 b
	A	73.34 ± 4.97 b	12.20 ± 0.22 b
150	B	93.34 ± 6.07 a	16.20 ± 0.32 a
	A	86.67 ± 5.93 a	15.80 ± 0.65 a

Values represent the average± Standard Error. *B: Before sterilization; A: After sterilization. ** Values followed by the same letter are not significantly different at $P<0.05$.

Effect of ZnO NPs on the percentage of contamination and vitrification.

The treated cultures of date palm with different concentrations of ZnO NPs showed significant variations ($p<0.05$) in the percentage of contaminations. The lowest percentage of contamination (no contamination) was found in MS media supplemented with 100 and 150 mg $l^{-1}$of ZnO NPs when added before and after sterilization of the media (Table 2) with

significant differences compared to other treatments. Followed by the treatment of ZnO NPs at 50 mg l^{-1} when added before and after sterilization of the media which were 13.34 and 20.0%; respectively. The highest percentage of contamination was recorded in control treatment without addition ZnO NPs which reached to 33.34%. Statistical analysis of results proved that the treatment of date palm cultures with ZnO NPs led to decrease the vitrification percent as shown in Table (2) The highest vitrification rate (20%) was examined in the control treatment, while the lowest rate (0%) was shown in the cultures treated with ZnO NPs before and after sterilization of the media.

Table (2): Effect of ZnO nanoparticles on percentage of contamination and vitrification of in vitro cultures of date palm during shooting stage.

ZnO NPs treatment (mg l^{-1})		Percentage of contamination (%)	Percentage of Vitrification (%)
Control	0.00	33.34 ± 2.18a**	20.00 ± 2.15 a
50	B*	13.34 ± 1.19 b	0.00 ± 0.00 c
	A	20.0 ± 4.81 b	0.00 ± 0.00 c
100	B	0.00 ± 0.00 c	0.00 ± 0.00 c
	A	0.00 ± 0.00 c	0.00 ± 0.00 c
150	B	0.00 ± 0.00 c	0.00 ± 0.00 c
	A	0.00 ± 0.00 c	0.00 ± 0.00 c

Values represent the average± Standard Error.
*B: Before sterilization; A: After sterilization.
** Values followed by the same letter are not significantly different at $P<0.05$.

Biochemical responses of date palm to ZnO treatment

The results presented in Figures (4 a-e) showed the biochemical responses of date palm shoots to different concentrations of ZnO nanoparticles (50, 100 and 150 mg l^{-1}) before (B) and after (A) autoclaving. The results revealed insignificant differences between ZnO NPs supplied before or after autoclaving on all examined biochemical features.

The results also showed that the high concentration of ZnO NPs (150 mg l^{-1}) caused a significant induction in carbohydrates production in treated date palm shoots (Figure 4 a); compared to other treatments, the carbohydrates concentration was increased from 5.34 mg g^{-1} in control treatment and reached to 8.70 and 8.35 mg g^{-1} FW before and after sterilization; respectively. Regarding the total soluble protein accumulation, results of Figure (4 b) proved that the treatment of ZnO NPs at 150 mg l^{-1} led to a significant increase in protein accumulation, two folds increase in protein accumulation was observed in abovementioned treatments compared to control which was 2.40 mg g^{-1} FW.

Similar trend of increase was observed in free amino acid content of cultured date palm tissues undergo ZnO NPs application. Free amino acid level was 1.20 mg g^{-1} FW in control treatment and reached the 2.46 and 2.12 mg g^{-1} FW in ZnO NPs at 150 mg l^{-1} before and after autoclaving; respectively (Figure4 c). Statistical analysis revealed that the treatment of ZnO NPs at all examined concentrations either before or after sterilization did not affect the proline production in treated date palm cultures as well as MDA accumulation (Figures 4d and e); insignificant differences were observed in all above-mentioned treatments with their corresponding control.

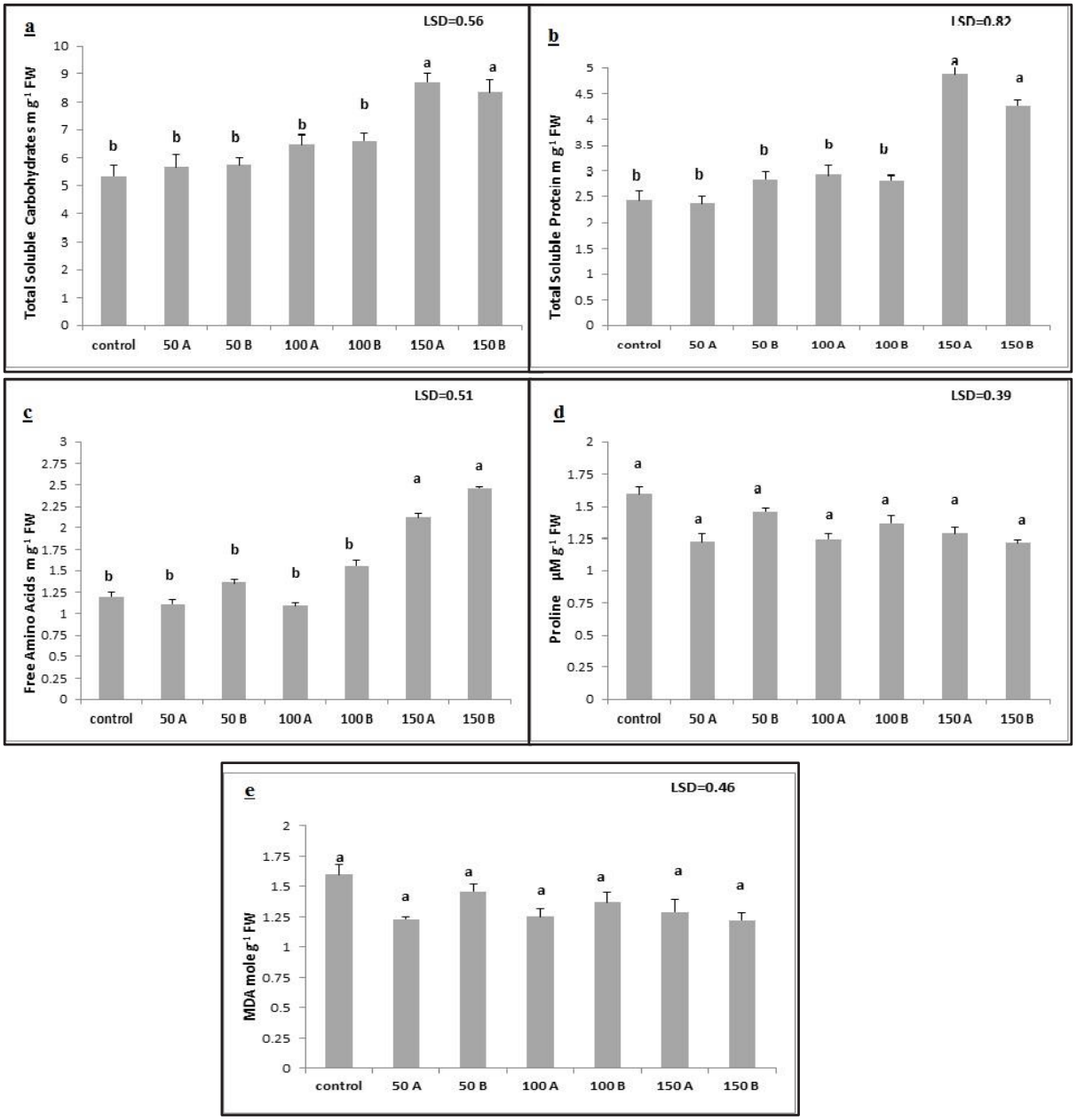

Fig. (4): Effect of Zn NPs at different concentrations in date palm shoots content of a:Total soluble carbohydrates (mg.g^{-1} FW); b: Total soluble protein (mg g^{-1} FW); c: Free Amino Acids (mg g^{-1} FW); d: Proline (µM g^{-1} FW); MDA (mole g^{-1} FW).Values represent the average of triplicates± standard deviation value. Different letters indicate significant differences between treatments according to LSD test (P<0.05). B and A before and after sterilization media.

DISCUSSION

Date palm is an important tree cultivated mainly for their nutrient-rich fruits. The role of *in vitro* multiplication is essential to meet the significant demand for date palm plants (Abass, 2016b). Nanomaterials have attracted attention because of their novel properties, especially in the fields of biotechnology and tissue culture. Our promising results on ZnO NPs application to *in vitro* cultures are consistent with several studies that elucidated the encourage effects of NPs on induction of callus and shoot regeneration of many other plants. The highest percentage of shoot regeneration (89.6%) was examined when nodal explants of *Stevia rebaudiana* were cultured on callus induction medium treated with ZnO NPs (Javed *et al.*, 2017). The significant induction of proliferation depended on the

ZnO NPs concentration. The increase in concentration from 50 mg L^{-1} to 150 mg L^{-1} of ZnO NPs was accompanied with a significant enhancement of multiplication. Similar trend was observed in Helaly *et al.* (2014) for the treatment of banana tissues with ZnO NPs, which results in increased shoot regeneration. The induction of date palm growth by ZnO NPs could be attributed to the enhancement of the plant nutrients absorption from medium. In addition, nanomaterials can act as catalytic cofactors in many vital enzymes, such as nitrate reductase (Alharby *et al.*, 2016). The present results indicated the efficiency of ZnO NPs in preventing microbial contamination in date palm cultured tissues. The fungal and bacterial contaminants are considered as limiting factors especially in woody plants (Sarmast *et al.*, 2011). Contaminants frequently accompany plant tissue cultures and cause the destruction of cultures due to endogenous microbial growth. Furthermore, most microbial contaminants produce phytotoxins, which could cause culture mortality and reduce shoot regeneration (Kane, 2003; Abass, 2013a and b). Our results are in accordance with many other studies showing the applicability of different NPs as antimicrobial agents in tissue cultures. The adding of 100 mg L^{-1} of Zn NPs or ZnO NPs into the MS medium led to the production of contaminant-free cultures (Helaly *et al.*, 2014). The efficiency of NPs in the elimination of microbial contaminations in cultured tissues of plants depended on several factors, such as size, distribution, and type of NPs (Applerot *et al.*, 2012). Metal and metal oxide NPs are useful in eliminating various microorganisms (Wang *et al.*, 2017). A wide range of NPs have been proven to have antimicrobial activities against different microorganisms (Beyth *et al.*, 2015). Many mechanisms have been suggested to elucidate the antimicrobial activity of NPs, such as their affinity to bind with the DNA, thereby interfering with microorganism replication, as well as to the sulfhydryl groups of the metabolic enzymes in the bacterial electron transport chain, leading to bacterial in activation (Slawson *et al.,* 1990) The addition of ZnO NPs decreased the vitrification in treated cultured date palm compared to control treatment (20%), The vitrification of cultured tissues is considered a serious problem that limits propagation of date palm in vitro during different stages, and frequently decrease the proliferation rate of tissue-cultured plantlets (Debergh *et al*., 1992). Vitrification induces the accumulation of water within the cultured tissues and reduces their growth; thus, micropropagated plantlets do not have the ability to survive in soil due to yellowing, swelling, glassiness and leaf curling of plantlets (Donelly and Vidaver, 1984; Alkhateeb, 2008). This disorder was detected in several plants propagated in vitro, such as apple, pears, grape and date palm (AI-Marie, 1996). NPs have advantages and disadvantages on the growth of plants and development. The effects of engineered NPs (ENPs) on plants varied according to the composition, concentration, and size, as well as physical and chemical properties of ENPs (Ma *et al*. 2010). Accumulative evidence suggests that ZnO NPs can enhance the growth and development in many plants, including the callus of date palm (Prasad *et al*., 2012; Amiri *et al*., 2016). Our results showed that ZnO NPs at 150 mg L^{-1} have a positive effect on date palm growth, including the accumulation of carbohydrates, total soluble proteins and free amino acids. The Zn element have a pivotal role in plant metabolism by influencing different enzyme activities such as hydrogenase and carbonic anhydrase, more efficiently stabilizing of ribosomal fractions and cytochrome synthesis. Activation of plant enzymes by Zn element are involved in metabolism of carbohydrates, maintenance of the cellular membranes integrity, and regulation of protein and auxins synthesis (Hafeez *et al*., 2013). Carbohydrates are carbon and energy sources in plant in vitro culture mainly to enhance cell division and enlargement. Priyanka and Venkatachalam (2016) suggest

that ZnO NPs might up regulate protein expression resulting in growth promotion. Proline and MDA accumulation are the most reliable markers of oxidative stress (Yang *et al*., 2012), MDA is the product of lipid peroxidation caused by reactive oxygen species and leads to peroxidation of polyunsaturated fatty acids (Shulaev and Oliver., 2006). Our results showed no significant change in proline and MDA levels in ZnO NPs at all examined concentrations compared to control treatment. Thus, ZnO NPs did not induce any toxic effect on treated date palm tissues even at high concentration (150 mg l^{-1})

CONCLUSIONS

This study showed that ZnO NPs has a positive effect on date palm cultured tissues. A significant increase in the multiplication rate of shoots was observed when 150 mg L^{-1} ZnO NPs were added either before or after media autoclaving. No contamination or vitrification was observed in all tested ZnO NP treatments compared to control.

The increase in shoot multiplication and number by ZnO NPs was accompanied with an increase in carbohydrate and protein contents in date palm tissues. The non-toxic effect of ZnO on date palm cultured tissues was confirmed by analysing proline and MDA contents. The results provided strong basis for the application of ZnO NPs (150 mg l^{-1}) to the media for in vitro multiplication of date palm.

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تقييم فعالية جزيئات أوكسيد الزنك النانوي في إكثار نخيل التمر (*Phoenix dactylifera* L.) خارج الجسم الحي

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الملخص

تعد الملوثات الميكروبية في مزارع الإكثار النسيجي من التحديات المهمة التي تواجه مراحل الإكثار المختلفة، وأثبتت جزئيات الزنك النانوية فعالية مضادة للميكروبات. جاءت الدراسة الحالية بهدف تقييم فعالية أوكسيد الزنك النانوي على التلوث الميكروبي وظاهرة التزجج وتشجيع نمو أنسجة النخيل المكثرة خارج الجسم الحي. وقُيمت الفعالية للجزئيات النانوية اعتمادا على عدد من المؤشرات؛ منها تكوّن وعدد الأفرع الخضرية ونسبة التلوث ومعايير بيوكيميائية متعددة.

أشارت النتائج إلى وجود زيادة بمقدار ضعفين في معدلات تضاعف المجموع الخضري؛ إذ بلغت المعدلات 88.67 و 93.34 % في معاملتي قبل وبعد التعقيم على التوالي، قياسًا بمعاملة المقارنة التي سجلت 46.6%، كما لوحظت الفعالية ذاتها في صفة الأفرع المتضاعفة في معاملة أوكسيد الزنك النانوي بتركيز 150 ملغم/ لتر. وبينت النتائج أي تلوث ميكروبي أو تزجج في مزارع أنسجة نخيل التمر المعامل قياسًا بمعاملة المقارنة التي بلغت فيها نسبة التلوث والتزجج 33.44 و 20 % على التوالي. لم تشر النتائج إلى أي نشاط سمي سلبي لجميع تراكيز جزئيات أوكسيد الزنك النانوي المدروسة في المعايير البيوكيميائية لأنسجة نخيل التمر المكثر خارج الجسم الحي مع تأثير تشجيعي لتركيز 150 ملغم/ لتر في معايير الكربوهيدرات والبروتين والأحماض الأمينية في الأنسجة المعاملة. وتشجع نتائج الدراسة الحالية إمكانية استخدام أوكسيد الزنك النانوي بتركيز 150 ملغم/ لتر في مزارع أنسجة نخيل التمر.

الكلمات المفتاحية: التكاثر الدقيق، تقنية النانو الحيوية، جزئيات أوكسيد الزنك النانوية، السمية الضوئية.