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EFFECT OF PLANT GROWTH REGULATORS AND ORGAN TYPE ON CALLUS INDUCTION, GROWTH DYNAMIC, POLYPHENOL, FLAVONOID AND TANNIN CONTENT IN MILK THISTLE (*SILYBUM MARIANUM L.GAERTN.*) TISSUE CULTURE

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ABSTRACT

Milk thistle (*Silybum marianum* L. Gaertn., Asteraceae) is an annual or biennial, broadleaf plant native in North African and Mediterranean with highly valued medicinal properties. The active compound in *S. marianum* is silymarin; which is an isomeric mixture of flavonolignans which used in pharmaceutical industries. Milk thistle is the most commonly used herbal product for chronic liver disease and may be beneficial for reducing the chances for developing certain cancers. Plant secondary metabolites, which have been extracted from *in vivo* grown plants, were used for a long time but the general trend new is to extract these secondary metabolites from *in vitro* grown plants. We cannot ignore the importance of using tissue culture techniques which gives the ease of almost all of plant researches, also there are so many other reasons such as protection from weather and from diseases, pests, soil problems and obtaining mass quantities with low cost.

As obtaining secondary metabolites from milk thistle plants is considered a tedious process, it became clear that replacing this by obtaining these products from *in vitro* might be better, so our successful attempt was made to produce calluses and extract the active ingredients from them. The result of the experiment showed that significances differences were found in the influence of organ and medium type on callus initiation, growth dynamic and chemical content from polyphenol, flavonoid and tannin. The best medium for callus induction was MS3 (supplemented with 2 mg Kin/L + 2 mg/L NAA) for leaf organ and MS1 (1.5 mg/L 2,4-D + 1.5 mg/L BA) for stem node organ. The highest callus fresh weight was 300.4±9.66 mg/culture and that achieved on MS3 from leaf organ. Stem node callus grow on MS3 showed the highest content of polyphenol and flavonoids while the leaf callus grow on MS1 and MS3 showed the highest content of tannin .

INTRODUCTION

The milk thistle *Silybum marianum* L. Gaertn belongs to Asteraceae family. An annual or biannual herbaceous plant is widespread in temperate American countries, Australia and areas of Mediterranean climate. In Egypt, it grows wild in most districts especially in Nile Delta. The great importance of this plant and consequently its active ingredients can be easily recognized from the list of diseases in which the plant is used such as anorexia disease, cancer disease, demulcent in catarrh and pleurisy, diabetes, and spleen disease. *S. marianum* is one of the most important medicinal plants cultivated worldwide. It can grow as a winter annual or a biennial herb **(Das *et al*., 2008)**. It is tolerant to a variety of climate situations **(Wallace *et al*., 2008)**, grows well in a variety of soil types, yields a decent harvest and often thought of as a plant that thrives along roadsides and in wastelands because it competes with crops for nutrients and water. It is also classified as a noxious weed in several countries **(Abenavoli *et al*., 2010)**. *S. marianum*, popularly known as milk thistle, is an edible plant in the Asteraceae family. The milky white veins on the leaves give origin to the common name "milk thistle," which discharges milky sap when cut apart. Sharp spines follow a solitary, big purple flower on each stem. The fruit of the plant is glossy, patchy brown, or dull in color **(Hogan *et al*., 2007)**.

Recently, great interest in the study of herbal drugs and traditional remedies has been emphasized worldwide and there has been an upsurge in scientific investigation in the area (**Jain and Defelli, 1991**). Currently, one fourth of all prescribed pharmaceuticals in industrialized countries contain compounds that are directly or indirectly derived from plants (**Rates, 2001**). The great importance of *S. marianum* and consequently its active ingredients can be easily recognized from the list of diseases in which the plant is used such as anorexia disease, cancer disease, demulcent in catarrh and pleurisy, diabetes estrogen-related diseases, hemorrhoids, hepatitis, cirrhosis, hydrophaints, malaria, and spleen disease (**Giese, 2001**). *S. marianum* has been used to treat liver disease as a hepatoprotective herb since 2000 years ago. The chemical compounds with medicinal value are found in the seeds of the plant. Seeds of milk thistle contain significant amounts of silymarin which is a mixture of flavonolignans such as silychristin, silydianin, silybin, and isosilybin, taxifolin (**Engelberth *et al.*, 2008**).

At present, the increasing demand for silymarin is endangering the *S. marianum* populations (**Ahmad *et al.*, 2008**). Moreover, the efficiency of seed germination and seedling growth, which highly depends on various biological and environmental factors, is very low (**Shinwari *et al*., 2014**). Besides, manual handling of the plants is very difficult because leaves and flowers are spiny and, as the plant is cultivated in rows, using harvesting machine causes damage and reduces the crop yield (**Hammouda *et al.*, 1993**). Although advanced microbial and chemical methods can synthesize medicinal and aromatic compounds, the cost in many cases is high. Unfortunately, traditional

agriculture of silybum plants has many agricultural problems which causes reduction of the total yield and that is due to the leaves of the plant having spiny margins and flowers are spiny also so, it is very difficult to manipulate the manual treatment with the plant during different stages of growth particularly during harvesting. The *in vitro* production of plant secondary metabolites is an excellent alternative. To meet the increasing need for plantlets, it is imperative to develop a protocol for mass propagation through tissue culture for the large-scale production of *S. marianum*.

Plants are known to produce a large number of natural products, which refers to as secondary metabolites. The secondary metabolites are economically important in medicinal and food industry. However, the concentration of various plant secondary metabolites is very low and depends on the physiological and developmental stages. Dried fruits of *S. marianum* contain the highest level of silymarin (4%). Silymarin amounts is low in different parts of *S. marianum* (such as young and old leaves, root, stem, inflorescence and seeds) during different months. The data obtained from HPLC analysis showed that the highest amount of silymarin was observed in the seeds (32.77 mg/g dry weight). In addition, stems collected during May month showed high amount of silymarin (17.83 mg/g dry weight) **Hassnloo *et al*.,(2008).**

Callus induction of *S. marianum* has been previously reported. **Rady *et al*., (2014)** in their study, reported that a protocol for initiation of callus and shoot cultures from leaves and shoot tips explants of different *silybum* genotypes collected from different locations in Egypt was established. Shoot cultures were initiated from shoot tips explants. Results obtained revealed that the optimum medium for growth and maintenance of friable callus was MS medium supplemented with 0.25 mg/L 2,4-Dichlorophenoxy acetic acid (2,4-D) + 0.25 mg Kinetin (Kin). They showed that flavonolignan content in 2 months old callus (1.5%) was lower than the values reported for seeds. **Hasanloo *et al*., (2008)** reported callus induction with cotyledons, shoot and root explants of *S. marianum* seedlings. They have also showed the positive effect of picloram on callus growth and flavonolignan production.

As we mentioned above, **Rady *et al*.,** (**2014)** reported that MS medium supplemented with 0.25 mg/L 2,4-D and Kin are a suitable medium for growth and maintenance of friable callus. Other researchers reported callus induction from cotyledon by different concentrations of mineral element, plant growth regulators, amino acids and vitamins on MS medium culture. Meanwhile, **Cimino *et al*., (2006)** found B5 medium supplemented with 0.05 mg/L of BA and 0.5 mg/L of 2,4-D is the best medium for callus production and biomass production of *S. marianum.* Studies on the production of silymarin using *in vitro* cultures reported the production of flavonolignan from callus and cell suspension cultures derived from cotyledons of *S. marianum* L. Higher accumulation of flavonolignan was observed in cell suspension cultures than in callus cultures. Explants from seedlings of *S. marianum* were used to produce either transformed (hairy root

culture) or untransformed (root culture) **(Cacho, *et.al*., 1999).**

The aims of this study is to establish a protocol for both callus initiation and calli cultures growth dynamic from *S. marianum* and to assess the effect of the organ and medium on polyphenol, flavonoids silymarin, and tannin content in calli cultures.

MATERIALS AND METHODS

All tissue culture experiments carried out during 2019-2023 seasons in Biotechnology and tissue culture lab. Genetics Dept. Faculty of Agriculture, Minia University. Leaves and young stems node of S. *Marinum* used as source of explants for callus initiation throughout this study.

Germination Experiment

Seeds of milk thistle (*Silybum marianum* L. Gaertn.) were collected/obtained from the agricultural areas of the El-Minia administrative Center of El-Minia Governorate, Egypt. Seeds were sterilized in 70% ethanol for 3 minutes then transferred to 20% Cholorax solution for 20 minutes. Then seeds were washed thoroughly with sterilized distilled water 3 times. After that, seeds were germinated on half MS medium (0.5 MS, Murashige and Skoog 1962: proudect No.0111307 , Company name:(TTTAN BIOTECH LTD) and incubated for 15 days until germinated. Seeds were germinated in the months from November to January of each year. Seeds placed in jars (250 ml) each containing 50 ml of 0.5 MS hormone-free medium). After 5-10 days, the seedlings appeared and their length was about from 7 to 10 cm. Then explants (young stems and leaves were dissected into small pieces and cultured in Erlenmeyer flasks containing 25 ml of callus establishment medium and incubated at

25 c^O in darkness. Callus formation were examined after 4 weeks from culturing. The explants were cultivated separately on three different MS callus establishment media as shown in Table (1). Through this experiment, the best medium and explant type were detected for callus initiation.

Maintenance Media

For callus maintenance, MS medium supplemented with various combinations of plant growth regulator were tested (2,4-Dichlorophenoxy acetic acid) Kin (kinetin); BA (6-benzyladinine); NAA (naphthalene acetic acid) as shown in Table (2). The initiated calluses were transferred to different MS maintaining media (**Murashige and Skoog 1962)** as shown in Table (2). Calli were subcultured to fresh maintenance media every four weeks and were maintained for 2 years at $25C^O$ in darkness.

Growth dynamic of callus

To measure the growth dynamic of callus; about 40-50 mg of the initiated callus were grown in 100 ml flasks containing 25 ml of each maintaining media separately (Table 2). Three pieces of callus in each flask, fresh weight and dry weight were measured weekly during the growing of 6 weeks period. To measure the dry weight, callus mass was dried for 24 hr at 70 c^O in thermostatic drier; callus growth activity was evaluated according to the method of

Maroti (1976). This method includes the measurement of:

1- Callus fresh weight (mg).
2- Callus dry weight (mg).
3- Growth ratio index (growth value) relative to fresh weight and dry weight.

Growth ratio index= pt – po / po whereas:

pt. = fresh or dry weight of callus at the end of each week.

po = the fresh weight or dry weight of callus at the beginning of the experiment.

This experiment was carried out with three replicates. Each experiment was repeated three time and the obtained results were statistically analyzed by LSD test according to **Little and Hills (1972) and Duncan (1955).**

Plant material and extract preparation.

In the agricultural areas of the El-Minia administrative Center of El-Minia Governorate, Egypt samples of the *S. marianum* wild type plants (roots, stems, and leaves) were collected from March to June. The collected *S. marianum* roots, stems, and leaves were carefully washed under running tap water, dried for a month at room temperature in the shade to avoid photo-oxidation, and then powdered using an electric blender **(Javeed *et al*., 2022)**.

Analysis of total phenolic contents (TPC).

The Folin-Ciocalteu reagent method was used to assess the quantitative analysis for phenols. In brief, 1 mg of the extract was dissolved in 1 mL of methanol. The resulting mixture was violently mixed before being separated for analysis. Folin-Ciocalteu reagent assay was used to determine the total phenolic content. 2.5 mL of 10% Folin-Ciocalteu reagent and 2 mL of a 2% sodium carbonate (Na_2CO_3) solution each received a total of 1 mL of solution (1 mg mL^{-1}) each. The subsequent combination was then incubated in the dark for 15 min in order to quantify the absorbance at 765 nm in comparison to a blank using a UV-Vis spectrophotometer. Gallic acid was used to create a standard curve at a concentration of 1 mg mL-1, and the following concentrations of 1, 0.50, 0.25, 0.10, 0.05, 0.02, 0.01, and 0 mg mL-1 were used to convert the results to mg of gallic acid equivalent/g of extract **(Wolfe *et al*., 2003)**.

Analysis of total flavonoids content (TFC).

The aluminum chloride colorimetric method was used to assess the total flavonoids content. In brief, 1 mL of the solution (1 mg mL^{-1}) was added to 3 mL of methanol, followed by the addition of 0.2 mL of potassium acetate (1 M), 0.2 mL of aluminum chloride (10%), and 5.6 mL of purified water. To determine the absorbance at 420 nm against the prepared reagent blank (without adding extract), the final combination was incubated in the dark for 30 min. To create a standard curve, quercetin was used as a standard at various concentrations (1, 0.50, 0.25, 0.10, 0.05, 0.02, 0.01, and 0 mg mL^{-1}). The data is given as mg quercetin equivalent/g extract **(Djeridane *et al*., 2006).**

Analysis of total tannins content (TTC).

The total tannins concentration was determined using the Folin-Ciocalteu technique. In brief, 1 mg of the extract was dissolved in 1 mL of methanol. 250 µL of Folin-Ciocalteu reagent was added to 500 µL of solution (1 mg mL^{-1}), which was then incubated for 30 minutes at 37°C. 1.25 mL of 20% Na_2CO_3 was then added, and the mixture was then incubated at 37°C for 10 minutes. At 725 nm, absorbance values were measured. A standard curve for gallic acid was created to estimate the overall tannin concentration. In mg gallic acid equivalent/g extract the results are expressed **(Makkar *et al*., 1993).**

RESULTS AND DISCUSSION

In the present study, MS media was used to initiate callus cultures from two different explants (Leaf and young stem node) of *S. marinum*. Friable creamish white tissues were produced on explants within four weeks of culturing on MS media (Fig1). The influence of explant on callus formation was evaluted as percentage of the formed callus relative to the total number of planted explants (Table 2). The obtained results will be summarised as follows:

The influence of explant type and MS medium content from growth rgulators on callus induction:

The percentage of the formed callus on leaf and young stem explants was different. As shown in Table (2), the percentage of the formed callus on leaf explants was found to be higher than that of young stem. Moreover, the percentages of callus iniation and formation were varied according the medium type and its content from growth regulators. The highest callus induction percentage from the leaf explant (93.33%) was achived on MS3 medium while the highest percentage of callus intiation form the young stem node (81.25%) was achived using MS1 medium. The results in Table 2 is in a good agreement with many auther researchers (e.g. **Hasanloo *et al*. (2008), Cimino *et al*., (2006) and Rady *et al*. (2014)** .

Growth dynamics of *Silybum marinum* callus tissues:

Results of growth dynamic of *S. marinum* callus tissues are presented in Fig.1 and Tables (3 and 4). Table (3) and Fig.1 shows the weekly growth dynamics of *Silybum marinum*_callus tissues of young stem node origin grown on **MS1** medium supplemented with 1.5 mg /L 2,4-D + 1.5 mg /L BA. The fresh and dry weights of callus tissues were increased considerably during the growing period of 6 weeks. At the end of sixth week, callus tissues of young stem origin grow 3.63 times the size of the starting fresh weight and 3.62 times the size of the starting dry weight (Table 3). The growth values relative to fresh and dry weights of culture of stem origin was increased gradually with increasing the incubation periods (Table 3). Callus cultures of 6 weeks old showed the highest growth value. In the case of callus cultures of young stem, origin the highest growth value relative to fresh weight was 3.63 and it was 3.62 relative to dry weight. The daily growth of callus tissues related to fresh weight showed

that the fourth week was the most intensive growth period in which callus cultures displayed the maximum value of daily was slightly slow down. The maximum daily growth in the case of callus tissues of young stem origin was ranged between 2.14 mg/d in the second week to 5.00 mg/day in the fifth week.

Table (3) and Fig.1 shows the weekly growth dynamics of callus tissues of young stem origin grown on **MS2** media supplemented with 2 mg /L 2,4 D + 2 mg /L Kin. The fresh and dry weights of callus tissues were increased considerably during the growing period of 6 weeks. At the end of sixth week callus tissues of young stem origin, grow 3.42 times of the size of the starting fresh weight and 3.14 times of the size of the starting dry weight (Table 3). The growth values relative to fresh and dry weights of culture of young stem origin was increased gradually with increasing the incubation periods (Table 3). Callus cultures of five weeks old were showed the highest daily increase. The daily growth of callus tissues showed that the in the sixth week was decreased and slightly slow down. The maximum daily growth in the case of callus tissues of young stem origin was 4.28 mg/day and that achieved in the fifth week on MS1 and MS3 medium,respectively.

Table (3) and Fig. 1 shows the weekly growth dynamics of *Silybum marianum* callus tissues of young stem node origin grown on **MS3** media supplemented with 2 mg /L NAA+ 2 mg /L Kin. The fresh and dry weights of callus tissues were increased considerably during the growing period until 6 weeks. At the end of sixth week, callus tissues of young stem origin grow 4.13 times the size of the starting fresh weight and 4.52 times the size of the starting dry weight (Table 3). The growth values relative to fresh and dry weights of culture of stem origin was increased gradually with increasing the incubation periods (Table 3). The daily growth of callus tissues related to fresh weight showed that the fourth and fifth weeks were the most intensive growth period in where callus cultures displayed the maximum value of daily increase per day and slightly slowdown in the sixth week. The maximum daily growth in the case of callus tissues of young stem origin was 5.00 mg/day and that achieved in the fourth and fifth weeks on MS1 and MS3 medium, respectively.

Table (4) and Fig.1 shows the weekly growth dynamics of callus tissues of leaf origin grown on **MS1** media supplemented with 1.5 ml/L 2,4-D + 1.5 ml/L BA. The fresh and dry weights of callus tissues were increased considerably during the growing period of 6 weeks. At the end of sixth week; callus tissues of leaf origin grow 3.4 times the size of the starting fresh weight and 3.18 times the size of the starting dry weight (Table 4). The growth values relative to fresh and dry weights of culture of leaf origin was increased gradually with increasing the incubation periods (Table 4). Callus cultures of 6 weeks old showed the highest growth value. The daily growth of callus tissues related to fresh weight showed that the fifth week was the most intensive growth period in which callus cultures displayed the maximum value of daily was slightly slow down. The maximum daily growth in the case of callus tissues of leaf origin was 6.00 mg/day and that achieved in the fifth week.

Table (4) and Fig.1 shows the weekly growth dynamics of *Silybum marinum* callus tissues of leaf origin grown on **MS2** media supplemented with 2 mg /L 2.4-D + 2 mg/L Kin. The fresh and dry weights of callus tissues were increased considerably during the growing period of 6 weeks. At the end of sixth week; callus tissues of leaf origin grow 3.35 times of the size of the starting fresh weight and 3.24 times of the size of the starting dry weight (Table 4). The growth values relative to fresh and dry weights of culture of leaf origin was increased gradually with increasing the incubation periods (Table 4). Callus cultures of 6 weeks old showed the highest growth value. The daily growth of callus tissues related to fresh weight showed that the sixth week was the most intensive growth period in which callus cultures displayed the maximum value. The maximum daily growth in the case of callus tissues of leaf origin was 4.28 mg/day and that achieved in the 4^{th} week.

Table (4) and Fig. 1 shows the weekly growth dynamics of *Silybum marinum* callus tissues of leaf origin grown on MS3 media supplemented with 2 ml/L NAA + 2 ml/L Kin. The fresh and dry weights of callus tissues were increased considerably during the growing period of 6 weeks. At the end of sixth week, callus tissues of leaf origin grow 6.40 times of the size of the starting fresh weight and 6.11 times of the size of the starting dry weight (Table 4). The daily growth of callus tissues related to fresh weight showed that the fourth week was the most intensive growth period in which callus cultures displayed the maximum value of daily was slightly slow down. The maximum daily growth in the case of callus tissues of leaf origin was ranged between 7.54 mg/day and that achieved in the fourth week.

The statistical analysis of ***S.*** marianum callus growth dynamic grown from 2 different explant types (stem and leaf) and 3 different MS media types (MS1, MS2 and MS3) using LSD test and Dancun test are shown in Tables (5). Significant differences were found between the organ types and medium types in all measurements (callus fresh weight, callus dry weight, callus dry matter content, growth value relative to fresh, growth value relative to dry weight and growth increase. The callus fresh weight at the end of growth 6 weeks period was ranged between 175 to 300 mg / culture and the dry weight in the same period was ranged from 7.70 to 16.30 mg/ culture. Moreover, the growth value relative to fresh weight was ranged from 3.4 to 6.4 times while the growth value relative to dry weight was ranged from 3.12 to 6.26 times. These results are in a good agreement with the results of many authors (**Cacho*, et.al*. 1999; Cimino *et al*., 2006; Hassnloo *et al*. 2008 and Rady *et al*., 2014)**

Phytochemical studies:

Phenolic compounds are found in many plants and aid in the prevention of chronic diseases such as heart disease, diabetes, and cancer **(Zamora-Ros *et al*., 2014)**. Flavonoids are phenolic compounds found in a variety of plant tissues. They have a C6-C3-C6 skeleton (2 aromatic rings connected by a 3-carbon bond) and are phytoconstituents **(Agati *et al*., 2011)**. Tannins (also known as tannoids) are polyphenolic

plant secondary metabolites that bind to and precipitate proteins and alkaloids **(Khanbabaee and van Ree, 2001)**. In our study, the total phenolic contents (TPC), total flavonoid content (TFC) and total tannin content (TTC) in various parts of *Silybum marianum* wild type plants or tissue culture derived calluses) are shown in **Figure 2**, which expressed as mg gallic acid equivalent GAE/g extract, mg of quercin equivalent QCE/g extract and mg gallic acid equivalent GAE/g extract, respectively.

TPC, TFC and TTC were 19.67±0.88, 24.33±0.66, 41.67±1.45, 22.67±0.66, 24.67±0.33, 43.00±0.57, 36.67±0.88, 21.67±0.88,4.90±0.05,7.66±1.20,21.00± 0.57,13.33±1.20,14.33±1.20,20.33±0.80, 5.66±1.20,5.00±1.15,3.53±0.12,4..03±0. 49,3.76±0.29,1.33±0.20,3.96±0.28,2.30± 0.05,3.80±0.30 and 3.56±0.23 in the roots, stems, leaves, MS1 stems, MS1 leaves, MS3 stems, MS3 leaves and **MS2 leaves**, respectively. The highest value of TPC (41.67±1.45) was found in *S. marianum* leaves of wild type plant while the highest amount of TPC (43.00±0.57) in callus cultured was found in *S. marianum* callus derived-stems cultured on MS3 medium. There were no significant differences ($p < 0.05$) in TPC of *S. marianum* stems of wild type plants and callus culture derived from steam explant. Furthermore, the level of TPC in *S. marianum* callus derived-leaves grown on MS3 medium was significantly higher ($p < 0.05$) than the level of TPC in *S. marianum* callus derived-leaves grown on MS1 medium by 32.73 % (**Figure 2A)**.

Similarly, the highest quantity of TFC (21.00±0.57) was identified in *S. marianum* leaves of wild type plants, while the highest amount of TFC from tissue cultured derived calluses (20.33±0.80) was observed in calluses of *S. marianum* derived from stems explants cultured on MS3 medium (Fig. 2B). There was no significant ($p < 0.05$) difference in TFC level between S. *marianum* callus derived-leaves cultivated on MS3 and M.S4 media and the similar observation was noticed between roots and stems of wild type plants (**Figure 2)**.

Furthermore, the TTC level (Fig. 2C) did not significantly; ($p < 0.05$) differ between *S. marianum* roots, stems, and leaves of wild type plants. A similar finding was noticed between callus cultures of *S. marianum* derived from leaves cultivated on MS1, MS3 and MS2 medium, respectively. The TTC level in *S. marianum* callus derived-stems cultured on MS3 medium was significantly ($p < 0.05$) increased by 42.18 % compared to *S. marianum* callus derived-stems cultured on MS1.

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Table (1): MS media types and their content of plant growth regulators, which used in the experiment for callus induction from seedling explants of S. *marianum*.

MS* media type	Growth regulators content (in mg/L)			
	2,4- D	Kin	NAA	BA
MS1	1.5	-	-	1.5
MS2	2	2	-	-
MS3	-	2	2	-

MS*: Murashige and Skoog (1962)

Table (2): The influence of organ type and different media on callus induction.

Organ	Media type*	No. of cultured explants	No. of callus formed	% of the formed callus
Leaf	MS1	90	75	83.33
	MS2	80	60	75.00
	MS3	75	70	93.33
Stem node	MS1	70	60	85.71
	MS2	75	50	66.66
	MS3	80	65	81.25

*MS1= MS medium +1.5 mg/L 2.4-D + 1.5 mg/L BA. MS2= MS medium + 2 mgl/L 2.4-D + 2 mg/L Kin. and MS3= MS medium + 2 mgl/L NAA + 2 mg/L Kin.

Table: (3) Young Stem node callus growth dynamic on MS1, MS2 and MS3 media.

Age in weeks	MS Media Type*	Callus F. W. in mg/ culture	Callus D. W. in mg/ culture	Dry matter %	Growth rate/F.W	Growth rate /D.W.	Daily increase in mg. /day
0	**MS1**	40.50 ±2.00	2.00±0.01	5.00	--	--	--
	MS2	40.30±1.05	2.10±0.03	5.25	--	--	--
	MS3	40.60±0.80	2.04±0.02	5.10	--	--	--
1	**MS1**	55.20±2.75	2.69±0.13	4.89	0.37	0.34	2.14
	MS2	53.22±1.30	2.90±0.04	4.47	0.33	0.38	1.85
	MS3	60.30±0.85	3.10±0.06	5.11	0.50	0.55	2.85
2	**MS1**	75.30± 3.75	3.64±0.15	4.85	0.82	0.83	2.85
	MS2	72.53±1.70	3.20±0.04	4.44	0.80	0.52	2.17
	MS3	85.90±1.60	4.36±0.18	5.08	1.13	1.18	3,54
3	**MS1**	105.50±5.25	5.35±0.14	5.02	1.63	1.67	4.28
	MS2	97.02±1.90	3.80±0.06	3.91	1.43	0.80	3.57
	MS3	115.03±2.75	5.61±0,28	4.87	1.88	1.58	4.28
4	**MS1**	140.60±7.00	6.79±0.33	4.85	2.50	2.39	5.00
	MS2	125.09±2.10	5.10±0.08	4.08	2.12	1.42	4.00
	MS3	150.05±2.50	6.89±0,34	4.59	2.75	2.44	5.00
5	**MS1**	165.70±6.66	8.00±0.40	4.84	3.13	3.00	3.57
	MS2	155.20±2.70	7.10±0.09	4.58	2.87	2.38	4.28
	MS3	185.70±2.75	9.03±0.45	4.88	3.63	3.52	5.00
6	**MS1**	185.80±7.40	9.25±0.37	5.00	3.63	3.62	2.85
	MS2	177.05±3.10	7.70±0.19	4.92	3.42	3.14	3.11
	MS3	205.70±3.55	11.08±0.55	5.03	4.13	4.52	2.85

*MS1= MS medium +1.5 mg/L 2.4-D + 1.5 mg/L BA. MS2= MS medium + 2 mg/L 2.4-D + 2 mg/L Kin. and MS3= MS medium + 2 mg/L NAA + 2 mg/L Kin.

Table (4) leaf callus of *S.marinum* growth dynamic on MS1, MS2 and MS3 media.

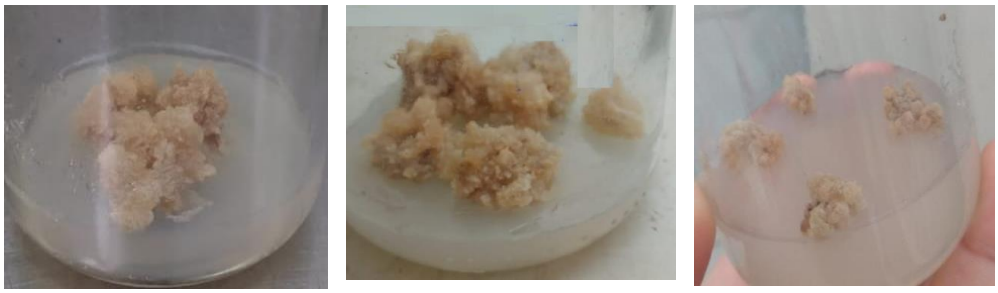
Age in weeks	MS Media Type*	Callus F. W. in mg/ culture	Callus D.W. in mg/ culture	Dry matter %	Growth rate/F.W	Growth rate /D.W.	Daily increase in mg. /day
0	**MS1**	40.00±0.01	2.02±0.05	5.00	--	--	--
	MS2	40.20±2.50	2.06±0.03	5.14	--	--	--
	MS3	40.50±2.31	2.15±0.11	5.03	--	--	--
1	**MS1**	55.10±1.09	2.80± 0.07	5.09	0.37	0.27	2.14
	MS2	56.33±4.90	2.75±0.05	4.89	0.40	0.33	2.30
	MS3	70.80±3.50	3.46±0.17	4.89	0.74	0.60	4.32
2	**MS1**	64.67±2.08	3.00±0,06	4.68	0.60	0.36	1.28
	MS2	77.60±4.40	3.74±0.07	4.82	0.93	0.82	3.04
	MS3	110.70±5.51	5.40±0.21	4.88	1.62	1.51	5.70
3	**MS1**	85.33±1.52	3.90±0.09	4.58	1.13	0.77	3.00
	MS2	100.00±2.10	4.80±0.07	4.80	1.48	1.33	3.20
	MS3	160..90±6.40	7.84±0.31	4.87	2.97	2.47	7.17
4	**MS1**	107.67±5.37	5.01±0.10	4.68	1.68	1.27	3.14
	MS2	130.00±2.69	6.28±0.08	4.83	2.23	2.04	4.28
	MS3	213.20±6.30	10.25±0.49	4.87	4.19	3.75	7.54
5	**MS1**	149.67±5.63	7.00±0.11	4.69	2.72	2.18	6.00
	MS2	155.33±2.56	7,59±0.09	4.89	2.86	2.68	3.61
	MS3	260.30±7.42	12.70±0.63	4.89	5.41	4.91	6.12
6	**MS1**	176.33±4.10	9.20±0.10	5.22	3.40	3.18	3.85
	MS2	175.06±4.50	8.75±0.09	5.00	3.35	3.24	2.81
	MS3	300.40±9.66	15.30±0.78	5.10	6.40	6.11	5.71

*MS1= MS medium +1.5 mg/L 2.4-D + 1.5 mg/L BA. MS2= MS medium + 2 mg/L 2.4-D + 2 mg/L Kin. and MS3= MS medium + 2 mg/L NAA + 2 mg/L Kin.

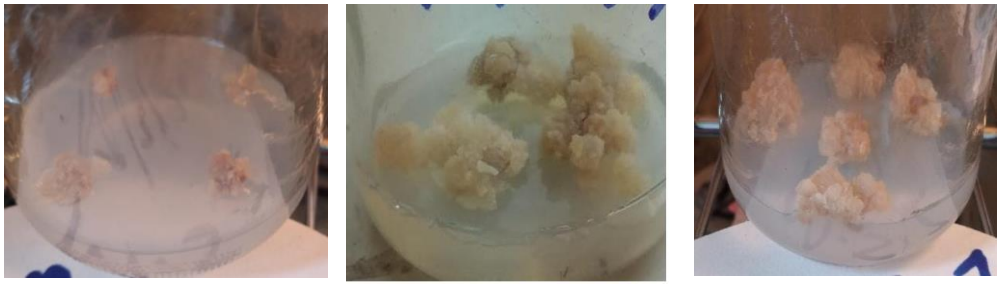
Table (5): Statically analysis of *S. marianum* callus growth dynamic grown from 2 different explant types (stem and leaf) and 3 different MS media types (MS1, MS2 and MS3).

Treatment	Stem			Leaf			Statistical data		
	MS1	MS2	MS3	MS1	MS2	MS3	F values	LSD 0.05	LSD 0.01
callus F.W.in mg/c	185^{c}	177^{d}	205^{b}	176^{d}	175^{d}	300^{a}	429.3360	7.427	10.56
callus D.W.in mg/c	9.25^{c}	7.70^{d}	11.12^{b}	9.20^{c}	8.75^{cd}	16.30^{a}	47.2096	1.422	2.023
Callus D.contnat%	5.00^{bc}	4.92^{c}	5.03^{abc}	5.22^{ab}	5.28^{a}	5.10^{abc}	3.1121	0.2441	0.3472
Growth g /F	3.63^{c}	3.42^{d}	4.13^{b}	3.40^{d}	3.55^{c}	6.40^{a}	2373.9959	0.08136	0.3660
Growth value g/D	3.62^{c}	3.14^{d}	4.52^{b}	3.12^{d}	3.24^{d}	6.26^{a}	398.6497	0.1908	0.2714
Growth rate mg/day	3.15^{d}	3.26^{c}	3.92^{b}	3.23^{c}	3.21^{c}	6.19^{a}	3394.3817	0.05753	0.08183

The means followed by the same letter are not significantly differences.



MS1 Leaf **MS2 Leaf** **MS3 leaf**



MS1Stem node **MS2 Stem node** **MS3 Stem node**

Fig. 1: Callus cultures one month age of *Silybum marinum* of leaf and stem node grown on different media.

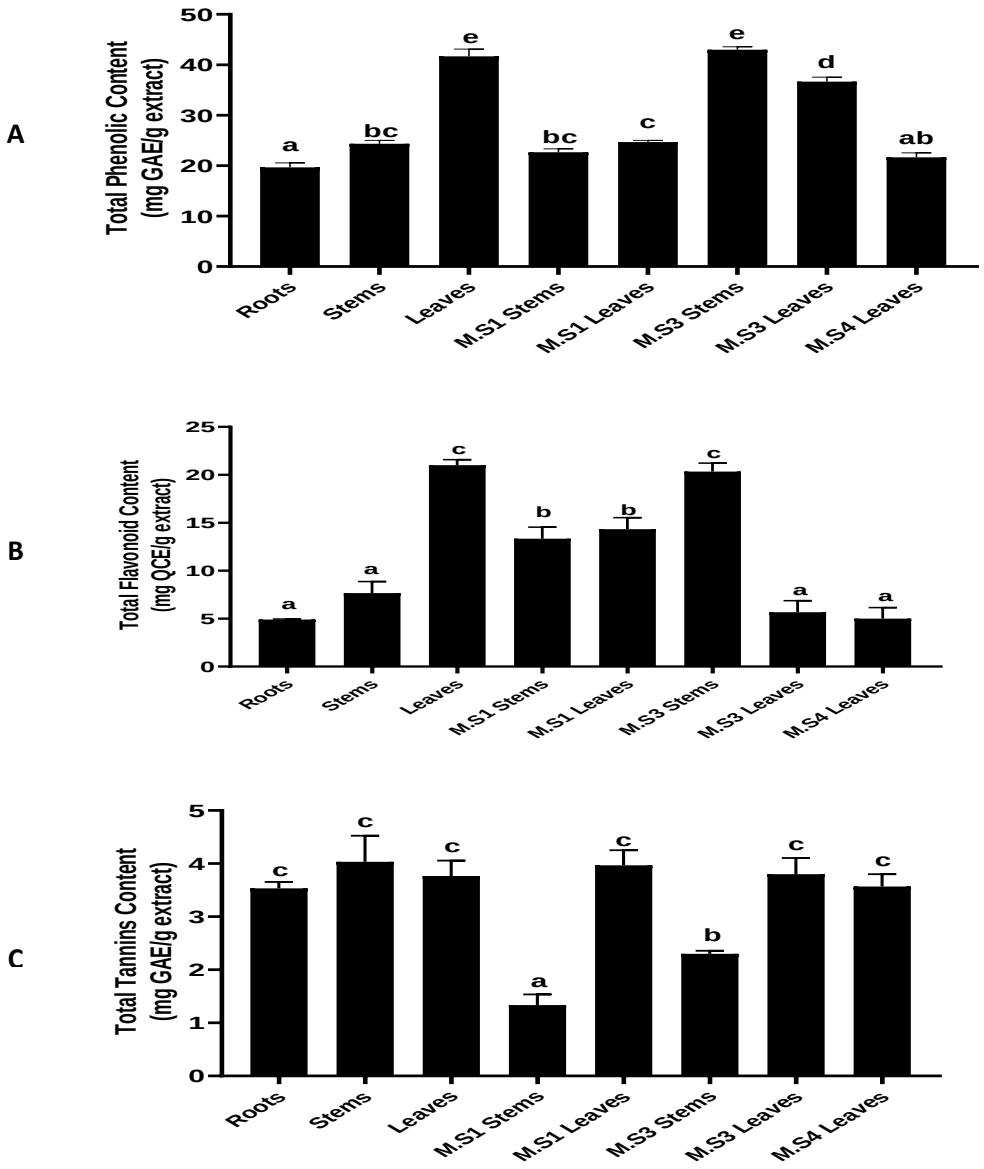


Figure (2): A: The total phenolic content (mgGAE/g extract); B: total flavonoid content (mgQCE /g extract) and C: total tannin content (mgGAE/g extract) in various *Silybum marianum* extracts (wild plant or callus cultures). Values are presented as mean ± SEM, Significant differences ($p < 0.05$) are indicated by different superscript letters.

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الملخص العربي

تأثير منظمات نمو النبات ونوع العضو على إنتاج الكالوس وديناميكية النمو ومحتوى البولي فينول والفلافونويد والتانين في زراعة أنسجة نبات شوك الجمل.(*Silybum marianum L.Gaertn.*)

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نبات شوك الجمل أو السليبين (*Silybum marianum* L. Gaertn., Asteraceae) هو نبات عريض الأوراق حولى أو ثنائى الحول موطنه الأصلى شمال أفريقيا والبحر الأبيض المتوسط وله خصائص طبية قيمة. المركب الفعال في الـ S. marianum هو السيليمارين. وهو عبارة عن خليط أيزوميري من مركبات الفلافونويد وهو يستخدم في الصناعات الدوائية. يعتبر شوك الجمل المنتج العشبي الأكثر استخداما فى علاج أمراض الكبد المزمنة وقد يكون مفيدًا لتقليل فرص الإصابة ببعض أنواع مرض السرطان. وقد تم استخدام المستخلصات النباتية الثانوية، والتي تم استخلاصها من النباتات المزروعة، لفترة طويلة ولكن الاتجاه العام الجديد هو استخلاص هذه المستخلصات الثانوية من النباتات المزروعة في القوارير. ولا يمكن أن نتجاهل أهمية استخدام تقنيات زراعة الأنسجة والتي لها دور مهم فى تسهيل الأبحاث النباتية، كما أن هناك العديد من الأسباب الأخرى مثل الحماية من الطقس المتغير ومن الأمراض والآفات ومشاكل التربة والحصول على كميات كبيرة من النباتات بتكلفة منخفضة. أظهرت نتيجة التجربة الحالية وجود اختلافات معنوية في تأثير نوع العضو (المنفصل) النباتي ونوع الوسط الغذائى على تكوين الكالوس وديناميكية النمو والمحتوى الكيميائي من البولي فينول والفلافونويد والتانينات. وقد وجد أن أفضل وسط غذائى لنمو الكالوس من الورقة هو MS3 (مزوده بـ 2mg Kin/L + 2 mg/L NAA) والوسط الغذائى MS1 (1.5 mg/L 2,4-D + 1.5 mg/L BA) بالنسبه للعقد الساقية. وقد وجد أن أعلى وزن طازج للكالوس كان 9.66±300.40 ملجم/مزرعة وذلك على الوسط الغذائى MS3 الناتج من الورقة. كما أوضحت الدراسة أن كالوس العقدة الساقية الذي ينمو على الوسط الغذائي MS3 أعلى في محتواه من البولي فينول والفلافونويد بينما أظهر كألوس الأوراق الذي ينمو على الوسط الغذائي MS1 والـ MS3 أعلى محتوى من التانينات.