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Full Length Research paper

Production of parthenolide in organ and callus cultures of *Tanacetum parthenium* (L.)

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The *in vitro* micropropagation of the seeds of *Tanacetum parthenium* (L.) Schultz-Bip. family Asteraceae was performed on half strength Murashige and Skoog (MS) medium containing 0.2% gibrellic acid. The explants were *in vitro* cultured on MS-medium using different plant growth regulators, culture media ingredients and carbon sources. Parthenolide content in the different established cultures was studied and compared with that present in the open field herb. Results revealed that using half strength MS-medium containing 50 g/l glucose or fructose as a carbon source and 0.5 mg/l benzyl aminopurine (BAP) resulted in significantly higher parthenolide content than that present in the open field herb. In addition, bud sprouting ability, number and length of shootlets and number of leaves of different explants under different treatments were studied. The *in vitro* callus formation was conducted on MS-medium using different plant growth regulators and culture media ingredients. Parthenolide was detected in callus culture for the first time in only two different hormonal treatments which were MS medium containing 0.5 mg/l BAP or 0.5 mg/l naphthalene acetic acid (NAA). In addition, callusing capacity and weight of callus of different explants under different treatments were also determined. Parthenolide content was determined in the explants as well as in callus using RP-HPLC on Luna C_{18} column and SPD-10A UV detector. The mobile phase used was acetonitrile: water (55 : 45) and the flow rate was 1.5 ml/min at the ambient temperature.

Key words: *Tanacetum parthenium,* plant growth regulators, culture media ingredients and carbon sources, parthenolide, HPLC, callus, micropropagation.

INTRODUCTION

Feverfew, *Tanacetum parthenium* (L.) Schultz-Bip. is one of the safest herbaceous members of the family Asteraceae, growing in Europe, has a long history of use as a herbal treatment for fever, migraine prophylaxis and arthritis due to presence of sesquiterpene lactones of which parthenolide was the main active constituent (Evans, 2002; Hobbs and Gram, 1989; Ross, 2001). The plant do not grow well under Egyptian environmental conditions since it needed full sun and temperature not exceeding 20°C. The conventional propagation methods for *T. parthenium* (L.) produced limited numbers under normal conditions in our country compared with *in vitro* culture. The rapidity and high quantity of produced plant by tissue culture and its acclimatization in Egyptian environmental conditions in a short time was our aim in this paper. Reviewing the current literature, it was found that all trials failed to make the callus of *T. parthenium* (L.) able to produce parthenolide (Banthorpe and Brown, 1989, 1990, 1993; Brown et al., 1993). So, the current study was achieved to study the effect of some factors on the behavior of the *in vitro* micropropagation, callus formation and productivity of parthenolide.

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EXPERIMENTAL

Plant material

Seeds of *T. parthenium* (L.) *Schultz-Bip.*, used in this study were imported from Schifeild Co., U.S.A., were divided into two groups, the first one was aseptically seeded into quarter strength Murashige and Skooge (MS) medium to produce *in vitro* seedlings and the se-

cond group was germinated under outside natural conditions to obtain open-field herbs at Beni-Sueif, Egypt.

Explants types

From the *in vitro* seedlings, the explants were aseptically excised and used to serve in the shootlets multiplication stage of the *in vitro* micropropagation and the *in vitro* callus formation trials. The used explants were shoot tip explants, leaf explants, nodal explants**,** hypocotyl explants, cotyledonary and root explant.

Material for phytochemical study

Different plant organs were separately air dried, powdered and kept in tightly closed amber colored glass containers and protected from light at low temperature.

Chemicals

Sucrose (SIGMA), glucose (SIGMA), fructose (SIGMA), starch (SIGMA), gebbrellic acid (GA_3) (SIGMA), naphthalene acetic acid (NAA) (SIGMA), benzyl aminopurine (BAP) (SIGMA), 2,4-dichlorophenoxy acetic acid (2,4-D) (SIGMA) and chemicals of MS medium (SIGMA) and reference sample of parthenolide (Aldrich, 97% pure) were used in the experiments.

Culture media

The basal MS-culture media used in this study was the formulation of Murashige and Skoog (1962).

Apparatus

Shimadzu class-LC 10 liquid chromatographic system equipped with Shimadzu SPD-10A UV detector and Phenomenex Luna® C_{18} column (25 cm x 4.6 mm internal diameter and 5 µm particle size) for HPLC determination of parthenolide using column type (Phenomenex® Luna 5u C_{18} column) with dimensions of 250 X 4.6 mm. and SPD-10A UV detector which was adjusted at λ_{max} of 214 nm. The mobile phase was Acetonitrile : deionized water (55:45) with flow rate of 1.5 ml/min, autoclave (Sterilimatic, Market Forge Co., U.S.A.), automatic media dispenser (Unispense, Wheaton Instruments, U.S.A.), laminar air flow (Clean-Ceil Fan Filter Module, double fan with HEPA filter, Canadian Cabinets Co. LTD., Canada), digital hot plate stirrer (Thermolyne, Branstead, U.S.A.), automatic shaker (Lab-Line Instruments, U.S.A.), digital balance (Denver Instruments, AC 400D, U.S.A.), pH meter (Corning Science Products, U.K.) incubation room which has the following conditions:

a) Room temperature**:** was adjusted 20 ± 2°C day/night cycle by "Power" air condition.
b) Photoperiod: automatically controlled by electronic timer to be 16 h light and 8 h darkness using day light florescent lamps (110 cm long – 40 w).
c) Light intensity**:** 3.5 k lux was measured by a luxmeter "Lt Lutron Lx- 101" instrument at the top of culture.

First experiment (*In vitro* micropropagation)

Effect of explants types and plant growth regulators (P.G.R.): In this trial, three types of explants (shoot tip, leaf nodal explants and cotyledonary nodal explants) were aseptically sectioned from *in vitro* seedlings. The explants of each type were plated into full strength MS-medium and provided with one of sixteen treatments of P.G.R. [benzyl aminopurine (BAP), naphthalene acetic acid (NAA) and their interactions].

Effect of explants types and culture media ingredient (C.M.I.): In this test, the same types of explants were excised from shootlets came from full strength MS-medium provided with BAP (0.5 mg/l) plus NAA (0.05 mg/l). Each type of explants was vertically inserted into one of four strengths of MS-medium.

Effect of explants types and carbon sources: In this test, the same types of explants were excised from shootlets cultured on full strength MS-medium provided with BAP (0.5 mg/l) plus NAA (0.05 mg/l). The explants from each type were cultured into full strength MS-medium provided with one of thirteen carbon sources. Two months after culturing, the parameters measured for shootlet multiplication behavior under the effect of explants types, P.G.R., C.M.I. and carbon sources were bud sprouting ability, shootlet number, shootlet length (in cm) and leaves number.

Second experiment:

***In vitro* callus formation:** In this experiment, the behavior of the *in vitro* callus formation was examined under the effect of explants types, P.G.R. and C.M.I. treatments. *In vitro* seedlings (2 ± 0.5 cm) resulting from *in vitro* seed germination stage were used as the source of different explants types for serving the *in vitro* callus formation experiment.

Effect of explants types and plant growth regulators: In this trial, the types of explants (shoot tip, node, leaves, cotyledons, hypocotyl and root) were aseptically excised from the *in vitro* seedlings. Each one of the six explants types was cultured on full strength MS-medium enriched with one of fifteen treatments of P.G.R. [2,4-dichlorophenoxy acetic acid (2,4-D), naphthalene acetic acid (NAA), benzyl aminopurine (BAP)].

Effect of explants types and culture media ingredients: In this trial, the same six types of explants were aseptically excised. Each one of the six explants types were cultured on one of three strengths of MS-media. After two months of culturing, the parameters recorded for the *in vitro* callus formation behavior under the effect of explants types, P.G.R. and C.M.I. treatments were callusing capacity (%), fresh weight of callus (in grams).

HPLC determination of parthenolide in the *in vitro* shoot and callus

Sample and stock solution preparation (Zhou et al., 1999): 1 g of the air-dried powdered organ was accurately weighed and introduced into a 120 ml bottle, 100 ml of acetonitrile : water (90:10) solution was added. Each bottle was subjected to sonication for 30 min for complete extraction of parthenolide. Each extract was filtered through 0.2 µm PTFE membrane filter before injection into the HPLC column. For tissue culture study, all explants used for formation of shoots and calli were taken from seeds germinated on MS media ¼ strength containing GA_3 (50 mg/L) which reported to induce germacrene, a precursor for parthenolide synthesis, in shoot and callus (Brown et al., 1993). One gram, of each treatment, of air dried powdered shoot or callus was subjected to the same extraction and analysis procedure in HPLC determination.

For the stock solution, 10 mg of parthenolide (Aldrich, 97% pure) was accurately weighed and dissolved in 10 ml volumetric flask using few ml of methanol and the volume was completed to the mark using methanol.

Table 1a. Effect of explants types, plant growth regulators and their interaction on leaves number and bud sprouting ability of explants *in vitro*.

P.G.R. treatment	Leaves number			Bud sprouting ability (%)		
	Cotyledonary explant	Shoot tip	Nodal explant	Cotyledonary explant	Shoot tip	Nodal explant
MS (control)	12.33^{a-f}	10.67^{a-f}	11.00^{a-f}	75.00^{ab}	75.00^{ab}	91.67^{ab}
0.5 mg/l BAP	10.33^{a-f}	11.33^{a-f}	10.67^{a-f}	73.67^{ab}	83.33^{ab}	70.00^{ab}
1.0 mg/l BA P	6.33^{f-h}	11.33^{a-f}	9.33^{b-f}	50.00^{bc}	91.67^{ab}	76.67^{ab}
2.0 mg/l BAP	11.33^{a-f}	9.33^{b-f}	11.00^{a-f}	86.67^{ab}	86.67^{ab}	90.00^{ab}
0.05 mg/l NAA	7.67^{d-h}	11.67^{a-f}	7.67^{d-h}	88.67^{ab}	85.00^{ab}	50.00^{bc}
0.1 mg/l NAA	5.33^{gh}	9.67^{b-f}	9.33^{b-f}	50.00^{bc}	90.00^{ab}	70.00^{ab}
0.2 mg/l NAA	9.00^{b-f}	12.00^{a-f}	12.67^{a-e}	55.33^{b}	80.00^{ab}	95.00^{ab}
0.5 mg/l BAP + 0.05 mg/l NAA	14.00^{a-c}	15.00^{ab}	16.00^{a}	96.37^{ab}	96.90^{ab}	96.74^{ab}
0.5 mg/l BAP + 0.1 mg/l NAA	10.67^{a-f}	12.33^{a-f}	12.33^{a-f}	61.00^{ab}	73.33^{ab}	76.67^{ab}
0.5 mg/l BAP + 0.2 mg/l NAA	11.33^{a-f}	12.67^{a-e}	8.33^{c-g}	83.33^{ab}	90.00^{ab}	90.00^{ab}
1.0 mg/l BAP + 0.05 mg/l NAA	12.33^{a-f}	13.67^{a-d}	12.67^{a-e}	86.67^{ab}	76.67^{ab}	85.00^{ab}
1.0 mg/l BAP + 0.1 mg/l NAA	8.67^{c-g}	10.33^{a-f}	8.00^{c-g}	41.67^{bc}	81.67^{ab}	81.67^{ab}
1.0 mg/l BAP + 0.2 mg/l NAA	5.67^{gh}	10.33^{a-f}	8.67^{c-g}	25.00^{bc}	88.33^{ab}	93.33^{ab}
2.0 mg/l BAP + 0.05 mg/l NAA	11.00^{a-f}	8.33^{c-g}	6.67^{e-h}	100.00^{a}	80.00^{ab}	83.33^{ab}
2.0 mg/l BAP + 0.1 mg/l NAA	6.33^{f-h}	7.00^{e-h}	11.67^{a-f}	33.33^{bc}	95.00^{ab}	83.33^{ab}
2.0 mg/l BAP + 0.2 mg/l NAA	4.00^{h}	8.00^{c-g}	9.33^{b-f}	41.67^{bc}	88.33^{ab}	68.67^{ab}
LSD 5% **A** Type of explants		1.54			10.46	
B Media strength		3.56			24.16	
A x B Interactions		6.17			41.8	

*(a,b,c,d,e,f) means with the same letters in each column are not significantly different at $p < 0.05$.
*LSD (least significant difference between means) calculated by one way ANOVA.

Construction of calibration curve: Different aliquots of the stock parthenolide solution equivalent to 0.2 to 250 ug parthenolide, were separately injected into HPLC column. Parthenolide found to appear at R_t 5.97 - 6.01 min under the specified conditions. Three replicates were performed for each spot, extraction and injection and the mean values of the absorbance or the peak areas corresponding to the concentration of parthenolide used were compiled. A calibration curve was plotted (the peak area versus the concentration used). The regression equation was computed for calculating the concentration of parthenolide. A linear relationship was obtained by plotting the recorded absorbance or peak area versus the concentration used of the authentic parthenolide. The regression equation for calculating parthenolide concentration in different plant organs was:

$\mathbf{A} = 0.0394\ \mathbf{C} + 0.0162 \quad \mathbf{r} = 0.99994$

Where "A" is the absorbance at λ_{max} 214 nm expressed in term of area under peaks, "C" is the respective concentration expressed in ug/ml and "r" is the correlation coefficient (Steel and Torrie, 1980).

The concentration of parthenolide was determined from the absorbance or peak area in the previous equation and the results were transformed to be in form of mg % (w/dry weight of each plant organ).

Determination of parthenolide: 10 µl of plant extract was analyzed adopting the same procedure as in calibration curve, then the peak area was determined by UV detector. The explants resulting from the effect of different plant growth regulators, culture media ingredients and carbon source treatments were compared with the open field herb (O.F.H.) for the amount of parthenolide produced. The *in vitro* callus tissues growing under the effect of different plant growth regulators and culture media ingredients treatments were applied for HPLC investigation of the presence of parthenolide and determination of its content.

Statistical analysis

The data of the two series of the experiments were averaged and statistically submitted to the analysis of variance. The data of the *in vitro* shootlets multiplication stage in the first experiment and the *in vitro* callus formation behavior in the second experiment were subjected to statistical analysis of completely randomized design (two factors). The least significant differences (LSD) test was applied for comparison among means according to method described by Steel and Torrie (1980).

The calibration curves found to have linearity of r = 0.99994, the relative standard deviation (RDS) found to be 0.098 % (n = 7) and the spike recovery (recovery %) found to be 99.4 %.

RESULTS AND DISCUSSION

In vitro micropropagation

Effect of explants types and plant growth regulators (P.G.R.): The effect of explants types and plant growth

Figure 1. Effect of explants types, plant growth regulators and their interaction on shootlet multiplication behavior.

Figure 2. Effect of explants types, culture media ingredients and their interaction on shootlet multiplication behavior

regulators is shown in Figure 1. A significant difference on bud sprouting ability of explants was found due to the effect of explants types tested (Table 1a). The data revealed that the shoot tips explants sprouted at a rate of (85.10%) which was significantly different from the rates of other explants. Regarding the effect of various P.G.R., it was found that the highest value (96.67%) of bud sprouting ability was recorded when the explants were cultured on 0.5 mg/l BAP + 0.05 mg/l NAA.

A significant difference on shootlets number of explants was found due to the effect of different explants types tested (Table 1b). The data revealed that the nodal explants and shoot tips had the highest shootlets numbers (1.98 and 1.81, respectively) which were significantly different from the cotyledonary explants. Regarding the effect of various P.G.R., it was found that the highest value (2.89) of shootlets number was recorded when the explants were cultured on 0.5 mg/l BAP + 0.05 mg/l NAA.

A significant difference on shootlets length of explants was found due to the effect of different explants types tested (Table 1b). The data revealed that the shoot tips and nodal explants had the tallest shootlets (1.97 and 2.02 cm, respectively) which were significantly different from the length of cotyledonary explants. Regarding the effect of various P.G.R., it was found that the highest value (4.64 cm) of shootlets length was recorded when the explants were cultured on 0.5 mg/l BAP + 0.05 mg/l NAA. A significant variation in leaves number of explants was found due to the effect of different explants types tested (Table 1a). The data revealed that the shoot tips effect of various P.G.R., it was found that the highest value (15.00) of leaves number was recorded when the explants were cultured on 0.5 mg/l BAP + 0.05 mg/l NAA. All the previous results were in agreement with previous data (Elena et al., 1996; Cervilli, 1987; Castillon and Cornish, 2000). Thus the MS medium containing 0.5 mg/l BAP + 0.05 mg/l NAA was the most favorable medium for inducing the highest number of leaves *in vitro*.

Effect of explants types and culture media ingredients (C.M.I.): The effect of explants types and culture media ingredients is shown in Figure 2. Data presented in Table 2a showed that the various C.M.I. tested and their interaction with the different explants types caused a significant effect on bud sprouting ability of the explants. However, explants types also had non significant influence. Regarding the effect of various C.M.I., it was found that the peak value (100%) of bud sprouting ability was recorded when the explants were cultured on half strength MS medium.

A significant difference on shootlets number of explants was found due to the effect of different explants types tested (Table 2a). The data revealed that the nodal explants had the highest shootlets number (3.08) which was significantly different from other explants. Regarding the effect of various C.M.I., it was found that the highest value (3.44) of shootlets number was recorded when the explants were cultured on half strength MS medium.

Results shown in Table 2b showed that the various C.M.I. tested and their interaction with the different explants types caused a significant effect on shootlets length. However, explants types had also non significant influence. Regarding the effect of various C.M.I., it was found that the highest value (2.13 cm) of shootlets length was recorded when the explants were cultured on half strength MS.

Data presented in Table 2b showed that the various C.M.I. tested and their interaction with the different explants types caused a significant effect on leaves number. However, the different explants types had also non significant influence. Regarding the effect of various C.M.I., it was found that the highest value (18.22) of leaves number was recorded when the explants were cultured on half strength MS medium. All the mentioned results were achieved by previous researchers (Pereira-Pinto et al., 1996; Mantell et al., 1998).

Effect of explants types and carbon source (C.S.): The effect of explants types and carbon source is shown in Figure 3. A significant difference on bud sprouting ability of explants was found due to the effect of different explants types tested (Table 3a). The data revealed that the shoot tips and nodal explants sprouted at rate of (94.23% and 92.31%, respectively) which was significantly different from the cotyledonary explants. Regard-

Table 1b. Effect of explants types, plant growth regulators and their interaction on shootlets number and length of explants *in vitro*.

P.G.R. treatment	Shootlets number			Shootelts length (cm)		
	Cotyledonary explant	Shoot tip	Nodal explant	Cotyledonary explant	Shoot tip	Nodal explant
MS (control)	1.00^{cd}	1.00^{cd}	1.00^{cd}	1.83^{i-l}	3.07^{c-g}	3.20^{c-f}
0.5 mg/l BAP	1.67^{bc}	1.33^{c}	1.67^{bc}	1.25^{j-l}	1.17^{j-l}	1.37^{i-l}
1.0 mg/l BA P	1.00^{cd}	2.00^{b}	2.00^{b}	0.60^{m}	1.30^{j-l}	1.17^{i-l}
2.0 mg/l BAP	1.67^{bc}	1.33^{c}	2.67^{ab}	0.93^{lm}	0.80^{lm}	1.77^{i-l}
0.05 mg/l NAA	1.67^{bc}	1.00^{cd}	1.33^{c}	1.47^{j-l}	3.97^{bc}	1.90^{h-l}
0.1 mg/l NAA	1.67^{bc}	1.00^{cd}	1.67^{bc}	1.30^{j-l}	3.63^{c-e}	2.97^{c-h}
0.2 mg/l NAA	1.00^{cd}	2.33^{ab}	2.67^{ab}	1.10^{j-l}	1.80^{i-l}	2.67^{e-i}
0.5 mg/l BAP + 0.05 mg/l NAA	3.33^{a}	2.33^{ab}	3.00^{ab}	3.80^{b-d}	5.33^{a}	4.80^{ab}
0.5 mg/l BAP + 0.1 mg/l NAA	1.33^{c}	2.33^{ab}	2.00^{b}	1.63^{i-l}	1.43^{j-l}	2.17^{f-j}
0.5 mg/l BAP + 0.2 mg/l NAA	1.67^{bc}	2.67^{ab}	1.33^{c}	1.27^{j-l}	1.90^{h-l}	1.33^{j-l}
1.0 mg/l BAP + 0.05 mg/l NAA	1.67^{bc}	2.33^{ab}	2.00^{b}	1.60^{i-l}	2.07^{g-k}	1.73^{i-l}
1.0 mg/l BAP + 0.1 mg/l NAA	1.33^{c}	2.33^{ab}	1.67^{bc}	0.97^{kl}	1.43^{j-l}	1.37^{j-l}
1.0 mg/l BAP + 0.2 mg/l NAA	1.00^{cd}	2.00^{b}	2.33^{ab}	0.37^{m}	1.17^{j-l}	1.90^{h-l}
2.0 mg/l BAP + 0.05 mg/l NAA	1.33^{c}	0.67^{d}	1.00^{cd}	0.83^{lm}	0.53^{m}	0.93^{lm}
2.0 mg/l BAP + 0.1 mg/l NAA	1.00^{cd}	1.33^{c}	2.67^{ab}	0.50^{m}	0.67^{m}	1.50^{j-l}
2.0 mg/l BAP + 0.2 mg/l NAA	1.00^{cd}	3.00^{ab}	2.67^{ab}	0.53^{m}	1.23^{j-l}	1.60^{i-l}
LSD 5% **A** Type of explants	**0.31**			**0.28**		
B Media strength	**0.72**			**0.65**		
A x B Interactions	**1.25**			**1.128**		

*(a,b,c,d,e,f) means with the same letters in each column are not significantly different at $p < 0.05$.
*LSD (least significant difference between means) calculated by one way ANOVA.

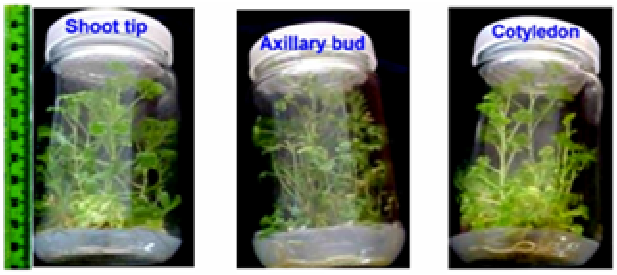

Figure 3. Effect of explants types, carbon source and their interact-tion on shootlet multiplication behavior.

ing the effect of various C.S., it was found that the peak value (100%) of bud sprouting ability was recorded when the explants were cultured on 12.5 g/l glucose, sucrose or starch.

A significant difference on shootlets number of explants was found due to the effect of different explants types tested (Table 3b). The data revealed that the nodal explants and shoot tips had the highest shootlets numbers (3.23 and 2.97 respectively) which were significantly different from the cotyledonary explants. Regarding the effect of various C.S., it was found that the highest values (5.11 and 4.67) of shootlets number were recorded when the explants were cultured on 12.5 g/l glucose or sucrose, respectively.

A significant difference on shootlets length of explants was found due to the effect of different explants types tested (Table 3b). The data revealed that the shoot tips and nodal explants had the tallest shootlets (1.66 and 1.6 cm, respectively) which were significantly different from the length of cotyledonary explants. Concerning the effect of various C.S., it was found that the highest values (2.94 and 2.90 cm) of shootlets length were recorded when the explants were cultured on 12.5g/l glucose or sucrose, respectively.

A significant variation in leaves number of explants was found due to the effect of different explants types tested (Table 3a). The data revealed that the shoot tips had the highest leaves number (8.18) which was significantly different from other explants. Regarding the effect of various C.S., it was found that the highest value (12.67) of leaves number was recorded when the explants were cultured on 12.5 g/l glucose. Similar results have also been reported (Ya and Reed, 1990; Ihsanul et al., 1998).

In vitro callus formation

In this part of experiments, the callus formation behavior of the explants *in vitro* were determined under the effect of different types of explants (i.e. leaves, roots, cotyle-

Table 2a. Effect of explants types, culture media ingredient and their interaction on shootlets multiplication behavior of explants *in vitro*.

Media treatment	Bud sprouting ability (%)			Shootlets number		
	Cotyledonary explant	Shoot tip	Nodal explant	Cotyledonary explant	Shoot tip	Nodal explant
MS full strength	55.33^{ab}	58.33^{ab}	38.67^{b}	1.00^{d}	2.33^{c}	2.67^{bc}
MS three quarter strength	75.00^{ab}	80.33^{ab}	44.33^{b}	2.33^{c}	2.33^{c}	2.67^{bc}
MS half strength	100.00^{a}	100.00^{a}	100.00^{a}	2.33^{c}	3.67^{ab}	4.33^{a}
MS quarter strength	49.67^{b}	57.00^{ab}	58.33^{ab}	2.00^{cd}	1.67^{cd}	2.67^{bc}
LSD 5% A Type of explants	**N. S.**			**0.588**		
B Media strength	**26.68**			**0.674**		
A x B Interactions	**46.21**			**1.167**		

*(a,b,c,d,e) means with the same letters in each column are not significantly different at $p < 0.05$.
*LSD (least significant difference between means) calculated by one way ANOVA.

Table 2b. Effect of explants types, culture media ingredient and their interaction on shoot lets length and leaves number of explants *in vitro*.

Media treatments	Leaves number			Shootlets length (cm)		
	Cotyledonary explant	Shoot tip	Nodal explant	Cotyledonary explant	Shoot tip	Nodal explant
MS full strength	9.00^{b-d}	12.00^{bc}	12.67^{b}	1.10^{c-e}	1.80^{ab}	1.53^{a-c}
MS three quarter strength	7.00^{cd}	9.00^{b-d}	8.67^{b-d}	1.23^{b-d}	1.63^{a-c}	1.67^{a-c}
MS half strength	18.00^{a}	18.67^{a}	18.00^{a}	2.10^{a}	2.17^{a}	2.13^{a}
MS quarter strength	4.33^{d}	5.00^{d}	6.33^{d}	0.47^{e}	0.57^{de}	0.80^{de}
***LSD 5% A** Type of explants	**N. S.**			**N. S.**		
B Media strength	**3.021**			**0.382**		
A x B Interactions	**5.233**			**0.662**		

*(a,b,c,d,e) means with the same letters in each column are not significantly different at $p < 0.05$.
*LSD (least significant difference between means) calculated by one way ANOVA.

Table 3a. Effect of explants types, carbon sources and their interaction on leaves number and bud sprouting ability of explants *in vitro*.

Carbon treatment	Leaves number			Bud sprouting ability (%)		
	Cotyledonary explant	Shoot tip	Nodal explant	Cotyledonary explant	Shoot tip	Nodal explant
MS (control)	2.33^{h}	4.33^{h}	3.33^{h}	50.00^{b}	91.67^{ab}	91.67^{ab}
12.5 g/l Glucose	12.00^{a-d}	12.67^{a-c}	13.33^{a}	100.00^{a}	100.00^{a}	100.00^{a}
25 g/l Glucose	9.33^{b-g}	8.33^{d-h}	6.67^{f-h}	91.67^{ab}	100.00^{a}	91.67^{ab}
50 g/l Glucose	6.67^{f-h}	5.00^{gh}	7.33^{e-h}	83.33^{ab}	83.33^{ab}	91.67^{ab}
12.5 g/l Fructose	5.00^{gh}	9.67^{b-g}	9.00^{c-h}	66.67^{ab}	91.67^{ab}	100.00^{a}
25 g/l Fructose	11.67^{a-e}	10.67^{a-f}	11.00^{a-f}	91.67^{ab}	91.67^{ab}	91.67^{ab}
50 g/l Fructose	3.33^{h}	6.33^{f-h}	3.00^{h}	58.33^{b}	75.00^{ab}	66.67^{ab}
12.5 g/l Sucrose	11.33^{a-f}	13.00^{ab}	11.00^{a-f}	100.00^{a}	100.00^{a}	100.00^{a}
25 g/l Sucrose	12.00^{a-d}	12.00^{a-d}	8.00^{d-h}	55.33^{b}	100.00^{a}	100.00^{a}
50 g/l Sucrose	8.33^{d-h}	9.00^{c-h}	6.00^{f-h}	100.00^{a}	100.00^{a}	83.33^{ab}
12.5 g/l Starch	3.33^{h}	5.67^{f-h}	5.67^{f-h}	100.00^{a}	100.00^{a}	100.00^{a}
25 g/l Starch	4.00^{h}	5.00^{gh}	4.00^{h}	83.33^{ab}	91.67^{ab}	83.33^{ab}
50 g/l Starch	4.00^{h}	4.67^{h}	4.00^{h}	83.33^{ab}	100.00^{a}	100.00^{a}
LSD 5 A Type of explants	**0.841**			**9.71**		
B Media strength	**1.943**			**22.49**		
A x B Interactions	**3.366**			**38.96**		

*(a,b,c,d,e,f,g,h,i) means with the same letters in each column are not significantly different at $p < 0.05$.
*LSD (least significant difference between means) calculated by one way ANOVA.

Table 3b. Effect of explants types, carbon sources and their interaction on shootlets number and length of explants *in vitro*.

Carbon treatment	Shootlets number			Shootlets length (cm)		
	Cotyledonary explant	Shoot tip	Nodal explant	Cotyledonary explant	Shoot tip	Nodal explant
MS (control)	1.33^{i}	2.33^{f-i}	1.67^{hi}	0.40^{h}	0.60^{h}	0.33^{h}
12.5 g/l Glucose	5.00^{ab}	4.33^{b-d}	6.00^{a}	2.97^{ab}	2.63^{a-e}	3.23^{a}
25 g/l Glucose	3.33^{c-f}	3.67^{b-e}	3.33^{c-f}	2.50^{a-f}	2.00^{c-h}	1.67^{e-h}
50 g/l Glucose	2.33^{f-i}	2.33^{f-i}	3.00^{d-g}	1.13^{gh}	0.90^{gh}	0.90^{gh}
12.5 g/l Fructose	2.00^{g-i}	2.67^{e-h}	3.67^{b-e}	1.10^{gh}	1.37^{f-h}	2.20^{b-g}
25 g/l Fructose	3.00^{d-g}	3.33^{c-f}	4.00^{b-e}	1.73^{e-h}	3.07^{ab}	2.63^{a-e}
50 g/l Fructose	1.33^{i}	2.33^{f-i}	1.67^{hi}	0.50^{h}	1.30^{f-h}	0.57^{h}
12.5 g/l Sucrose	4.33^{b-d}	4.67^{ab}	5.00^{ab}	2.80^{a-c}	3.23^{a}	2.67^{a-d}
25 g/l Sucrose	1.67^{hi}	2.33^{f-i}	2.67^{e-h}	1.80^{d-h}	1.80^{d-h}	2.57^{a-f}
50 g/l Sucrose	3.00^{d-g}	3.67^{b-e}	3.33^{c-f}	1.57^{f-h}	2.37^{a-g}	2.47^{a-g}
12.5 g/l Starch	1.00^{i}	2.33^{f-i}	2.33^{f-i}	0.27^{h}	0.87^{gh}	0.50^{h}
25 g/l Starch	1.67^{hi}	1.67^{hi}	2.33^{f-i}	0.33^{h}	0.70^{h}	0.40^{h}
50 g/l Starch	2.00^{g-i}	3.00^{d-g}	3.00^{d-g}	0.27^{h}	0.80^{gh}	0.60^{h}
LSD 5 A Type of explants	**0.365**			**0.234**		
B Media strength	**0.843**			**0.542**		
A x B Interactions	**1.461**			**0.939**		

*(a,b,c,d,e,f,g,h,i) means with the same letters in each column are not significantly different at $p < 0.05$.
*LSD (least significant difference between means) calculated by one way ANOVA.

Table 4. Effect of explants types, plant growth regulators and their interaction on callusing capacity (%) of explants *in vitro*.

P.G.R. treatment	Leaves	Root	Cotyledon	Hypocotyl	Node	Shoot tip
0.0 (control)	---	0.00^{e}	---	---	90.00^{a-c}	11.00^{e}
0.5 mg/l 2,4-D	56.67^{c-e}	50.00^{c-e}	77.33^{a-d}	63.33^{a-d}	80.00^{a-d}	50.00^{c-e}
1.0 mg/l 2,4-D	76.67^{a-d}	43.33^{de}	36.67^{e}	60.00^{b-d}	60.00^{b-d}	53.33^{c-e}
0.5 mg/l NAA	80.00^{a-d}	73.33^{a-d}	40.00^{de}	70.00^{a-d}	73.33a-d	60.00^{b-d}
1.0 mg/l NAA	86.67^{a-d}	22.00^{e}	46.67^{de}	80.00^{a-d}	60.00^{b-d}	60.00^{b-d}
0.5 mg/l BAP	3.33^{e}	88.67^{a-c}	86.67^{a-d}	22.00^{e}	76.67^{a-d}	70.00^{a-d}
1.0 mg/l BAP	17.00^{e}	70.00^{a-d}	16.67^{e}	44.00^{de}	80.00^{a-d}	76.67^{a-d}
0.5 mg/l 2,4-D + 0.5 mg/l BAP	73.33^{a-d}	70.00^{a-d}	83.33^{a-d}	66.67^{a-d}	76.67^{a-d}	73.33^{a-d}
0.5 mg/l 2,4-D + 1.0 mg/l BAP	66.67^{a-d}	83.33^{a-d}	53.33^{c-e}	66.67^{a-d}	93.33^{ab}	86.67^{a-d}
1.0 mg/l 2,4-D + 0.5 mg/l BAP	70.00^{a-d}	80.00^{a-d}	83.33^{a-d}	88.67^{a-c}	85.33^{a-d}	85.33^{a-d}
1.0 mg/l 2,4-D + 1.0 mg/l BAP	55.33^{c-e}	56.67^{c-e}	33.33^{e}	66.67^{a-d}	60.00^{b-d}	66.67^{a-d}
0.5 mg/l NAA + 0.5 mg/l BAP	53.33^{c-e}	80.00^{a-d}	80.00^{a-d}	66.67^{a-d}	60.00^{b-d}	70.00^{a-d}
0.5 mg/l NAA + 1.0 mg/l BAP	73.67^{a-d}	46.67^{de}	50.00^{c-e}	88.67^{a-c}	78.67^{a-d}	75.33^{a-d}
1.0 mg/l NAA + 0.5 mg/l BAP	100.00^{a}	46.67^{de}	63.33^{a-d}	83.33^{a-d}	93.33^{ab}	85.33^{a-d}
1.0 mg/l NAA + 1.0 mg/l BAP	50.00^{c-e}	100.00^{a}	100.00^{a}	100.00^{a}	100.00^{a}	100.00^{a}
LSD 5% **A** Type of explants	**13.51**					
B Media strength	**21.17**					
A x B Interactions	**38.41**					

*(a,b,c,d,e) means with the same letters in each column are not significantly different at $p < 0.05$.
*LSD (least significant difference between means) calculated by one way ANOVA.

dons, hypocotyls, nodes and shoot tips), various P.G.R. (that is, auxins of 2,4-D and NAA and cytokynins of BAP alone or in combinations in different concentrations) and several C.M.I. (that is, differentstrengths of macro- and micro-elements of MS medium).

Effect of explants types and plant growth regulators: The effect of explants types and plant growth regulators is shown in Figure 4. Data presented in Table 4 showed that the various explants types, the different P.G.R. and their interaction caused significant influences on callusing

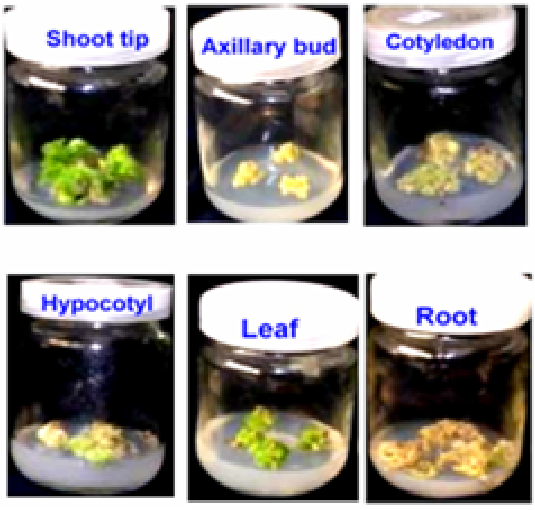

Figure 4. Effect of explants types, plant growth regulators and their interaction on callus formation *in vitro*

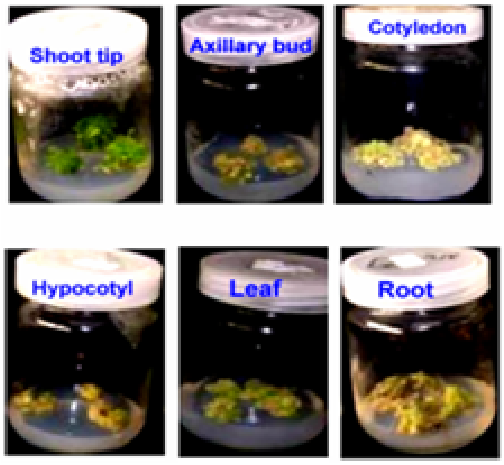

Figure 5. Effect of explants types, culture media ingredients and their interaction on callus formation *in vitro*

capacity of the explants. Taking into account the effect of P.G.R., introducing the combination of 1 mg/l NAA + 1 mg/l BAP into the culture medium induced the explants to form callus tissues in the highest rate (91.67%). These results indicate that the most promising P.G.R. which allowed the explants to form callus in the maximum rate was the combination form between high 1 mg/l NAA + high 1 mg/l BAP concentration and this might be due to the highest physiological effect of such combination on callus formation.

Relating the influence of explant types and P.G.R. interaction, the peak callusing capacity (100%) was recorded when the leaves explants are cultured on medium provided with combination of 1 mg/l NAA + 0.5 mg/l BAP and when the roots, cotyledons, hypocotyls, nodes and shoot tips explants are incubated on medium enriched with combination of 1 mg/l NAA + 1 mg/l BAP.

These results proved that the medium supplemented with combination of high (1 mg/l) NAA + low (0.5 mg/l) BAP concentration for leaves explants and the medium provided with combination of high (1 mg/l) NAA + high (1 mg/l) BAP concentration for root, cotyledon, hypocotyls, node and shoot tip explants were the most suitable ones for inducing the callus formation in the maximum rates.

The data illustrated in Table 5 indicate that the fresh weight of callus tissues was significantly affected by different types of explants, various P.G.R. and their interaction. Regarding the effect of different P.G.R., using 1 mg/l NAA + 1 mg/l BAP resulted in the heaviest callus fresh weight (1.11 g). From the obtained results, it could be affirmed that the combination of high (1 mg/l) concentration of NAA plus BAP was the most favorable treatment for producing the heaviest fresh weight of callus tissue and this might be due to that such combination of high concentration of auxin NAA plus cytokinin BAP had a pronounced effect on increasing the growth of fresh callus in term of weight. The interaction effect of explants types and P.G.R. showed that callus tissues initiated from shoot tips explants grown on medium enriched with combination of 1 mg/l NAA + 1 mg/l BAP exhibited the heaviest fresh weight (1.74 g). This means that the maximum value of fresh weight of callus could be obtained when the shoot tip explants are cultured on MS medium supplemented with combination of high concentration (1 mg/l) of NAA plus BAP. All results agree with the previous data (Stojakowska and Kisiel, 1997; George and Sherrington, 1984).

Effect of explants types and culture media ingredients: The effect of explants types and culture media ingredients is shown in Figure 5. Data presented in Table 6 shows that the different explants types tested and their interaction with the C.M.I. caused a significant effect on callusing capacity of the explants. However, the various C.M.I. had non significant influence. Regarding the effect of explants types and C.M.I. interaction, the explants of leaves, roots, nodes and shoot tips planted on all the tested MS media strengths showed the peak capacity (100%) for producing callus tissues. In case of hypocotyls explants, planting on one and half strength of MS medium produced the highest callusing capacity (100%). From these results, it could be concluded that the highest callusing capacity (100%) of leaves, roots, nodes and shoot tips explants were obtained when all the C.M.I. are applied, while the cotyledons explants inserted into half strength of MS medium and hypocotyls explants cultured on one and half and full strength of MS medium produced the maximum callusing rates (from 83.30 to 100%). Such high callusing capacity resulting from these treatments might be interpreted that the specialized cells of explants tissues had maximum ability to change easily into unspecialized parenchymatous cells.

Table 5. Effect of explants types, plant growth regulators and their interaction on callus fresh weight (g) of explants *in vitro*.

P.G.R. treatments	Leaves	Root	Cotyledon	Hypocotyl	Node	Shoot tip
0.0 (control)	---	---	---	---	0.10^{n}	0.03^{n}
0.5 mg/l 2,4-D	0.61^{gh}	0.12^{n}	0.60^{g-i}	0.87^{ce}	0.40^{j-l}	0.18^{m}
1.0 mg/l 2,4-D	0.10^{n}	0.39^{k-m}	0.21^{lm}	0.42^{j-l}	0.52^{h-j}	0.41^{j-l}
0.5 mg/l NAA	0.10^{n}	0.97^{b-d}	0.11^{n}	0.34^{k-m}	0.15^{m}	0.16^{m}
1.0 mg/l NAA	0.25^{lm}	0.17^{m}	0.17^{m}	0.58^{g-i}	0.32^{k-m}	0.19^{m}
0.5 mg/l BAP	0.002^{n}	0.34^{k-m}	0.24^{lm}	0.12^{n}	0.17^{m}	0.09^{n}
1.0 mg/l BAP	0.002^{n}	0.06^{n}	0.07^{n}	0.11^{n}	0.04^{n}	0.07^{n}
0.5 mg/l 2,4-D + 0.5 mg/l BAP	0.61^{gh}	0.23^{lm}	0.42^{j-l}	0.24^{lm}	0.22^{lm}	0.40^{j-l}
0.5 mg/l 2,4-D + 1.0 mg/l BAP	0.33^{k-m}	0.37^{k-m}	0.16^{m}	0.08^{n}	0.05^{n}	0.32^{k-m}
1.0 mg/l 2,4-D + 0.5 mg/l BAP	0.30^{k-m}	0.37^{k-m}	0.17^{m}	0.22^{lm}	0.19^{m}	0.31^{k-m}
1.0 mg/l 2,4-D + 1.0 mg/l BAP	0.05^{n}	0.50^{i-k}	0.02^{n}	0.12^{n}	0.06^{n}	0.22^{lm}
0.5 mg/l NAA + 0.5 mg/l BAP	0.35^{k-m}	0.30^{k-m}	0.29^{lm}	0.12^{n}	0.26^{lm}	0.23^{lm}
0.5 mg/l NAA + 1.0 mg/l BAP	0.16^{m}	0.56^{hi}	0.07^{n}	0.70^{f-h}	0.50^{i-k}	0.47^{j-l}
1.0 mg/l NAA + 0.5 mg/l BAP	0.29^{lm}	0.75^{ef}	0.50^{i-k}	0.74^{e-g}	0.29^{lm}	0.54^{h-j}
1.0 mg/l NAA + 1.0 mg/l BAP	0.85^{de}	0.96^{b-d}	1.00^{bc}	1.05^{b}	1.05^{b}	1.74^{a}
LSD 5 % **A** Type of explants	**0.049**					
B Media strength	**0.076**					
A x B Interactions	**0.139**					

*(a,b,c,d,e,f,g,h,l,j,k,l,m,n) means with the same letters in each column are not significantly different at $p < 0.05$.
*LSD (least significant difference between means) calculated by one way ANOVA.

Table 6. Effect of explants types, culture media ingredients and their interaction on callusing capacity (%) of explants *in vitro*.

Treatments	Leaves	Root	Cotyledon	Hypocotyl	Node	Shoot tip
MS 1.5 strength	100.00^{a}	100.00^{a}	66.67^{c}	100.00^{a}	100.00^{a}	100.00^{a}
MS full strength	100.00^{a}	100.00^{a}	66.67^{c}	88.67^{ab}	100.00^{a}	100.00^{a}
MS half strength	100.00^{a}	100.00^{a}	83.33^{b}	66.67^{c}	100.00^{a}	100.00^{a}
LSD 5 % **A** Type of explants	**11.71**					
B Media strength	**N. S.**					
A x B Interactions	**15.23**					

*(a,b,c) means with the same letters in each column are not significantly different at $p < 0.05$.
*LSD (least significant difference between means) calculated by one way ANOVA.

Table 7. Effect of explants types, culture media ingredients and their interaction on callus fresh weight (g) of explants *in vitro*.

Treatments	Leaves	Root	Cotyledon	Hypocotyl	Node	Shoot tip
MS 1.5 strength	0.95^{a}	0.38^{f}	0.18^{h}	0.43^{f}	0.22^{g}	0.57^{cd}
MS full strength	0.97^{a}	0.61^{c}	0.52^{de}	0.60^{c}	0.25^{g}	0.54^{de}
MS half strength	0.80^{b}	0.22^{gh}	0.20^{gh}	0.16^{h}	0.18^{h}	0.49^{e}
LSD 5 % **A** Type of explants	**0.039**					
B Media strength	**0.030**					
A x B Interactions	**0.051**					

*(a,b,c,d,e,f,g,h) means with the same letters in each column are not significantly different at $p < 0.05$.
*LSD (least significant difference between means) calculated by one way ANOVA.

Table 8. Different concentrations of parthenolide and their respective area under peaks (AUPs).

Concentration (ug/ml)	AUPs (x 10^6)
0.244	0.011
0.976	0.049
3.906	0.199
15.625	0.632
62.5	2.471
150	5.903
250	9.876

Each figure is the mean of 3 determinations.

Table 9. Effect of plant growth regulators on shoot production of parthenolide.

Treatment	Parthenolide concentration (mg %)
Open field herb (O.F.H.)	0.098
Control Ms free	0.032
0.5 mg/l BAP	0.116
1.0 mg/l	0.092
2.0 mg/l BAP	0.118
0.05 mg/l NAA	0.061
0.1 mg/l NAA	0.08
0.2 mg/l NAA	0.04
0.5 mg/l BAP + 0.05 mg/l NAA	0.068
0.5 mg/l BAP + 0.1 mg/l NAA	0.064
0.5 mg/l BAP + 0.2 mg/l NAA	0.062
1.0 mg/l BAP + 0.05 mg/l NAA	0.079
1.0 mg/l BAP + 0.1 mg/l NAA	0.051
1.0 mg/l BAP + 0.2 mg/l NAA	0.039
2.0 mg/l BAP + 0.05 mg/l NAA	0.067
2.0 mg/l BAP + 0.1 mg/l NAA	0.067
2.0 mg/l BAP + 0.2 mg/l NAA	0.07

Table 10. Effect of culture media ingredient on shoot production of parthenolide.

Treatment	Parthenolide concentration (mg%)
Open field herb (O.F.H.)	0.098
Full strength MS	0.068
Half strength MS	0.119
Quarter strength MS	0.078
Three quarter strength MS	0.098

The data illustrated in Table 7 indicate that the fresh weight of callus was significantly influenced by different explants types, C.M.I. and their interaction. Regarding to the effect of different C.M.I., using full strength of MS medium produced the heaviest callus fresh weight (0.582 g). It means that full strength of MS medium was the most suitable culture media ingredients for obtaining the heaviest callus fresh weight comparing with either one and half or half strength of MS medium. The interaction effect of types of explants and C.M.I. showed that callus derived from leaves grown on one and half or full strength of MS medium characterized by the heaviest values of fresh weight (0.95 and 0.97 g, respectively). These results affirm that callus tissues initiated from leaves explants growing on one and half or full strength of MS medium had the most potentiality to grow up in higher rates resulting in the heaviest fresh weight comparing with the other treatments. Results agree with previous data (Arafa et al., 1993; Gad, 1997).

Table 11. Effect of carbon sources on shoot production of parthenolide.

Treatment	Parthenolide concentration (mg%)
Open field herb (O.F.H.)	0.098
1. Control Ms free	0.05
2. 12.5 g/l Glucose	0.034
3. 25 g/l Glucose	0.089
4. 50 g/l Glucose	0.127
5. 12.5 g/l Fructose	0.055
6. 25 g/l Fructose	0.096
7. 50 g/l Fructose	0.129
8. 12.5 g/l Sucrose	0.032
9. 25 g/l Sucrose	0.068
10. 50 g/l Sucrose	0.05
11. 12.5 g/l Starch	0.023
12. 25 g/l Starch	0.029
13. 50 g/l Starch	0.036

HPLC determination of parthenolide in the *in vitro* shoot and callus

From Table 9, it was found that the best plant growth regulators treatment which significantly increased parthenolide production higher than that produced in open field herb was MS + BAP (0.5 mg/l) which not significantly differed from MS + BAP (2 mg/l). While from Table 10, it can be concluded that the best culture media ingredient treatment which significantly increased parthenolide production higher than that produced in open field herb was MS half strength. From Table 11, it was found that the best carbon source treatments which significantly increased parthenolide production higher than that produced in open field herb was MS + glucose (50 g/l) which not significantly differed from MS + fructose (50 g/l) meaning that the plant preferred to utilize monosaccharides rather than disaccharides and polysaccharides for production of secondary metabolites.

Table 12. Effect of plant growth regulators on callus production of parthenolide.

Treatment	Parthenolide concentration (mg%)
1. Control Ms free	0.003
2. 0.5 mg/l 2,4-D	0.000
3. 1.0 mg/l 2,4-D	0.000
4. 0.5 mg/l NAA	0.099
5. 1.0 mg/l NAA	0.013
6. 0.5 mg/l BAP	0.074
7. 1.0 mg/l BAP	0.024
8. 0.5 mg/l 2,4-D + 0.5 mg/l BAP	0.004
9. 0.5 mg/l 2,4-D + 1.0 mg/l BAP	0.004
10. 1.0 mg/l 2,4-D + 0.5 mg/l BAP	0.003
11. 1.0 mg/l 2,4-D + 1.0 mg/l BAP	0.002
12. 0.5 mg/l NAA + 0.5 mg/l BAP	0.016
13. 0.5 mg/l NAA + 1.0 mg/l BAP	0.002
14. 1.0 mg/l NAA + 0.5 mg/l BAP	0.003
15. 1.0 mg/l NAA + 1.0 mg/l BAP	0.002

From Table 12, it was found that nearly all hormonal treatments could not produce or produce trace amount of parthenolide except two hormonal treatments, MS + 0.5 mg/l NAA and MS + 0.5 mg/l BAP, which could produce reasonable amounts of parthenolide, for the first time. This could be attributed to the fact that high hormonal concentrations (1 or 2 mg/l) have increased callus formation to form large friable non-productive callus since all the callus cells working for cell mitosis rather than secondary metabolite production. Therefore low hormonal concentration (0.5 mg/l) produced small hard productive callus, and slow increase in callus led the cells to produce secondary metabolites.

Conclusion

It could be concluded that addition of monosaccharides (glucose or fructose) and low concentration of the plant growth regulator (BAP) in half strength MS medium could increase the production of parthenolide in *in vitro* culture to meet demands for market. Culture media ingredient had non significant effect on callus production of parthenolide.

REFERENCES

Arafa AS, Mohamed BR, Ibrahim IA, Nor El-Din TM (1993). Tissue culture study of some plants belonging to family compositae. Egypt J. Agric. Res. 71(4): pp 987-996.

Banthorpe DV, Brown GD (1990). *In vitro* production of parthenolide in Tanacetum parthenium (L.). Plant Sci. 67: 107-113.

Banthorpe DV, Brown GD (1989). Two unexpected coumarin derivatives from tissue cultures of compositae species. Phytochemistry 28: 3003-3007.

Banthorpe DV, Brown GD (1993). Trials for production of parthenolide and other sesquiterpene lactones from some compositous plants. Agric. For. 24: 361-372.

Brown GD, Bernath J, Craker LE, Levy A (1993). Coumarins and sterols from hairy root cultures of *Tnancetum parthenium* (L.) Acta Hort. 330: 269-232.

Castillon J, Cornish K (2000). *In-vitro* Cellular and Developmental Biology Plant, 36 (3): 215-219.

Cervilli R (1987). Characteristics of shoot multiplications for some herbaceous plants in North Africa. Hort. Sci. 22(2): 304-306.

Elena Gonzalez-Benito M, Tapia J, Rodrigues N, Iriondo JMJ (1996). Tissue culture study of some plants belong to family Zingiberaceae. Hort. Sci. 71 (1): pp 11-15.

Evans WC (2002). Trease and Evans Pharmacognosy, 15th Ed., WB Sauners Co. Ltd., London, Philadelphia, Torento, Sydney, Tokyo, pp. 320-321.

Gad MMA (1997) Some Physiological Studies on Some Woody Trees, Ph.D. Thesis, Faculty of Agriculture, Cairo University.

George EF, Sherrington PD (1984). Plant Preparation by Tissue Culture, 1st edition, Longmann Press, London, pp. 146-185.

Hobbs C, Gram H (1989) Feverfew monograph 20: 26-35.

Ihsanul H, Khan J, Khattak MS (1998) Comparison between different method of callus formation for some desert plants. Sarhad J. Agric. 14(3): pp 211-213

Mantell SH, Boggetti B, Bessa AMS, Lemos EP, Mpunami AA (1998). Proceeding of The Intercashew and Coconut Conference, pp. 95-107.

Murashige T, Skoog F (1962). Plant Tissue Culture: Effect of Hormones on Tobacco Explants. Physiol. Plant. 15: 473-497,

Pereira-Pinto JEB, Fonseca A, Pereira-Pinto CAB Pereira-Barbosa MH (1996). Tissue culture study of some plants belong to family Labiatae. Ciencia Rural 26 (1): 57-61.

Ross IA (2001). Medicinal Plants of the World", Humana press, Totowa, New jersey, 2(1): pp 397-409,

Steel RGD, Torrie JH (1980). Principles and Procedures of Statistics. A Biochemical Approach. 2nd Ed., McGraw-Hill Kogakusha, LTD. pp 633-334.

Stojakowska A, Kisiel W (1997). "Plant-cell, Tissue and Organ Culture", 47 (2): 159-162,

Ya KL, Reed MF (1990). Effect of macro-, micro-elements and carbon sources on the morphological characters of two Chrysanthemum species. Plant Cell Rep. 9(6): 269-274.

Zhou JZ, Kou X, Stevenson D (1999). Rapid extraction and high-performance liquid chromatographic determination of parthenolide in feverfew *(Tanacetum parthenium)*. J. Agric. Food Chem. 47: 1018-1022,.