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Effect of extracellular products extract of cyanobacteria *Oscillatoria tenuis* on micropropagation of date palm *Phoenix dactylifera* L. cv. "Barhee"

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Abstract. The aim of the study was to determine the effect of the extract of extracellular products of cyanobacteria *Oscillatoria tenuis* as an alternative or complementary component of the MS medium prepared for the date palm *Phoenix dactylifera* shoot proliferation growing by in vitro culture. The results shows that the medium supplemented with a half-strength of MS salts and the extract of cyanobacteria extracellular products (CEP) gave significantly increase of the percentage response to shoot proliferation and rooting, number of shoot, primary and secondary root, fresh and dry weight of shoot and root, total chlorophyll content, N%, phosphorus and potassium content compared with other two treatments. But this treatment gave the lowest value in shoot length. Also the results indicate that the medium supplemented with the full strength of MS salts recorded the lowest values in the percentage response to shoot proliferation and shoot length, fresh and dry weight of shoot, total chlorophyll content, N% and phosphorus content. But the medium supplemented with the cyanobacteria extracellular products (CEP) extract gave the lowest value in number of shoots. While this treatment gave the highest value in length of the shoot compared to the other two treatments. While the medium supplied with the cyanobacteria extracellular products (CEP) extract gave the lowest value in leaf content of total. After the plants were obtained from the shoot proliferation by the micro propagation method of *P. dactylifera* cv. "Barhee" were acclimatized by a success rate of 65.2%.
Key Words: auxin, chlorophyll, cytokinin, in vitro, shoot, proliferation.

Introduction. Date palm (*Phoenix dactylifera*) trees are evergreen fruit trees also cultivated in southern and middle of Iraq. The species of *P. dactylifera* belong to more than 600 agricultural cultivars in Iraq. The finest *P. dactylifera* cultivar is the cv. "Barhee", which is grown commercially in Iraq (Muter 1991). The traditional propagation methods of this plant are seeds and offshoots. The disadvantages of seed propagation are to produce plants that are not genetically similar to the mother plant as a result of cross-pollination. The offshoots are few in number and expensive to buy. Many researchers have resorted to in vitro culture technique to overcome these problems. This technique of propagation gives the large number and true to type with mother plant (Ibrahim et al 2013). The micropropagation of plants depends mainly on the type of medium and growth regulators. In several studies, cyanobacterial extract has been used as an alternative to the high-cost media for plant tissue culture (Zaccaro et al 2006; Banerjee & Sharivastava 2008). It has been found that cyanobacteria may produce some bioactive substances such as growth regulators that can be used for propagation of horticultural plants. These substances include gibberellins, auxins, cytokinins, jasmonic acid, ethylene and abscisic acid. Keerthiga et al (2012) found in his study that cyanobacteria have the ability to produce these bioactive substances from their cells, accumulate and release. In a study with addition of extracts of cyanobacteria (*Scytonema* sp., *Mastigocladus laminosus* and *Aulosira fertilissima*) with different concentrations (10-90%), Seema et al (2011) noted that the concentrations of 70% gave the highest rate of

shoot proliferation compared with the other concentrations. In other study, Keerthiga et al (2012) also found that the addition of the extracellular products extract of cyanobacteria *Phormidium subincrustatum* with concentrations of 12.5 and 25.0% to the prepared MS medium for the micropropagation of the Singapore daisy (*Wedelia trilobata* L.) plant showed that the 25.0% concentration gave the best results in shoot proliferation. Another study by Ghasolia et al (2013) indicated that the addition of 4% of the extract of the extracellular products of the cynobacteria (*Nostoc muscorum*, *Chroococcidiopsis*, *Spirulina* sp. and *Tolypothix* sp.) and 2 mg L^{-1} of kinetin to the MS medium prepared for the micropropagation of the Brahmi (*Bacopa monnieri* L.) plant resulted in the best values for the shoot proliferation.

The main aim of this study is to determine the effect of the extract of extracellular products of cyanobacteria *Oscillatoria tenuis* as an alternative or complementary component of the MS medium prepared for the *P. dactylifera* shoot proliferation growing by in vitro culture.

Material and Method. The study was conducted in the Plant Tissue Culture Laboratory of the Agricultural Company Fadak in the area of Bahadriya, district of Abu al-Khassib, the province of Basrah for the period 2015-2017. *P. dactylifera* offshoots of cv. "Barhee" were obtained from 3-4 years aged plants from one of the *P. dactylifera* orchards of Abu Al-Khassib for use as a source of explant plant parts. The offshoots were sliced to the shoot tips. After that, the shoot tips were placed for 24 hours in the refrigerator in the antioxidant solution consisting of citric and ascorbic acid and distilled water at a concentration of 150 and 100 mg L^{-1}, respectively. The following day, the shoot tips were sterilized for 60 min with the mercury chloride ($HgCl_2$) sterilized solution at a concentration of 0.001% with the addition of several droplets of Tween 20 to increase the efficiency of sterilization of the outer surfaces of explants. The shoot tips were then grown in the MS medium (Murashige & Skoog 1962). Some chemicals and growth regulators were added to the MS medium at a concentration of 185.5 mg L^{-1} $Na_2H_2PO_4.2H_2O$, 20.0 mg L^{-1} D-pantothenate calcium, 80.0 mg L^{-1} adenine sulfate, 2.0 g L^{-1} Calcium nitrate tetrahydrate, 100.0 mg L^{-1} inositol, 0.4 mg L^{-1} Thiamine, 500.0 mg L^{-1} Polyvinyl pyrrolidone, 200.0 mg L^{-1} Glutamine, 60 g L^{-1} Sucrose, 6.5 g L^{-1} Agar, 0.2 mg L^{-1} BA, 0.2 mg L^{-1} 2ip, 0.2 mg L^{-1} Kinetin and 6.0 mg L^{-1} NAA. Direct adventitious bud initiation was induced after eight weeks of in vitro culture.

Cultivation and development of O. tenuis. *Oscillatoria tenuis* was grown in sterile glass basins of 30 x 50 x 40 cm, containing the sterile BG11 liquid medium. These cultures were then placed in a growth chamber at a temperature of 25±2°C and under the light intensity of 60 µmol m^{-2} s^{-1}. These blue-green algae were developed using a fluorescent light of 24 hours light. The cultures have been continuously shaken for the purpose of obtaining a growing mass of algae. The date of the algae harvest was in the Stationary Phase (Mackinney 1941). The extracellular products were extracted from cyanobacteria by separation from biomass using the filter papers (Whatman No.1). These extracts were kept in sterile containers in the refrigerator at 4°C until use.

The cyanobacteria extracellular products (CEP) extracts were added to the media prepared for the two stages of the shoot proliferation and rooting according to the following treatments:

1. Control treatment: Shoots of *P. dactylifera* were cultured on the full-strength MS medium.
2. Shoots of *P. dactylifera* were cultured on the half-strength MS medium supplemented with cyanobacteria extracellular products (CEP) of extract.
3. Shoots of *P. dactylifera* were cultured on the medium supplemented with cyanobacteria extracellular products (CEP) of extract (Without MS salts).

The same MS medium components were used above (first paragraph), with the exception of sucrose, agar and growth regulators, which were added as follows: 50 g L^{-1} sucrose, 6.5 g L^{-1} agar, 0.1 mg L^{-1} for each of the 2ip, kinetin and benzyl adenine for the shoot

proliferation stage and 40 g L^{-1} sucrose, 6.7 g L^{-1} agar and 0.5 mg L^{-1} NAA for the rooting stage.

After the formation of the plantlets resulting from shoot proliferation of *P. dactylifera* cv. "Barhee", subjects were acclimatized after transfered from culture containers and washed with sterilized distilled water to remove the residual effect of the medium. After that, the plantlets were diluted for 5 minutes with the Hymazol 0.5 mg L^{-1} with 10 mg L^{-1} of the tetracycline antibiotic with several drops of the Tween20 to reduce the surface tension and improve the sterilization efficiency. The plants were cultured in plastic pods containing a mixture of peat moss and perlite by 1:2, and covered with plastic cups to maintain the surrounding humidity.

Recorded characteristics:

1. Percentage of response to the shoot proliferation;
2. Number of formed shoots/explant;
3. Shoot length (cm);
4. Total chlorophyll content in shoot leaves: Total chlorophyll was estimated in mg g^{-1} as described in Goodwin (1976);
5. The content of leaves of nitrogen, phosphorus and potassium: The dry samples of the leaves of the shoots were digested according to the method described by Cresser & Parsons (1979). Total nitrogen concentration in leaves (%) was estimated using Micro Kjeldahl. The content of the phosphorus in leaves of the shoot was estimated in g kg^{-1}. The amount of potassium in the leaves of the shoots was estimated in mg kg^{-1} unit according to the methods described by Page et al (1982);
6. Percentage of shoot response to rooting;
7. Number of primary adventitious roots per shoot.

Experimental design and statistical analysis. All simple experiments for the current study were designed according to Complete Randomized Design (CRD). Each treatment in the experiments under study was repeated five times. The data were analyzed statistically using the analysis of variance. Compare the mean of treatments using Revised Least Significant Difference (R-LSD) at a 5% probability level based on Al-Rawi & Khalaf Allah (2000).

Results and Discussion. The results from Table 1 show that the medium supplemented with a half-strength of MS salts and the cyanobacteria extracellular products (CEP) extract gave significantly increased percentage in response to shoot proliferation, the number of shoots and fresh and dry weight of the shoot compared with the other two treatments. This treatment recorded 97%, 58.0 shoots/explant, 13.18 g and 1.14 g, respectively. But this treatment gave the lowest value in shoot length which reached 3.83 cm.

Table 1

Effect of adding the extract of cyanobacteria extracellular products (CEP) to the medium in some physical characteristics of *Phoenix dactylifera* shoots

Treatment (%)	*Response to proliferation (%)*	*No. of shoots/explant*	*Shoot length (cm)*	*Fresh weight (g)*	*Dry weight (g)*
Full strength of MS	81.5	50.75	4.18	6.99	0.68
Half strength of MS + CEP	97.5	58.00	3.83	13.18	1.14
CEP	92.5	31.50	4.45	8.39	0.74
R-LSD (P≤0.05)	2.44	5.01	0.12	1.21	0.02

The results from the Table 1 indicates that the medium supplemented with the full strength of MS salts recorded the lowest values in the percentage response to shoot proliferation, shoot length, and fresh and dry weight of the shoot. This treatment recorded 81.5%, 4.18 cm, 6.99 g and 0.68 g, respectively. But the medium supplemented with the cyanobacteria extracellular products (CEP) extract gave the lowest value in number of shoots which it reached 31.5 shoots per explant. While this treatment gave the highest value in length of the shoot compared to the other two treatments, reaching 4.45 shoots per explant.

Table 2 shows that the medium supplemented with a half-strength of MS salts and the cyanobacteria extracellular products (CEP) extract led to a significant increase in leaf content of total chlorophyll, nitrogen, phosphorus and potassium compared with the other two treatments. This treatment recorded 0.405 mg g^{-1} as a fresh weight, 4.482%, 8.161 g kg^{-1} and 0.242 mg kg^{-1}, respectively. But the medium supplemented with the full strength of MS salts recorded the lowest value in leaf content of total chlorophyll, nitrogen and phosphorus which it reached 0.323 mg g^{-1} as a fresh weight, 2.942% and 7.962 g kg^{-1}. While the medium supplied with the cyanobacteria extracellular products (CEP) extract gave the lowest value in leaf content of total potassium which it reached 0.089 mg kg^{-1}.

Table 2

Effect of adding the extract of cyanobacteria extracellular products (CEP) to the medium in some chemical characteristics of *Phoenix dactylifera* shoots

Treatment (%)	*Total chlorophyll (mg g^{-1} FW)*	*Nitrogen (%)*	*Phosphorus (g kg^{-1})*	*Potassium (mg kg^{-1})*
Full strength of MS	0.323	2.942	7.962	0.145
Half strength of MS + CEP	0.405	4.482	8.161	0.242
CEP	0.378	3.222	8.158	0.089
R-LSD (P≤0.05)	0.023	0.201	1.520	0.011

The results from Table 3 indicate significant differences between the treatments. The medium containing half the strength of the MS salts and the cyanobacteria extracellular products extract was increased significantly in the number of primary and secondary roots and the fresh and dry weight of the roots compared with the other two treatments.

Table 3

Effect of adding the extract of cyanobacteria extracellular products (CEP) to the medium in some rooting characteristics of *Phoenix dactylifera*

Treatment (%)	*Response to rooting (%)*	*No. of primary roots per shoot*	*No. of secondary roots per shoot*	*Fresh weight (g)*	*Dry weight (g)*
Full strength of MS	80.00	2.00	2.50	1.60	0.242
Half strength of MS + CEP	87.59	3.75	3.00	1.66	0.256
CEP	76.25	2.50	2.50	1.64	0.242
R-LSD (P≤0.05)	1.65	0.25	0.12	0.004	0.010

As the values reached in this treatment, 3.75 primary roots per plantlet, 3.0 secondary roots per plantlet, 1.66 g and 0.256 g, respectively. While the medium supplemented with the full strength of MS salts gave the lowest values. As the values reached in this

treatment 2.0 primary root per plantlet, 2.5 secondary roots per plantlet, 1.60 g and 0.242 g, respectively. After the plants were obtained from the shoot proliferation by the micro propagation method of *P. dactylifera* cv. "Barhee" were acclimatized by a success rate of 65.2%.

The reason for the significant increase in the percentage of response to shoot multiplication, shoot and root number and the fresh and dry weight of the developing on the half strength of MS medium supplemented with extract of cyanobacteria extracellular products is due to the chemicals found in the algae extract, such as vitamins and antibiotics, which have positively affected the division, multiplication of cells and growth in the shoot and root tissues (Zaccaro et al 2006; Banerjee & Sharivastava 2008). The cyanobacteria extracellular products extract also contain the bioactive substances produced by these algae as growth regulators such as auxins, gibberellins, cytokinins and jasmonic acid, which have been positive in encouraging the growth and multiplication of shoot and root tissues (Gupta & Agarwal 1973; Strik et al 2002; Molnar & Ordog 2005). The reason for the significant increase in the treatment of the medium containing the extract of cyanobacteria extracellular products in the length of shoot is due to the lack of competition of these shoots on food due to the decrease in number, which helped to increase the cell division and elongation of shoots. Cyanobacteria have the ability to fix nitrogen by using the ATP energy from photosynthesis to ammonia by an enzyme called nitrogenase (Waterbury et al 1979; Wolk 1980). The use of the water extract of cyanobacteria extracellular products with MS salts at half the strength has completed the shortage of nitrogen, phosphorus and potassium, and has helped to support and grow the adventitious buds formed in this medium (Mazri et al 2016). The addition of the extract of the extracellular products of the cyanobacteria was positively reflected on the improvement of characteristics of the shoot and root which contributed to the increase of total chlorophyll content in leaves and important mineral elements such as nitrogen, phosphorus and potassium. The results of the present study agree with the results of studies on the micro propagation of other plants when adding the extract of extracellular products of different species of cyanobacteria to the media, which in turn improved the shoot and root traits at the shoot proliferation (Shanab et al 2003; Manickavelu et al 2006; Seema et al 2011; Ghasolia et al 2013).

Conclusions. The addition of the cyanobacteria extracellular products extract to the nutrient medium, which is half the strength of MS salts, has led to improved shoot multiplication in *P. dactylifera* trees cv. "Barhee". The extract of these extracellular products can also be used as an alternative or complement to the food culture prepared for in vitro culture.

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