Adv. Hort. Sci., 2020 34(3): 255-263 DOI: 10.13128/ahsc-7767

AHS
Advances in Horticultural Science

Enhancement of Pentacyclic Triterpenoids (Betulinic and Oleanolic acids) production from callus cultures of *Lantana camara* L.

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Key words: elicitation, growth regulators, heavy metals, *in vitro,* Lantana, *Lantana camara* L., sugars.

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Citation:
AL-ASHOUSH A.M., SHIBLI R.A., TAHTAMOUNI R.W., AL-QUDAH T.S., ABU-IRARMAILEH B.B., 2020 - *Enhancement of Pentacyclic Triterpenoids (Betulinic and Oleanolic acids) production from callus cultures of Lantana camara L.* - Adv. Hort. Sci., 34(3): 255-263

Data Availability Statement:
All relevant data are within the paper and its Supporting Information files.

Competing Interests:
The authors declare no competing interests.

Received for publication 30 december 2019
Accepted for publication 26 May 2020

Abstract: *Lantana camara* L. is an ornamental plant with high medicinal value. This study aimed to investigate the possibility of enhancing the production of betulinic and oleanolic acids in *Lantana* callus by adding different types and levels of chemical elicitors (NaCl, sugars, growth regulators and heavy metals) to Murashige and Skoog (MS) medium. Data revealed that, adding NaCl to the culture medium affected callus fresh weight and color negatively, but it increased the extracted amounts of oleanolic and betulinic acids significantly to reach maximum levels of 0.702 and 0.051 mg/g DW at 120 mM NaCl compared to 0.659 mg/g DW and 0.014 mg/g DW obtained in the control. Meanwhile, increasing glucose level to 36.02 g/l in the medium maximized oleanolic acid accumulation to 0.829 mg/g DW, while betulinic acid accumulation reached 0.038 mg/g DW at 54.03 g/l glucose. In growth regulators experiment, highest callus fresh weight was observed in the control medium, while it declined to the minimum at 0.50 mg/l of Thiadiazuron (TDZ). Maximum values of both acids (0.685 and 0.033 mg/g DW) were recorded in MS medium plus 1.0 mg/l TDZ. Callus fresh weight decreased significantly in response to heavy metals addition, while adding chromium at 0.08 mg/l improved production of oleanolic acid to reach the maximum of 0.676 mg/g DW. Meanwhile betulinic acid was maximized at 0.057 mg/g DW in callus cultures exposed to 0.08 mg/l cobalt.

1. Introduction

Lantana camara L. is a popular flowering ornamental plant (Charitha and Ranwala, 2018), belonging to the family Verbenaceae (Mishra, 2015). It is native to tropical and sub-tropical areas of America (Singh and Saxena, 2016), West Africa (Waoo *et al.,* 2015 a) and tropical Asia

(Ghisalberti, 2000). This plant is routinely used as an evergreen aromatic ornamental or hedge shrub (Bhakta and Ganjewala, 2009; Zoubiri and Baaliouamer, 2012). It's commonly named as Lantana, red sage, Surinam tea plant, Spanish flag and West Indian lantana (Kalita *et al*., 2012).

Lantana camara L. is also listed as one of the most important medicinal plants, as it possesses many distinguished medicinal properties (Waoo *et al.,* 2014). The interest in *Lantana camara* L. has recently, increased because it is an excellent source of many chemical compounds of medicinal potential (Srivastava *et al.,* 2011; Saxena *et al.*, 2012; Waoo *et al.,* 2014) like pentacyclic triterpenoids which includes many active compounds such as betulinic and oleanolic acids (Kensa, 2011; Venkatachalam *et al.,* 2011; Kazmi *et al.,* 2012; Mariajancyrani *et al*., 2014).

Betulinic acid has recently gained increased attention as it possesses a remarkable variety of biological and medicinal properties (Moghaddam *et al.,* 2012; Pandey *et al.,* 2015). Oleanolic acid was also reported to possess many important biological activities (Ghosh *et al.,* 2010; Xia *et al.,* 2011; Singh *et al.,* 2012). So producing such valuable compounds in commercial amounts is of great importance.

In vitro culture was utilized as an efficient approach for production of secondary metabolites. Currently, *in vitro* culture techniques are used to facilitate the possibility of making quantitative and qualitative elicitation of the production of plant secondary metabolites by changing the culturing media composition (Affonso *et al.,* 2007; Alenizi *et al.,* 2020). According to Taiz and Zeiger (2002), adding elevated levels of growth regulators, sugars and heavy metals was found to drive the plant cell machinery to produce more secondary metabolites rather than cell division. For example, Pasqua *et al.* (2005) reported successful enhancement of anthocyanin production in *Camptotheca acuminata* cell cultures by adding different types and levels of growth regulators and sugars to the growth media. In addition, Lee *et al.* (2011) were able to produce more rutin from callus and adventitious roots of white mulberry tree by adding auxins, cytokinins, and nitrogen to the growth medium.

Heavy metals were also found to increase the production of bioactive compounds in many plants (Verpoorte *et al.,* 2002). So, this study was carried out to enhance the possibility of production of betulinic and oleanolic acids in *Lantana* callus cultures by modifying the culture medium using different types and levels of growth regulators, sugars, NaCl, and heavy metals in callus culture media.

2. Materials and Methods

Mother stock culture establishment and maintenance

In vitro grown callus cultures of *Lantana camara* L. were implemented by the Plant Tissue Culture and Microbiology Laboratories at Hamdi Mango Center for Scientific Research (HMCSR). The cultures were subcultured on callus maintenance semi solid medium consisting of 4.4 g/l of Murashige and Skoog (Murashige and Skoog, 1962) MS premix (Duchefa Biochemie) plus 34.2 g/l sucrose in addition to 1.0 mg/l Kinetin and 2.0 mg/l 2,4-D. Cultures were maintained in the growth room under dark conditions at 24± 1°C. Subculturing of callus was performed every 3-4 weeks by subdividing callus under sterile conditions.

Enhancement of betulinic and oleanolic acids production

To study the possibility of enhancing production of betulinic and oleanolic acids in *Lantana camara* L. callus cultures, different types and levels of chemical elicitors (NaCl, sugars, growth regulators, heavy metals) were added to the culture medium.

In NaCl experiment, callus clumps of 1.0 g were subcultured onto fresh MS callus maintenance medium described earlier and supplemented with NaCl at different concentrations (0, 40, 80, 120 and 160 mM). Meanwhile, the effect of sugars on betulinic and oleanolic acids was experimented by transferring 1.0 g of callus clumps onto maintenance medium supplemented with different types and levels of sugars: sucrose (34.2, 68.4 and 102.6 g/l); glucose or fructose (18.01, 36.02 and 54.03 g/l).

In heavy metals experiment, lead (Pb), cobalt (Co) or chromium (Cr) were prepared from their salts; lead nitrate Pb $(NO_3)_2$, potassium chromate (K_2CrO_4), and cobalt (II) chloride ($CoCl_2.6H_2O$) respectively. A stock solution of 100 mg/l was prepared from each salt. After that the requested amount of each heavy metal (0.08, 0.16 and 0.24 mg/l) was added to the callus medium prepared as described earlier.

To test the effect of plant growth regulators thidiazuron (TDZ) and kinetin on betulinic and oleanolic acids production, the explants were transferred onto hormone free MS medium for one week to remove any carry over effect of callus maintenance media. Next, callus clumps of 1.0 g were subcultured onto full strength MS solid medium supplemented with different levels (0.5, 1.0 and 1.5 mg/l) of either TDZ

or Kinetin in combination with 2.0 mg/l 2,4-D.

All cultures were maintained in the growth room under dark conditions at 24±1°C, and data were collected after 6 weeks for callus fresh weight and color before oven drying.

Extraction

Calluses from each treatment were oven dried at 50°C for 2 days. Then, the dried callus was ground using liquid nitrogen. Next, 100 mg were taken from the dried matter of each treatment and soaked in 10 ml of methanol and water in a ratio of 1:1. The samples were then placed on a shaker at slow speed for at least 72 hours at room temperature (Hussain *et al*., 2012). The plant extract was filtered and centrifuged at 5000 g for 10 min. The resulting supernatant was dried in a rotary evaporator at 40°C. Next, the dried extract was resuspended in the mobile phase for HPLC analysis.

Preparation of betulinic and oleanolic acids stock solutions and working standards

Betulinic and oleanolic acid stock solutions at concentration of 1000 mg/l were prepared by weighing 5 mg of each in 5 ml volumetric flask, dissolved and brought to volume by methanol HPLC grade. Both stock solutions were stored at 4°C in dark. Working solutions were prepared by serially diluting stock solutions using the mobile phase at concentrations of 800, 400, 200, 100, 50, 25 and 12.5 mg/l. Fresh working standards were prepared daily. Betulinic and oleanolic acids were eluted at 15.8 and 19 min, respectively. Calibration curves were constructed for betulinic and oleanolic acids before starting chemical analysis (r^2= 0.9999).

HPLC instrumentation and conditions

HPLC analysis was performed using a Shimadzu-LC system (Shimadzu Corporation, Kyoto, Japan) equipped with a CBM-20A controller, LC-20AT pump, and SPD-20AV UV/VIS detector Chromatographic separations were achieved using an Agilent Eclipse Plus C18 column and were carried out using an isocratic flow rate of 1 ml/min, a column temperature of 25° C, a mobile phase of acetonitrile: methanol: acidified water (70:20:10 v/v), with pH adjusted to 2.8 using 85% phosphoric acid. The ultraviolet (UV) detection was set at 210 nm. The injection volume was 20 µl of sample solution. Total run time was 19 min for each injection. Data were acquired and processed with LC-Solution software (Shimadzu Corporation, Kyoto, Japan).

Statistical analysis

Each treatment was arranged in a complete randomized design (CRD) and replicated ten times with two callus clumps/replicate. HPLC analysis was conducted on three replicates for each treatment with two samples/replicate. The collected data were statistically analyzed using SPSS analysis system. The analysis of variance (ANOVA) was used and mean separation was done according to the Tukey's HSD at probability level of 0.05.

3. Results

Effect of NaCl

After 6 weeks of incubation under different levels of NaCl, the obtained results indicated that, callus fresh weight and color (callus quality) declined in response to NaCl and the effects became more severe as NaCl level increased (Table 1). The maximum fresh weight 8.6 g was recorded in the control, while brownish yellow callus with minimal fresh weight value of (2.6 g) was obtained on the medium supplemented with 160 mM NaCl (Table 1).

On the other hand, adding NaCl to the growth medium enhanced the production of oleanolic acid in the callus cultures in response to NaCl level reaching the maximum level of 0.702 mg/g DW at 120 mM

Table 1 - Effect of NaCl on callus fresh weight, color and levels of oleanolic and betulinic acids in callus culture of *Lantana camara*

NaCl (mM)	Weight (g)	Color	Oleanolic acid (mg/g DW)	Betulinic acid (mg/g DW)
Control	8.60 ± 0.36 a *	White	0.659 ± 0.011 d	0.014 ± 0.0003 e
40	5.90 ± 0.35 b	White	0.668± 0.0028 c	0.021± 0.0531 d
80	4.70 ± 0.59 bc	Yellow	0.674± 0.0124 b	0.043 ± 0.0081 b
120	4.11 ± 0.32 c	Yellow	0.702 ± 0.0088 a	0.051 ± 0.0083 a
160	2.62 ± 0.53 d	Yellow to brown	0.656± 0.0096 d	0.035± 0.0057 c

* Values represent means ± standard error. Control: represents callus maintenance medium consisting of solid MS medium + 34.2 g/l sucrose+ 2.0 mg/l 2, 4-D + 1.0 mg/l kinetin. Means with different letters are significantly different according to Tukey HSD range test at P≤0.05.

NaCl compared to 0.659 mg/g DW extracted from the control treatment (Table 1). Also, the production of betulinic acid was enhanced by adding NaCl. The maximum value 0.051 mg/g DW was obtained at 120 mM NaCl compared to 0.014 mg/g DW extracted from the control (Table 1).

Effect of sugars

Sucrose. Our results revealed that, increasing sucrose concentration in the MS media led to a dramatic decline in fresh weight and quality of *Lantana camara* L. callus cultures (Table 2). Fresh weight of the white callus decreased significantly from 8.6 g in control treatment to reach the minimum (3.1 g) at 102.6 g/l sucrose where it turned dark brown (Table 2). Meanwhile, in response to level of sucrose, we observed significant increases in oleanolic and betulinic acids production. The maximum amounts of oleanolic acid (0.97 mg/g DW) and betulinic acid (0.035 mg/g DW) were recorded in media with 102.6 g/l of sucrose. This level of sucrose enhanced the production of betulinic and oleanolic acids in callus cultures to levels that exceeded values of those extracted from naturally growing plants (Table 2). On the other hand, callus was not healthy and the growth was very limited in all treatments compared to the control (Table 2).

Glucose. Adding glucose resulted in a significant decline in callus growth and quality as shown in Table 2. Instead increasing glucose level in the medium resulted in increasing level of oleanolic acid to reach the maximum at 36.02 g/l glucose (0.829 mg/g DW) compared to control (0.659 mg/g DW). Meanwhile, betulinic acid accumulation increased to 2.5 times when 54.03 g/l glucose was added to the media compared to the control (0.014 mg/g DW) as shown in Table 2.

Fructose. The obtained data indicated that, fructose was not a good choice for *Lantana camara* L. callus cultures in terms of growth, color and secondary metabolite production. Callus fresh weight decreased gradually with increasing fructose levels in the medium and minimum fresh weight of the brown callus (1.55 g) was obtained on 54.03 g/l fructose (Table 2). Meanwhile, adding fructose to the culture medium at levels higher than 18.01 g/l negatively affected oleanolic accumulation compared to the control, and the same for betulinic acid (Table 2).

Effect of plant growth regulators

Effect of Kinetin. Data obtained revealed a significant increase in fresh weight (8.6 g) at Kinetin concentration of 1.0 mg/l (Table 3). Meanwhile maximum amounts of oleanolic acid and betulinic acids (0.67 and 0.021 mg/g DW) were extracted from callus cultures grown on media supplemented with low rate of kinetin (0.5 mg/l), while the amount of fresh weight and both acids declined significantly as kinetin level increased in the media (Table 3).

Table 2 - Effect of sucrose; glucose and fructose on callus fresh weight, color and production of betulinic acid and oleanolic acid in callus culture of *Lantana camara*

Carbohydrate (g/l)	Weight (g)	Color	Oleanolic acid (mg/g DW)	Betulinic acid (mg/g DW)
Sucrose				
Control (34.2)	8.60 ± 0.36 a*	White	0.659± 0.011 c	0. 014 ± 0.0003 c
68.4	3.21 ± 0.16 b	Yellow	0.824 ± 0.0082 b	0.027± 0.013 b
102.6	3.11 ± 0.32 b	dark brown	0.970± 0.0081 a	0.035± 0.0291 a
Glucose				
Control	8.60 ± 0.36 a	White	0.659± 0.011 c	0.014 ± 0.0003 c
18.01	4.49 ± 0.197 b	Yellow	0.765± 0.0093 b	0.025 ± 0.0084 b
36.02	3.80 ± 0.56 bc	Yellow start to be brown	0.829± 0.0089 a	0.036± 0.0189 a
54.03	3.00 ± 0.46 c	Yellow start to be brown	0.764± 0.0077 b	0. 038 ± 0.0095 a
Fructose				
Control	8.60± 0.36 a	White	0.659 ± 0.011 a	0.014 ± 0.0003 a
18.01	4.40 ± 0.22 b	Yellow	0.661 ± 0.0035 a	0.012 ± 0.0066 ab
36.02	2.34 ± 0.47 c	Yellow	0.514 ± 0.0092 b	0.010 ± 0.0090 b
54.03	1.55 ± 0.13 d	Brown	0.380 ± 0.0060 c	0.010± 0.0089 b

* Values represent means ± standard error. Control: represents callus maintenance medium consisting of solid MS medium + 34.2 g/l sucrose+ 2.0 mg/l 2, 4-D + 1.0 mg/l kinetin. Means with different letters are significantly different according to Tukey HSD range test at P≤0.05.

Table 3 - Effect of different growth regulators in combination with 2.0 mg/l of 2, 4-D on callus fresh weight, color and production of betulinic and oleanolic acids in callus culture of *Lantana camara*

Growth regulator (mg/l)	Weight (g)	Color	Oleanolic acid (mg/g DW)	Betulinic acid (mg/g DW)
Kinetin				
0.5	4.63 ± 0.55 b*	White	0.672 ± 0.0091 a	0.021 ± 0.0067 a
1.0 (control)	8.60 ± 0.36 a	White	0. 659 ± 0.011 b	0.014 ± 0.0003 b
1.5	4.72 ± 0.44 b	Yellow	0. 621 ± 0.0001 c	0.014 ± 0.0010 b
TDZ				
Control	8.60 ± 0.36 a	White	0.659 ± 0.011 c	0.014 ± 0.0003 c
0.5	1.40 ± 0.14 c	Yellow	0. 651 ± 0.0088 d	0.025±0.023 b
1.0	3.44 ± 0.63 b	White	0.685 ± 0.0082 a	0.033±0.009 a
1.5	3.68 ± 0.23 b	White	0.668±0.0045 b	0.012± 0.0072 c

* Values represent means ± standard error. Control: represents callus maintenance medium consisting of solid MS medium + 34.2 g/l sucrose+ 2.0 mg/l 2, 4-D + 1.0 mg/l kinetin. Means with different letters are significantly different according to Tukey HSD range test at P≤0.05.

Effect of TDZ. The highest fresh weight of callus (8.6 g) was obtained in the control treatment followed by that obtained on 1.5 mg/l TDZ in combination with 2.0 mg/l 2,4-D (Table 3). However, the resulted callus was of good quality as it remained white in all treatments levels (Table 3). Moreover, a significant increase in oleanolic acid and betulinic acids was obtained in response to TDZ, and maximum values of both acids 0.685 and 0.033 mg/g DW were recorded in callus cultures grown in MS medium plus 1.0 mg/l TDZ, while adding higher levels of TDZ negatively impacted on oleanolic and betulinic acids (Table 3).

Effect of heavy metals

Effect of cobalt. Callus fresh weight and color were negatively affected by adding cobalt to the media at levels higher than 0.08 mg/l. Minimum weight of 5.32 g was recorded at Co level of 0.24 mg/l (Table 4). On the other hand, callus remained healthy white despite of Co addition at all levels (Table 4, Fig. 1). Meanwhile, amounts of oleanolic acid were negatively affected by adding Co to the media at all levels compared to the quantity extracted from the control (Table 4). On the other hand, production of betulinic acid was significantly improved by adding Co to the medium. At 0.24 mg/l

Table 4 - Effect of heavy metals on callus fresh weight, color and production of betulinic and oleanolic acids in callus culture of *Lantana camara*

Heavy metal (mg/l)	Weight (g)	Color	Oleanolic acid (mg/g DW)	Betulinic acid (mg/g DW)
Cobalt				
Control	8.60 ±0.36 a *	White	0.659 ± 0.011 a	0.014 ± 0.0003 d
0.08	6.87 ± 0.59 ab	White	0.501 ± 0.0066 b	0.020 ± 0.0003 c
0.16	5.41 ± 0.71 b	White	0.472 ± 0.0046 c	0.033± 0.0072 b
0.24	5.32 ± 0.75 b	White	0.215 ± 0.0082 d	0.057± 0.0102 a
Lead				
Control	8.60± 0.36 a	White	0.659 ±0.011 a	0.014 ± 0.0003 b
0.08	3.80 ± 0.76 b	White with red spots	0.344 ± 0.001b	0.020 ± 0.009 a
0.16	3.42 ± 0.90 b	Yellow with red spots	0.343 ± 0.006 b	0.021 ± .0001 a
0.24	1.57 ± 0.10 b	Yellow with red spots	0.117± 0.0085 c	0. 016 ± 0.008 b
Chromium				
Control	8.6 ±0.36 a	White	0.659 ± 0.011 c	0.014 ± 0.0003 a
0.08	3.60 ± 0.54 b	Yellow with brown spots	0.676 ± 0.001 a	0.013 ± 0.0003 a
0.16	2.81 ± 0.66 b	Yellow with brown spots	0.665 ± 0.0083 b	0.010 ± 0.0085 b
0.24	0.99 ± 0.13 c	Yellow with brown spots	0.435 ± 0.0071 d	0.010± .0069 b

* Values represent means ± standard error. Control: represents callus maintenance medium consisting of solid MS medium + 34.2 g/l sucrose+ 2.0 mg/l 2, 4-D + 1.0 mg/l kinetin. Means with different letters are significantly different according to Tukey HSD range test at P≤0.05.

it was four times higher than the control as shown in Table 4.

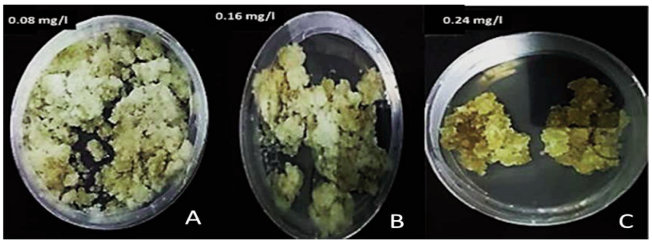

Fig. 1 - Effect of cobalt on callus fresh weight and color in callus culture of *Lantana camara* L. after 6 weeks of incubation in A medium containing 0.08; B medium containing 0.16 and C medium containing 0.24 mg/l cobalt.

Effect of lead. Data revealed that, callus growth, color and oleanolic acid production decreased dramatically with increasing Pb level in the media (Table 4, Fig. 2). The minimal value for fresh weight (1.57 g) was recorded in cultures grown in media supplemented with 0.24 mg/l Pb. Furthermore, adding Pb to the medium determined the appearance of red spots on the white callus at all Pb levels (Table 4, Fig. 2). Meanwhile, production of betulinic acid was improved significantly by adding either 0.08 or 0.16 mg/l Pb while it tended to decline at 0.24 mg/l Pb (Table 4).

Effect of chromium. The results obtained showed that callus growth was negatively affected by adding Cr in the medium (Table 4). Furthermore, adding Cr to the medium resulted in development of brown spots on the white callus at all levels (Table 4). Moreover, chromium had improved the production of oleanolic acid to reach the maximum (0.676 mg/g DW) at 0.08 mg/l, while it tended to decline at higher Cr levels (Table 4). Meanwhile, amounts of betulinic acid decreased in response to Cr to reach minimum level of (0.010 mg/g DW) at either 0.16 or 0.24 mg/l, respectively (Table 4).

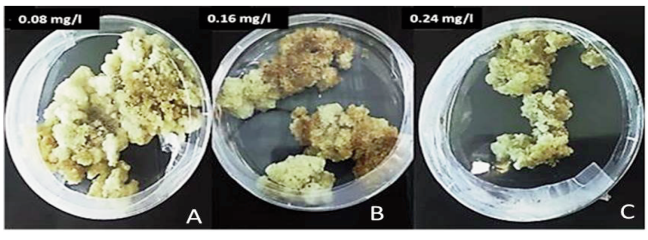

Fig. 2 - Effect of lead on callus fresh weight and color in callus culture of *Lantana camara* L. after 6 weeks of incubation in A medium containing 0.08; B medium containing 0.16 and C medium containing 0.24 mg/l lead.

4. Discussion and Conclusions

Effect of NaCl

Adding NaCl to the culture medium resulted in reduction in most growth parameters in callus cultures of *Lantana camara* which agrees with Nikman, *et al.* (2004) and Nikman *et al.* (2006) studies on *Nicotiana* seedlings and on calli and seedlings of two *Trigonella* species on culture media supplemented with elevated levels of NaCl, where growth and quality of the cultures declined sharply at levels high levels. Generally, quality parameters including color, declined in tissue cultured plant material of many plant species when exposed to osmotic stress due to addition of salts or sugars to the media (Taiz and Zeiger, 2002). This could be due to the continuous accumulation of osmotica, dehydration and phenolic compounds accumulation that results in a shift in color toward brown which might determine, with time, cell death.

Meanwhile, enhanced accumulation of oleanolic and betulinic acids in *Lantana camara* callus cultures was obtained at high NaCl levels agrees with Parida and Das (2005) who reported that salt stress might induce the production of secondary metabolites to maintain cell turgidity and to reduce dehydration. Also, Wang *et al.* (2015) confirmed in their study on cotton that salinity stress resulted from adding NaCl to the growth medium induced the production of some secondary metabolites such as gossypol, flavonoids and tannin.

Effect of sugars

It was clear from our data, that increasing sugar concentration in the medium inversely affected callus fresh weight and color. Elevated levels of sugars would make them act as osmotic agents instead of energy sources, and this would hinder growth rate of the explants (Tahtamouni *et al.,* 2016). Consequently cell volume would decrease as a result of low turgor pressure which would be translated in a form of growth reduction (Taiz and Zeiger, 2002).

Meanwhile, according to our results increasing sucrose level in the medium led to significant increase in production of oleolinic acid and betulinic acid. Generally, high levels of sucrose were reported to increase the secondary metabolite production in plant tissue cultures (Bandhakavi and Kamarapu, 2016). Similar results were also recorded in different species such as, grape berries (Dai *et al.,* 2014), *Ginkgo biloba* (Park *et al.*, 2004), and *Clematis pitcher* (Kawa-Miszczak *et al.,* 2009).

Enhancement of secondary metabolites production in response to glucose was also obtained by Verma *et al.* (2012) in their study about improving alkaloid content in callus culture of *Catharanthus roseus.*

Effect of plant growth regulators

Callus fresh weight was influenced by the level of Kinetin in the medium. This was in full agreement with Singh and Saxena findings (2016) where maximum callus growth was obtained when 2, 4-D was used in combination with Kinetin to induce callusing in yellow variety of *Lantana camara* L.

Effect of heavy metals

Our results showed that, increasing levels of the experimented heavy metals in the culture medium led to a gradual decline in callus fresh weight and color. A decline in cultures growth in response to addition of chromium to the medium was reported by Waoo *et al*. (2015 a) who observed that increasing chromium in the medium decreased shoot length and percentage of survival in *Lantana camara* L. Similar response was also reported in Waoo *et al*. (2015 b) study about the toxic effects of different lead concentrations on *in-vitro* grown shoot cultures of *Lantana camara*.

Our results had also indicated that production of oleanolic and betulinic acids varied with type and level of the added heavy metal. Chemical elicitation was commonly combined with osmotic stress that usually reduces growth (Taiz and Zeiger, 2002). In many studies, increasing biosynthesis of secondary metabolites by stressing the plant using chemical and/ or physical elicitation agents was possible. But adding such elicitors usually resulted in decreasing biomass of the plant, and consequently the levels of secondary metabolites was unchanged or declined, although the concentration is strongly enhanced at tissue or cell level (Gershenzon, 1984; Selmar and Kleinwächter, 2013; Paulsen and Selmar, 2016). Therefore, although biosynthesis of these compounds increased significantly in plant material like callus cultures or microshoots (Paulsen and Selmar, 2016) due to chemical elicitation, the total biomass sharply decline because of the negative effects of the elicitors. So, the total biosynthesis of secondary metabolites is dramatically decreased.

It can be concluded from our study that enhancement of oleanolic and betulinic acids production in *Lantana camara* callus cultures is possible by adding some types of chemical elicitors to the culture medium. Meanwhile, obtaining enough plant material is crucial to guarantee sustainable production of secondary metabolites in commercial amounts. This would open the gate for conducting research to improve our methodology to maintains proper amounts of plant material with enhanced levels of both acids.

Acknowledgements

Authors would like to thank the University of Jordan, The Deanship of Academic Research, for granting this research fund. Further thanks are extended to Hamdi Mango Center for Scientific Research, the Faculty of Agriculture: the Department of Horticulture and Crop Sciences at the University of Jordan.

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