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Evaluation of *Ziziphora tenuior* growth, oil yield and pulegone content *after in vitro* slow growth storage

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ABSTRACT

Ziziphora tenuior is a medicinal plant which has been used to treat many diseases due to the presence of many valuable medicinal ingredients such as, pulegone. This valuable plant has become extinct from its natural environment due to illegal collection, urbanization, overgrazing and drought. In this study, *in vitro* slow growth storage was applied to study the possibility of conserving *Ziziphora tenuior* L. microshoots using elevated levels of different carbon sources (sucrose, sorbitol and mannitol) at (0.1, 0.2, 0.3 and 0.4 M) or ABA at different concentrations (0, 3.8, 7.6, 11.4 µM). Data were recorded after two periods (6 and 12 weeks) of cultivation for growth parameters, survival and recovery percentages. Recovery percentage was used as a survival indicator for shoots after 12 weeks of conservation. Furthermore, effect of slow growth storage on the total oil content and pulegone concentration of the microshoots was investigated after 3 months conservation using Gas chromatographic method (GC/MS) for the identification and quantification of the marker compounds pulegone in *Ziziphora tenuior*. The current study revealed that, microshoots prestored in MS media + (0.2 M) sucrose or ABA were able to reduce the all measured growth parameters and to maintain recovery after storage. On the other hand, using sorbitol or mannitol had a strong negative impact on recovery after storage. Meanwhile, there were differences between the amounts of total oil yield and pulegone extracted from the wild type plants and the *in vitro* prestored microshoots. Maximum total oil yield content (5%) and pulegone (0.0312 M) were obtained from the wild type plants. On the other hand, the amount of total oil yield extracted from the *in vitro* stored microshoots were one third the amounts of oil obtained from the wild type plants. Also, the concentration of pulegone found in the dried *in vitro* stored samples (0.0041-0.0046 M) was about the same in all *in vitro* prestored microshoots but much lower than those obtained from the wild plants samples.

Keywords: Medicinal plant, Pulegone, Slow growth storage, Total oil yield, *Ziziphora tenuior* L.

1. INTRODUCTION

Secondary metabolites are chemical compounds arising from primary products (amino acids, fatty acids, carbohydrates,.. etc). Secondary metabolites are not essential for organism or plant normal growth, development, or reproduction, but essential for survival. Secondary metabolites main classes are: carbohydrates, alkaloids, steroids terpenoids, aliphatic, polyketides, aromatic, heteroaromatic, organic acids, volatile oils,

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phenolics, balsams, resins, and saponins (Hopkins, 1995). Reasons for producing secondary metabolites are: toxic materials providing defense against herbivores, competitors, and microorganisms as well as they are growth regulators and as volatile attractants. Additionally, secondary metabolites are responsible for plant characteristics, like odors, pungencies and colors, as well as most of pharmacologically active natural products are provided by secondary metabolism (Dewick, 2002; Kingston, 1996).

Z. tenuior is a dwarf (3-10 cm) annual plant with a simple or branched necked stem. The branches are erect or ascending, while the leaves of the sterile part of stem are not numerous. The floral leaves are lanceolate to linear-lanceolate, prominently veined and often longer than flowers., while the flowers are spike-like sessile inflorescences (10-12 mm long), with narrow calyx and pink corolla (Zohary and Feinbrun, 1978). Due to its medicinal activities, *Z tenuior* has been used in folk medicine for the treatments of many diseases (Ozturk, *et al*., 1995). The most important medicinal component in *Z. tenuior* is the essential oil (Mahboubi, *et al*., 2012), in which Polygon *is* the major constituent as it comprises about 87 % of the oil yield (Sezik, *et al*., 1991). *Z. tenuior* has been used for treatment of dysentery, fever, diarrhea, coughing, bladder stone, painful menstruation, stomach tonic and abortifacient (Naghibi, *et al*., 2005). Furthermore it has been used a as herbal tea due to its odor (Al-Rawashdeh, 2011).

In the past century, a serious and dramatic decline in plant diversity worldwide was noticed, as some plant species become extinct from their natural environment. This was due to several reasons, including illegal collection, agricultural expansion and urbanization, deforestation, overgrazing, soil erosion, and depletion of water resources (Hatough-Bouran*, et al*., 1998, Tatamouni et al., 2015). Slow growth storage can be referred as "inhibition", or "growth limitation", that implies use of several techniques such as, low temperature, low light intensity, modification nutrient in growth medium by adding osmotic agents or abscisic acid, in addition to storage under low pressure/low oxygen (Bajaj, 1991; Botau, *et al.,* 2005; Tahtamouni et al., 2015). Slow growth storage principle permits a safe utilization of *in vitro* cultures up to 1-15 years without periodic sub-culturing (Rao, 2004, Ciobanu and Constantinovici, 2012; Tahamouni et al., 2016).

Meanwhile, plants prestored under low growth conditions were reported to show some variations in terms of phenotypic and biochemical characteristics after storage (Hemant *et al*., 2012). Most of these variations were due to the carryover effect resulted from the exposure to either of growth regulators or osmotic stresses during the *in vitro* storage (Engelmann, 1991; Silva and Pereira 2011). This study was conducted to find out the how would *Z tenuior* microhoots perform after three months storage under slow growth conditions in terms of growth, total oil yield and Pulegone concentration.

2. Materials and Methods

2.1. *In vitro* establishment of *Z. tenuior*.

The establishment media consisted of hormone free (HF) MS salt (4.4 g\L) (Murashige and Skoog, 1962) (Sigma Aldrich Murashige and Skoog Basal salt mixture) pluse (0.1 M) sucrose. The, pH of the medium was adjusted to (5.7) using 1N NaOH and 0.1N HCl with nonstop stirring, and then the agar (Oxoid Bacteriological agar No.1) was added by 8.0 g/L to sloidifie the media. After that, media was dispensed in 250 ml flasks (100 ml media/vessel or flask), then closed and sterilized by autoclaving at 121□C and 15 kg/cm^2 pressure for 20 min.

Z. tenuior seeds were collected from AL-Hisha- Al-Shoubak region in southern part of Jordan with (30°31′53″N 35°33′39″E). After that, seeds were surface-sterilizated by dipping them in 70% ethanol solution for 30s, then soaking in a 20% aqueous solution of 5.4% sodium hypochlorite for 20 min with gentle shaking, and then followed by three washes with sterilezed distilled

water under laminar air-flow cabinet. After that, the sterilized seeds were inoculated into flasks containing Murashige and Skooge (MS) media, and cultures were left in the growth room conditions in dark at 25±1 °C until seed were germinated. Then seedling were transferred to normal conditions growth room and maintained under daily light regime of 16-h (photosynthetic photon flux density (PPFD) = 40-45 μ mol. m^{-2} sec^{-1}) and 8 h dark at 24 ± 1 °C. Cultures were subcultured periodically every (4 weeks) to the media of in vitro establishment described above until enough plant material was established.

2.2.1. Slow growth conservation using osmotic agents

About 1.0 cm shoot length of *Z. tenuior* microshoots were sub-cultured on a hormone free medium (MS- HF) provided with different concentrations of either sucrose, sorbitol or mannitol (0.1, 0.2, 0.3 and 0.4 M) with 20 replicates for each treatment and incubated under similar growth room conditions described earlier. After three month storage period, data were recorded on shoot hieght, number of shoots, rooting and recovery precentages. Recovery percentage was recorded one month after transferring the prestored microshoots to fresh MS - HF medium provided with 0.1 M sucrose at culture room conditions (at 25±1°C under a 16/8 (light/dark) photoperiod of 45-50 μmol $m^{-2}s^{-1}$).

2.2.2. Slow growth conservation using Abscisic Acid (ABA)

Microshoots were stored for 3 months on (HF) MS media pluse (0.1 M) sucrose in addition to different concentrations of ABA (0, 3.8, 7.6 and 11.4 μM), with 20 replicates / treatment and kept under similar growth room conditions described earlier. Data were recorded as described above.

2.3. Volatile oil extraction and volatile oils' determination of *Z. tenuior*

2.3.1. Plant Material

For oil extraction, samples from wild plants of *Z. tenuior* aerial parts of plants were collected from AL-Hisha, Al-Shoubak site in June. Also, samples from the control microshoots (grown in MS + 0.1M sucrose), in addition to microhoots prestored in treatments that were best for storage in terms of growth parameters and recovery (microshoots prestored in MS media + 0.2 M sucrose, or MS media + 3.8 μM abscisic acid). The tested samples were dried separately on paper sheets in the oven at 30 ˚C for two weeks. Then these samples were kept in refrigerator until the analysis time for volatile oil. Then dried samples were grounded separately, and 20 g powder from each sample was taken with three replicates for each treatment.

2.3.2. Volatile oil extraction and Standard Preparation

Volatile oils were extracted from dry plant material by steam distillation using volatile oil distillation apparatus. Distillation was performed by using Clevenger-type apparatus (20 g of dry plant material in 750 ml distilled water for 2 h). The essential oil was recovered from the distillate by shaking with mixture of hexane-dichloromethane (1:1) using a separatory funnel. The essential oils which was obtained over anhydrous sodium sulfate and stored in dark glass vials at 4 ˚C until required for analysis. The percent of oil yield was calculated as volume (v) of essential oil per g of plant dry material.

2.3.3. Determination of Pulegone in Z. tenuior volatile oils

Gas chromatographic method (GC/MS) was used for the identification and quantification of the marker compounds pulegone in *Z. tenuior* essential oil using external reference standard. The type of GC used was (Varian Chrompack CP 3800) supplied with MS detector (Varian Saturn 2200) and DB- 5 low bleed GC-MS capillary column (HP-5, 30 m length x 0.25mm ID x 0.25μM df, Phenomenex, USA). The temperature of oven was raised from 60°C to 250°C with a 2°C/min slope. Also the temperature of the detector and the injector was set at 250°C. Extra pure Helium was used as gas carrier at 1ml/min flow rate with total run time of 17.5 min. The

MS detector had the following qualifications: full scan mode from 35-650 *m/z* and emission current of 70 eV.

Curve of standard calibration was prepared by diluting pulegone stock solution (Sigma-Aldrich) at four points (1, 2, 3, 4 µl) in 1ml n- hexane. Also, (1 µl) from each collected oil sample was diluted in n-hexane with a final volume of (1 ml) before (1 µl) from the final volume was injected into GC. Pulegone concentration was determined according to the following equation provided by the calibration curve.

$$Y = ax + b$$

Where y = area, a = constant, x= pulegone concentration and b = constant.

2.4. Experimental design

Treatments in each experiment were arranged in a complete randomized design (CRD). Each treatment replicated twenty times for slow growth experiments, while for secondary metabolites experiments, each treatment had three replicates. The collected data from the experiments described above was statistically analyzed using SAS (Statistical Analysis System, Cary, NC). Analysis of variance (ANOVA) was used to analyze the obtained results and means were separated at probability level of 0.05 according to the LSD.

3. Results and Discussions

3. 1. Slow growth *in vitro* storage of *Ziziphora tenuior* microshoots

3.1.1 Influence of sucrose concentrations

After three month storage periods, results showed that increasing sucrose level reducing in vitro microshoots growth. Shoot height decreased from (9.17 cm) on media supplemented with 0.1 M sucrose (control) to (2.08 cm) on media supplemented with 0.4 M sucrose (Table 1). Moreover, maximum number of shoots (11.95) was obtained in the control, while increasing sucrose level in the media was able to reduce number of new shoot developed where to reach a minimum value of (1.66) at sucrose level of (0.4M) sucrose (Table 1). The results in the current study similar to previous finding by Shibli, *et al*., (1999), on bitter almond. Similar results were recorded for different species such as *Achillea fragrantissima* (Younes, 2012) and *Teucrium polium* L. (Rabba'a, *et al.,* 2012).

The decline in growth was also observed in rooting percentage of the *in vitro* conserved *Z. tenuior* microshoots, as rooting decreased dramatically with increasing sucrose concentrations in the media. Full rooting percentage was recorded in the control microshoots, while rooting was completely inhibited in microshoots stored in media with 0.4 M sucrose (Table 1). Sharaf, *et al*., (2012) reported that, rooting of *Artemisia herba-alba* microshoots were formed by the same responses under different concentrations of sucrose in growth media. This may be due to the nature of the *Artemisia herba-alba* plant which can survive stress conditions.

Regrowth of the pre stored shoot tips or Recovery rate was recorded one month after the end of the storage period. Microshoots which were prestored in the control treatment were back to full capacity of growth (100% regrowth); (Fig. 1). On the other hand; the regrowth of the prestored microshoots on media with elevated levels of sucrose was declined after being transferred to normal growth conditions. For example, the recovery rate recorded in microshoots prestored in media with 0.2 M sucrose was (75%), while increasing sucrose up to 0.4 M sucrose had decreased recovery rate (Fig. 1). Our results were in agreement with Srivastava, *et al.,* (2013), who reported that complete survival and recovery percentages of *Glycyrrhiza glabra* were obtained when low sucrose concentration were used in the storage media, while no survival and recovery were obtained when sucrose increased in the storage media.

Table 1: Effects of sucrose concentrations on *in vitro* conserved of *Z. tenuior* microshoots.

Sucrose conc.(M)	Shoot Height (cm)	Number of Shoots	Rooting %
0.1 M	9.17 ±0.42 A[y]	11.95 ±0.83 A	100 ±0 [z]
0.2 M	2.19 ±0.47 B	5.18 ±0.93 B	55 ±11.41
0.3 M	2.08 ±0.52 B	4.76 ±1.04 B	15 ±8.19
0.4 M	2.26 ±1.09 B	1.66 ±2.16 C	0 ±0

[y] Means within groups followed by same letter(s) are not significantly different according to Least Significant Difference (LSD) ($P = 0.05$). [z] Means tested by standard error of means ±.

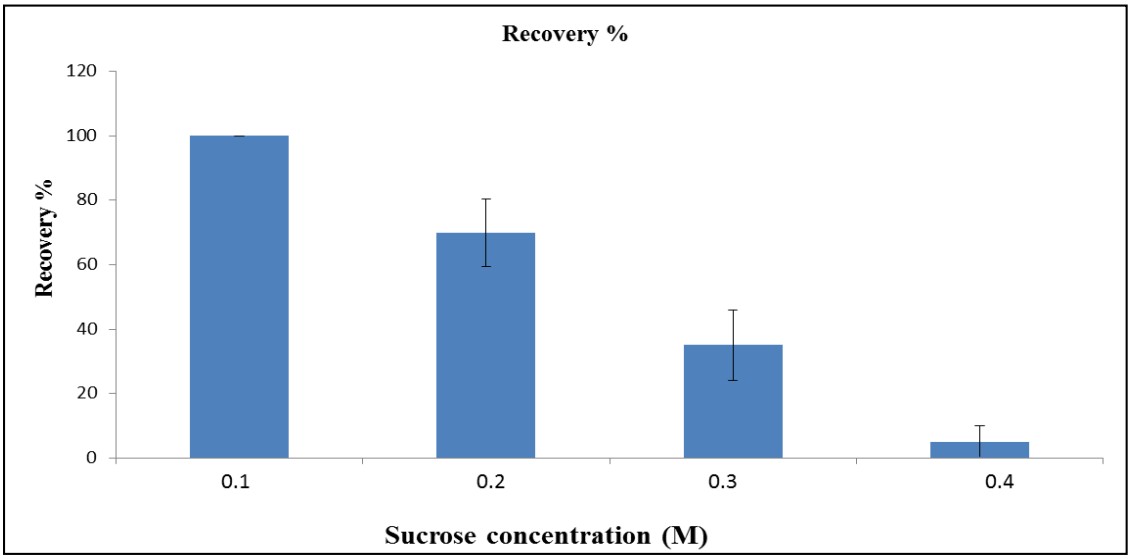

Figure 1: Effect of sucrose concentrations on recovery rate of *Z. tenuior* L microshoots after three month storage in slow growth media.

Adding osmotic agents to culture media was found to be very efficient in the reduction of growth or in the inhibition of growth and increasing storage life of the *in vitro* tissues (Rathore and Singh, 2013; Tahtamouni, *et al*., 2016). Besides that, osmotic agents increase the turgor pressure inside plant cells and so the cell division will be reduced and this will cause the inhibition of cell growth parameters (Brown, *et al*., 1979; Subaih, *et al*., 2007).

3.1.2. Influence of mannitol concentrations

Data recorded in (Table 2) display the growth of parameters of presorted shoot tips after 3 months of storage on different mannitol concentrations. A decline in all tested growth parameters of shoot tips was observed with increasing mannitol concentrations in culture media. No significant difference was observed in regrown shoots heights after 3 months of storing (Table 2, Fig. 2). Furthermore, neither new shoots nor root were developed in mannitol stored microshoots at all levels (Table, 2). The reduction in growth in response to elevated levels of mannitol was also reported by Charoensub and Phansiri, (2004), as mannitol supplemented to the culture media at levels of (40 and 60 g/L) caused reduction in the stem height, and number of shoots per plantlet of *in vitro* conserved shoots of rose colored leadwort plantlets stored for 8 months. The regrowth percentage of prestored shoo tips was declined from (15% at 0.1 M to 0% at 0.4 M of mannitol (Fig. 3).

The severe decline in regrowth percentage was reported to be accompanied with adding elevated mannitol concentrations to the media during *in vitro* storage of *Achillea fragrantissima* microshoots (Younes, 2012). Also, Sharaf et al., (2012) who worked on *Artemisia hrba-alba,* and Moges, *et al*, (2004) who worked on African violet, reported a complete loss of recovery on media supplemented with 0.3 or 0.4 M of mannitol, which strongly agreed with our findings.

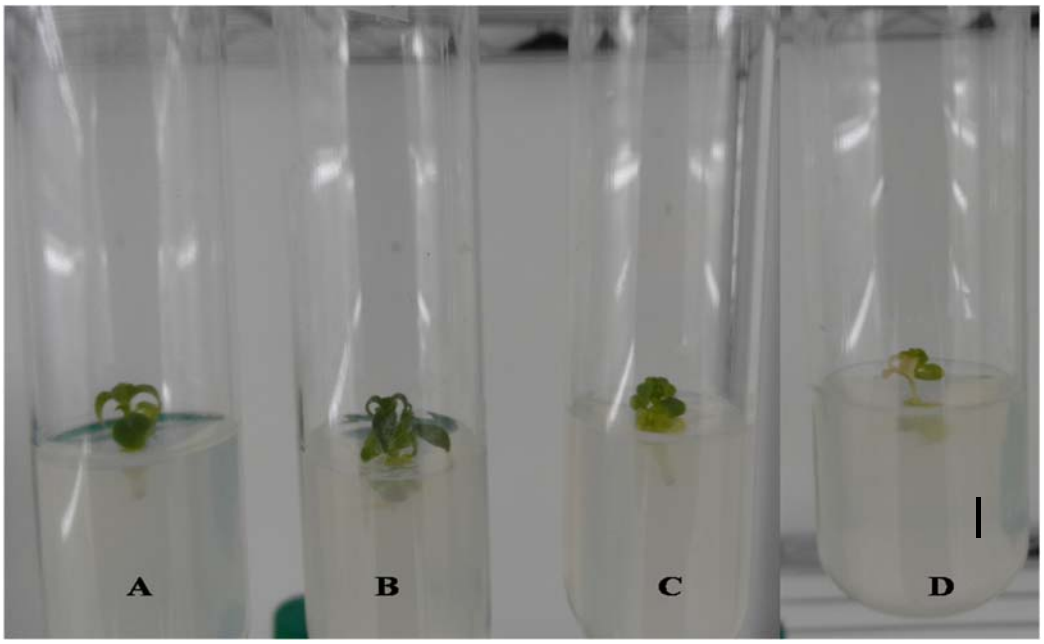

Figure 2: Effect of mannitol concentrations on shoot height of *Z. tenuior* microshoots after three months storage period. A: 0.1 M, B: 0.2 M, C: 0.3 M, D: 0.4 M). Bar represents 1.0 cm.

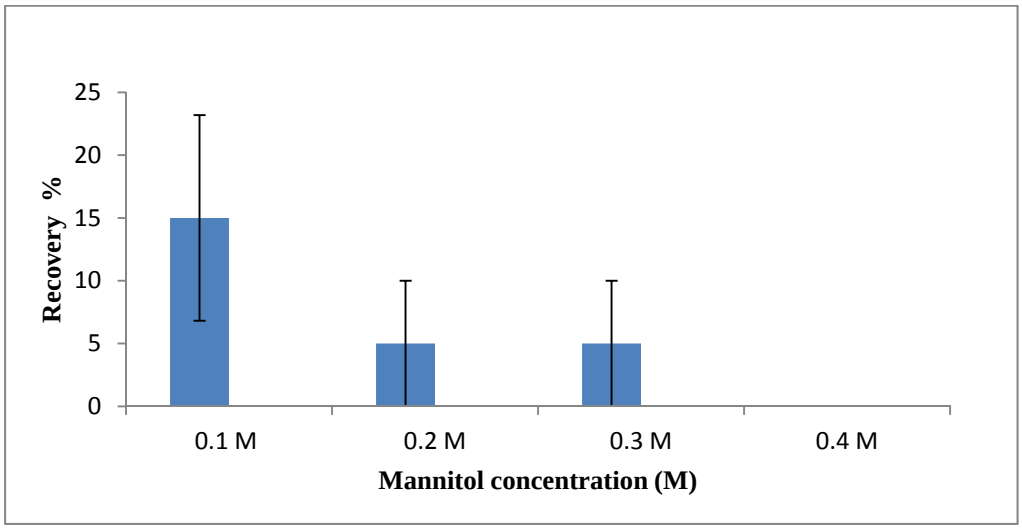

Figure 3: Effect of mannitol concentrations on recovery rate of *Z. tenuior* microshoots after three months storage period in slow growth media.

Table 2: Effects of Mannitol concentration on *in vitro* preserved *Z. tenuior* microshoots

Mannitol conc. (M)	Shoot Height (cm)	Number of new shoots	Rooting %
0.1	1.68 ±0.10 A[y]	0.0 ±0.0	0.0 ±0.0[z]
0.2	1.52 ±0.12 A	0.0 ±0.0	0.0 ±0.0
0.3	1.40 ±0.15 A	0.0 ±0.0	0.0 ±0.0
0.4	1.40 ±0.27 A	0.0 ±0.0	0.0 ±0.0

[y] Means within groups followed by same letter(s) are not significantly different according to Least Significant Difference (LSD) (*P = 0.05*).[z] Means tested by standard error of means.

3.1.3. Influence of sorbitol concentrations

A significant decline of shoot tips regrowth was obtained when the sorbitol level was increased up to 4.0 M in the media. Similarly, the regrowth of *Thymbra spicata var. spicata* microshoots was declined when they were stored in media with high sorbitol levels up to 0.4 M (Tahtamouni et al., 2016). On the other hand, increasing sorbitol level had improved shoot multiplication to reach maximum (3.0) at in microshoots prestored in 0 .2 M sorbitol, but this parameter decreased at higher sorbitol level (Table 3).

Moreover, high sorbitol levels mor than 0.2 M had affected number of the regrown shoot tips and rooting adversely (Table 3). This result agrees with Sharaf, *et al.*, (2012) on *Artemisia hrba-alba*, who reported some rooting occurred after 12 weeks on media supplemented with 0.1M sorbitol only. Meanwhile, recovery percentage declined with increasing of sorbitol concentrations beyond (0.2 M), from 35% to 0% at (0.4 M) sorbitol (Figure 4). Also, survival and recovery percentages of *Crocus moabiticus* and *Crocus hyemalis* calli were completely lost when they were incubated on media supplemented with 0.4 M sorbitol for twelve weeks (Baghdadi, *et al*., 2011).

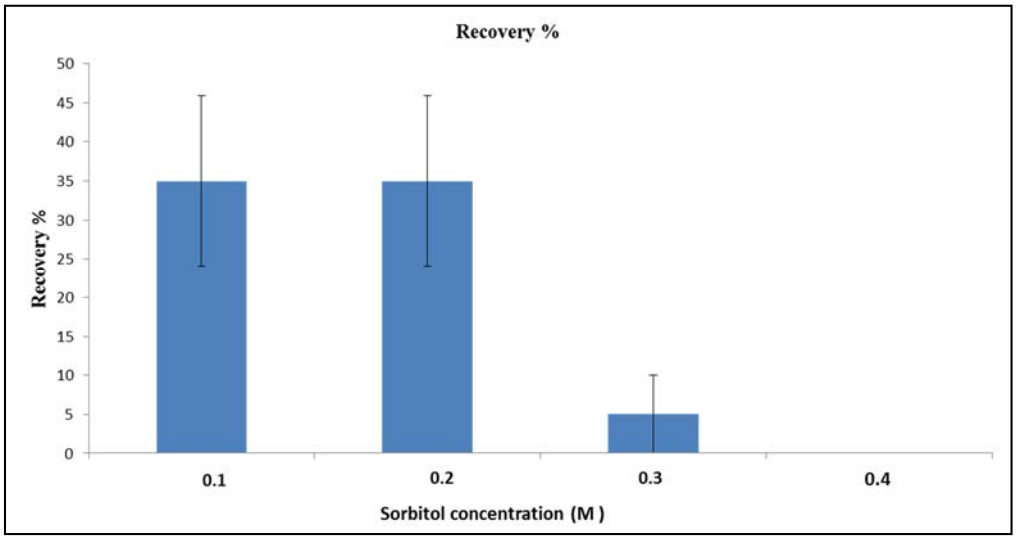

Figure 4: Effect of sorbitol concentrations on recovery rate of *Z. tenuior* L . microshoots after three months storage period in slow growth media.

Table 3: Effects of sorbitol concentration on *in vitro* preserved *Z. tenuior* microshoots

Sorbitol conc. (M)	Shoot Height (cm)	Number of shoots	Rooting %
0.1	1.68 ±0.05 A[y]	1.38 ±0.60 C	5 ±5[z]
0.2	1.71 ±0.05 A	3.00 ±0.54 A	30 ±10.51
0.3	1.52 ±0.09 AB	2.68 ±0.97 B	0 ±0
0.4	1.36 ±0.11 B	0 ±1.26 D	0 ±0

[y] Means within groups followed by same letter(s) are not significantly different according to Least Significant Difference (LSD) (*P = 0.05*).[z] Means tested by standard error of means ±.

3.1.4. Influence of ABA concentrations

The current study revealed that, application of ABA was very effective in reducing shoot height of the *in vitro* stored microshoots at all levels compared to the control (Table 4). All the ABA concentrations had similar effect on shoot height and number of shoots and lower than control treatment (without ABA). The minimum value for shoot height was (2.34 cm) recorded in microhoots stored in (11.4 µM) ABA. Meanwhile, microshoots response to ABA application was quite opposite in terms of number of new shoots, as there were no significant differences between values taken for the newly developed microshoots (number of shoots) in the control and ABA treated microshoots (Table 4). On the other hand, rooting decreased in ABA treated microshoots to reach 75% at (7.6 and 11.4 µM) ABA (Table 4). Meanwhile complete recovery percentages were obtained in all ABA prestored microshoots one month after being transferred to normal growth condition), which contrasted the results obtained by Tahtamouni *et al.,* (2016) reported that, adding ABA to the storage media had lethal effects on *Thymbra spicata* L. *in vitro* stored microshoots at all levels. ABA may be interact with *Thymbra spicata* plant contents of secondary metabolites cycle and this may be converted to lethal compounds which cause the death of plant.

Table 4: Effects of ABA concentration on *in vitro* stored *Z. tenuior* microshoots

ABA conc. (M)	Shoot Height (cm)	Number of Shoots	Rooting %
0 µM(control)*	8.82 ±0.24 A[y]	9.75 ±0.24 A	90 ±6.88[z]
3.8 µM	2.61 ±0.24 B	8.90 ±0.24 A	85 ±8.19
7.6µ M	2.57 ±0.24 B	8.10 ±0.24 A	75 ±9.93
11.4 µM	2.34 ±0.24 B	8.10 ±0.24 A	75±8.19

*Control represents hormone free MS media + 0.1 M sucrose. [y] Means within groups followed by same letter(s) are not significantly different according to Least Significant Difference (LSD) (*P = 0.05*).[z] Means tested by standard error of means ±.

3.2 Effect of in vitro slow growth storage on total oil yield and pulegone concentration

Our results showed that, the greatest oil yield (5 %) was obtained from *Z. tenuior*, samples which were collected from the wild. On the other hand, *Z. tenuior*, samples taken from the *in vitro* prestored microshoots yielded lower total oil yield compared to the wild samples. For example, the value of extracted total oil yield was (2 %) in microshoots prestored in 0.2 M sucrose, while it was (1.83 %) in microshoots prestored in 0.1 M sucrose (control) (Table 5). Moreover, (1.5%) was the total oil yield value extracted from 3.8 µM ABA

prestored microshoots (Table 5). Our results were in agreement with Bertoli, *et al*., (2012) who reported that the *in vitro* samples of *Mentha piperita* produced lower essential oil than of the parent plants in the field, which was due to high moisture content found in the *in vitro* grown samples. Also, our results were consistent with Al-Qudah, *et al*., (2011), found that oil yield extracted from wild *Teucrium polium L.* plants were higher than those extracted from *in vitro* samples.

Meanwhile, in the current study the maximum amount of total oil yield was extracted from microshoots prestored in elevated level of sucrose (0.2 M) (Table 5). Also, based on the standard calibration curve (Fig. 6), the current study showed that pulegone concentration was very low in the *in vitro* prestored microshoots compared to pulegone level found in wild plants (Table 5). This agreed with a previous study by Bertoli, *et al.,* (2012), who reported that the concentration was very low in the *in vitro* plants of *Mentha piperita,* and attributed their results to the transformation of pulegone precursor (limonene) into piperitenone instead of pulegone in the *in vitro* plant as a result to high moisture content in the plant cells.

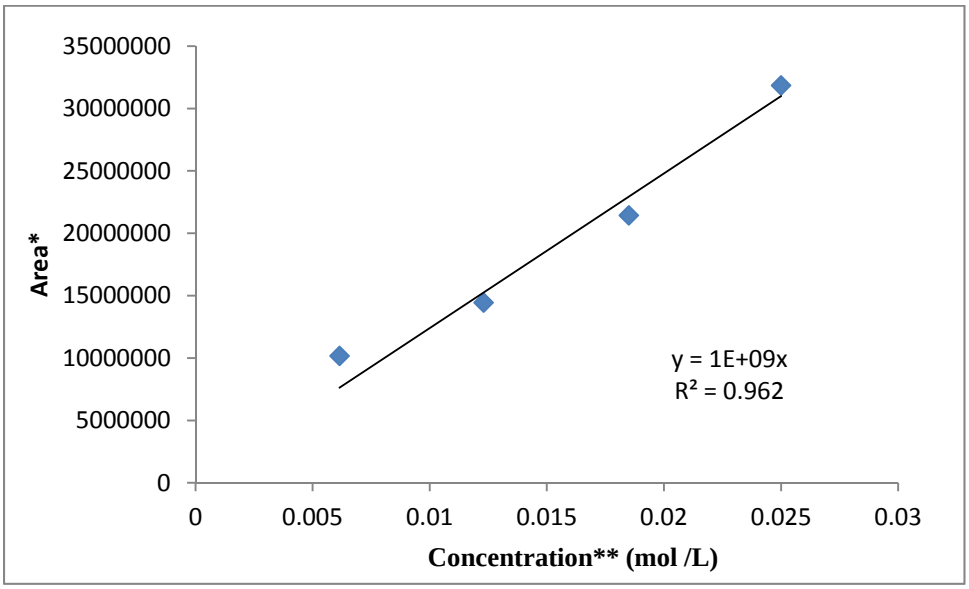

Figure 6: Standard calibration curve of Pulegone. * Area (X axis): area under the peak of the standard used; Concentration (Y axis): concentration of the standard used.

Table 5: Total oils yield and Pulegone concentrations of *Z. tenuior* L., microshoots as affected by slow growth conservation treatments.

Treatment	Total oil yield % (v/w)	Pulegone concentration (M)
Wild	5.00 ±0.74^{z}A^{y}	0.0312 ± 0.000632^{z} A^{y}
Tissue culture (0.1M sucrose)	1.83 ±0.60 B	0.0046 ±0.000632 B
Sucrose (0.2M)	2.00 ±0.60 B	0.0041 ±0.000632 B
ABA (3.8µM)	1.50 ±0.60 B	0.0045 ±0.000632 B

Means within groups followed by same letter(s) are not significantly different according to Least Significant Difference (LSD) (*P = 0.05*). z means tested by standard error of means ±.

Our study indicated that, slow growth storage of *Z. tenuior* L. is possible under *in vitro* conditions, using elevated levels of sugars or ABA. However, adding 0.2 M sucrose or 3.8 µM ABS to MS media resulted high microshoot storage in terms of reduction of growth and recovery rates after storage, but this was on the expense of total oil yield and pulegone concentrations which were lower than those extracted from the wild type plants.

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تقييم النمو وكمية المردود الزيت ومادة البوليغون في نبات النعنع البري (*Ziziphora tenuior* L.) بعد الحفظ المبطىء للنمو

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ملخص

يعتبر نبات النعنع البري (*Ziziphora tenuior* L.) من النباتات الطبية التي تم استخدامها لعلاج العديد من الأمراض بسبب وجود العديد من المكونات الطبية القيمة مثل، البوليغون. وقد تعرض هذا النبات القيم إلى خطر الانقراض من بيئته الطبيعية بسبب الجمع غير القانوني والتحضر العمراني والرعي الجائر والجفاف. في هذه الدراسة، تم تطبيق تقنية الحفظ البطيء داخل الأنابيب وذلك لدراسة إمكانية الحفاظ على سويقات نبات النعنع البري (*Ziziphora tenuior* L.) باستخدام مستويات عديدة من السكريات المختلفة أو من حمض الابسيسك اسيد (ABA). بالإضافة إلى ذلك تم دراسة تأثير الحفظ المبطيء للنمو على محتوى الزيت الكلي في النبات وتركيز مادة البوليغون في السويقات بعد تخزينه لمدة 3 أشهر. وأظهرت النتائج أن السويقات المخزنة في الوسط الغذائي MS مع (0.2 مولار) من السكروز أو حمض الابسيسك اسيد (ABA) كانت قادرة على تقليل جميع مقاييس النمو والحفاظ على استعادة نمو النبات بعد الحفظ. من ناحية أخرى، كان استخدام السوربيتول أو مانيتول له تأثير سلبي قوي على استعادة نمو النبات بعد الحفظ. وفي الوقت نفسه، كانت هناك اختلافات بين الكميات من إجمالي العائد من الزيت والبوليغون المستخرجة من سويقات النباتات داخل الأنابيب أو من نباتات البرية. تم الحصول على أقصى قدر من إجمالي عائد الزيت (5٪) وبوليغون (0.0312 م) من النباتات البرية. من ناحية أخرى، فإن كمية إجمالي محصول الزيت المستخرجة من النباتات داخل الأنابيب بعد الحفظ كانت ثلث كمية الزيت التي تم الحصول عليها من النباتات البرية. أيضا، كان تركيز بوليغون في العينات المجففة من سويقات النباتات داخل الأنابيب بعد الحفظ (0.0041–0.0046 مولار) و ذلك في جميع السويقات المحفوظة ولكنها كانت أقل بكثير من تلك التي تم الحصول عليها من عينات النباتات البرية.

الكلمات الدالة: نبات طبي، بوليغون، الحفظ متوسط الأمد ، إجمالي مردود الزيت، *Ziziphora tenuior* L.

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