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The effect of some treatments on development, growth and differentiation of chinese clove (*Dianthus chinensis* L) callus and the accumulation of some secondary compounds

Shaimaa Hashim Hussein[1] and Nadhim Salim Ghanim[2]

Department of Horticulture / University of Tikrit College of Agriculture

***Corresponding author e-mail**: Bmx5383@gmail.com

Abstract:

Experiment was conducted in tissue culture laboratory of Department of Horticulture and Landscape Engineering at College of Agriculture, Tikrit University, for the period 2022-2023 to develop callus from the Chinese clove plant (*Dianthus chinensis* L). The callus was developed from seedlings resulting from tissue culture, experiment was carried out according to design RCD.Parts of a real leaf were grown on MS medium supplemented with the growth regulator dichlorophenoxy acid (2,4-D) at concentrations (0, 0.5, 1, 1.5, 2) mg.L^{-1} mixed with Kinin (0.5) mg.L^{-1}. Results of callus induction showed significant differences in the volume of callus at a concentration of 1.5 mg L^{-1}, which gave highest callus volume of 1,550 g. As for wet weight, the concentration of 1 mg L^{-1} achieved highest wet weight of 1,364 g, while the concentration achieved 2 mg L^{-1} highest percentage of callus-forming parts.With regard to callus stimulation, it was found that addition of different concentrations of jasmonic acid (0, 25, 50, 75 µmol) mixed with sorbitol acid at concentrations (0, 20 mg L^{-1}) gave a significant increase in studied characteristics (wet weight, callus volume, weight dry) as treatment JA_2 at a concentration of 25 multiplicationes and SOR_2 at a concentration of 20 mg L^{-1} achieved highest values for studied traits. Some secondary metabolic compounds were estimated from the callus leaves of Chinese clove plant resulting from tissue culture, and their percentages were compared with the use of growth regulators at different concentrations. It was found that adding jasmonic acid at a concentration of 75 multiplication with sorbitol acid at a concentration of 20 mg L^{-1} recorded best amount of active substances (Eugenol) , Caryophyllene, Terpinen, Linalool, Pinen) were (63.33, 27.50, 2.50, 2.00, 2.70%), respectively.

Keywords: Jasmonic acid, sorbitol acid, callus, secondary metabolic compounds.

تاثير بعض المعاملات في نمو وتطور وتمايز القرنفل الصيني (*Dianthus chinensis* L) وتراكم بعض مركبات الايض الثانوي .

شيماء هاشم حسين و ناظم سالم غانم

قسم البستنة وهندسة الحدائق / جامعة تكريت كلية الزراعة

الخلاصة

أجريت هذه التجربة في مختبر الزراعة النسيجية التابع لقسم البستنة وهندسة الحدائق في كلية الزراعة جامعة تكريت للفترة 2022-2023 لاستحداث كالس نبات القرنفل الصيني (***Dianthus chinensis* L**) حيث تم استحداث الكالس من بادرات ناتجة من الزراعة النسيجية،نفذت التجربة وفق تصميم RCD. زرعت أجزاء من ورقة حقيقية على وسط MS المزود بمنظم النمو ثنائي الكلورو فينوكسي الحامضي (2.4-D) بالتراكيز (0 و0.5 و 1 و 1.5 و 2) ملغم .لتر$^{-1}$ متداخلاً مع Kin(0.5) ملغم .لتر$^{-1}$ ، اذ اظهرت نتائج استحثاث الكالس فروقاً معنوية في حجم الكالس عند تركيز 1.5ملغم لتر-1 اذ اعطى اعلى حجم

للكالس بلغ 1.550 غم ، اما الوزن الرطب فقد حقق التركيز 1 ملغم لتر$^{-1}$ اعلى وزن رطب بلغ 1.364 غم ، بينما حقق التركيز 2 ملغم لتر$^{-1}$ اعلى نسبة مئوية للاجزاء المكونة للكالس. وفيما يخص تحفيز الكالس فقد وجد ان اضافة تراكيز مختلفة من حامض الجاسمونيك (75،50،25،0 مايكرومول) متداخلا مع حامض السوربيتول بتراكيز (0، 20 ملغم لتر$^{-1}$) اعطى زيادة معنوية للصفات المدروسة (الوزن الرطب،حجم الكالس ، الوزن الجاف) اذ حققت المعاملة Ja_2 بتركيز 25 مايكرومول وSor_2 بتركيز 20 ملغم لتر$^{-1}$ اعلى القيم للصفات المدروسة ، وتم تقدير بعض مركبات الايض الثانوية من اوراق الكالس لنبات القرنفل الصيني الناتج من الزراعة النسيجية ومقارنة نسبتها مع استخدام منظمات النمو بتراكيز مختلفة اذ وجد ان اضافة حامض الجاسمونيك بتركيز 75 مايكرومول مع حامض السوربيتول بتركيز 20 ملغ لتر$^{-1}$ سجل افضل كمية من المواد الفعالة (Eugenol ، Caryophyllene، Terpinen، Linalool، Pinen) بلغت (27.50، 63.33، 2.50، 2.00، 2.70 %) على التوالي.

الكلمات المفتاحية: حامض الجاسمونيك ، حامض السوربيتول ، الكالس ، مركبات الايض الثانوي.

Introduction

Chinese carnation or rainbow carnation *Dianthus chinensis* L. is a perennial herbaceous plant with a height of 30-50 cm. It belongs to the carnation family (Caryophyllaceae), which includes 88 genera and more than 2,000 species, native to northern China, Korea, Mongolia, and southeastern Russia. It is one of most popular ornamental plants. In the world, which is characterized by beauty of its flowers and its great medicinal value [16] ,it is widely cultivated in gardens and decorated because of its variety of colors and attractive shades, in addition to its permanent flowering nature and availability of a wide range of multiple colors, and cultivation of cloves is spread all over the world except for the humid tropics [9] [23].Plant tissue culture means isolating a cell, plant organ, or tissue under conditions free of pathogens and cultivating it in sterile nutritional media. This results in development of transplanted part under ideal conditions in terms of light and heat [2]**.** Through tissue culture, it is possible to produce large numbers of plants that bear same characteristics as mother and are free of pathogens, in a short period of time and throughout year in a small area. This technique can be used in research fields, for applied purposes, and in breeding and improving plants. This can be done using many methods, including : Cell culture, process of merging protoplasts, and inducing callus cells to form somatic embryos that develop into vegetative growths [20].In recent decades, scientific and applied aspects of tissue culture techniques (ex vivo cultivation) have spread widely and rapidly in various countries of world, as some developed countries have resorted to using them in the production of secondary metabolic compounds from plants, which are primary source of drug production [3] [20]**.** Jasmonic acid, whose formula is C_{12} H_{18} O_3, is one of natural compounds found within the plant. It is one of volatile organic compounds that plays an important role in the regulation and development processes in plant, as well as its role in stimulating the defense system against mechanical stresses and various pathogens and environmental stresses. Such as drought, high and low temperatures, and it also has a role in improving the quality of fruits after harvest and reducing their susceptibility to physiological damage [1] [24] ,have shown that jasmonic acid has a role in the biosynthesis of secondary metabolites, so its use as a catalyst It is preferred for increasing the production of pharmaceutically active compounds in a short time and at a lower cost.

Carbohydrates, including sugars, are among important components of any nutrient medium, and their effect on cultivated plant part is determined by their being a source of energy and carbon, as well as their role in regulating osmosis of the medium [11]. Glucose and sorbitol are mannitol as a source of carbon to the culture medium. Alcoholic sugars are an essential component of plant tissue culture media and play a secondary role as a source of carbon (energy) and as an osmotic factor [8] .Sorbitol sugar inhibits growth of branches but increases the cell content of solutes [24],sorbitol and mannitol also have an important role in increasing the secondary metabolite compounds and increasing the dry matter of callus [28]. This study aims at possibility of creating callus from growing apex and stimulating production of secondary metabolite compounds from callus.

Materials and Methoods :

Experimental location:

Experiment was carried out in Plant Tissue Culture Laboratory in the Department of Horticulture and Landscape Engineering - College of Agriculture, Tikrit University. To develop and stimulate callus and stimulate the active compounds of Chinese clove plant, experiment was carried out according to a completely randomized design (C.R.D) arrangement. Complete random design (with ten replicates for each treatment, and differences between means were compared using Duncan's multinomial test at a 5% probability level, and ready-made program was used [27].

Plant parts used in agriculture :

In this experiment, various parts of Chinese clove seedlings, 5 cm long, were used, and their tissues were multiplied in order to obtain plant parts free of contaminants to conduct experiments on. A part of leaf, 1 cm long and 0.5 cm wide, was taken after cutting its edges, containing the central vein, with the aim of creating callus.

Preparing the nutrient medium used in experiment:

The MS nutrient medium from the Indian company (HIMEDIA) recommended by [29**]** was used, with some modifications in the growth regulators according to the aim of the study.

I prepared stock solutions of used plant growth regulators and kept them in the refrigerator at a temperature of 4° in glass containers. To prepare one liter of medium, put distilled water in a glass container on a rotating heater (magnnetic stirrer hot plate), then add 9 grams of agar to it until it boils. After complete dissolution of agar and homogeneity of solution, 4.43 grams of MS were added to it, then 30 grams of sucrose were added to it, then volume was completed to 1000 ml , then regulators were added to it according to goal of study, then the pH was adjusted to 5.8±0.1 by adding drops of 1 standard sodium hydroxide (NaOH) or 1 standard hydrochloric acid. Standard HCl using a pH meter, and the food medium was distributed at the rate of 20 ml per vial for glass bottles of 100 ml, and nozzles of bottles were covered with heat-resistant aluminum foil.

Sterilization:

After distributing the nutritional medium in glass bottles according to quantities required for the experiment, they were sterilized with an autoclave device at a temperature of 121 °C and a pressure of 1.04 atmosphere for a period of 20 minutes.

Growth regulators used in experiment :

I used growth regulators from (HI Media- India) in different concentrations, which are Jasmonic acid (Ja), Sorbitol acid (D- glucitol), Furfuryl ammino purine (Kintein-6) .

Callus induction experiments

Leaf was chosen to create callus by culturing it on MS medium supplied with different levels of growth regulator 2,4-D at concentrations (0, 0.5, 1, 1.5, 2 mg L^{-1}) interfering with Kin kinine at a concentration (0.5 mg L^{-1}). After four weeks of cultivation, the following measurements were taken (percentage of parts that formed callus, callus volume, callus wet weight) and then callus was propagated by culturing it on the best concentration of (2.4-D), which is a concentration of (1) mg.L^{-1}.

Experiments with the production of secondary metabolites :

After completing callus generation process, the best concentration was chosen and callus was grown on MS medium supplemented with sorbitol acid at a concentration of (0, 20 mg L^{-1}) mixed with jasmonic acid at a concentration of (0, 25, 50, 75 µmol). After four weeks had passed, the following measurements were taken: Callus volume, callus wet weight, callus dry weight).

Estimation of some secondary metabolite compounds using a GC device, separation method:

Oil extraction:

Taken was (5 g) from fresh sample and placed in a beaker, and (50 ml) of sterilized distilled water was added to it and placed in a cliffhanger for 3 hours. The oil was collected and 5 ml of hexane was added to it to separate oil from water droplets collected with oil. The oil was collected and stored in dark bottles in the refrigerator until analysis was performed.

Analysis method:

Examination was conducted in the science and technology laboratories / Department of Environment and Water using a gas chromatography device, model Shimadzu 2010, of Japanese origin, using a flame ionization detector (FID) and using a capillary separation column type (DM-5Ms) with lengths (30m * 0.25um * 0.25mm), where temperature was injection area and the detector, respectively: (280, 340 C), while temperature of separation column was gradual, starting from (300-100) C at a rate of increase of 10 degrees/min. Use inert nitrogen gas as carrier gas at a rate of 100Kpa. [32]

Concentration of substance was analyzed according to following equation:

$$\text{Concentration of substance} = \frac{\boldsymbol{sample\ area \times Standard\ area\ concentration}}{\boldsymbol{Standard\ material\ area}} \times \frac{\boldsymbol{Dilution}}{\boldsymbol{Sample\ volume}}$$

Results and Discussions:

Callus induction experiment:

Effect of different concentrations of 2,4-D and kin on induction of leaf callus of Chinese clove plants propagated by tissue culture.

It was found through the data of Table (1) that different concentrations of 2,4-D and kin for callus induction led to a significant increase in volume of callus, as concentrations (0.5, 1.5, 2, and 1 mg L^{-1}) respectively gave highest values (2.300). And 1.550, 1.450 and 1.450 gm) respectively, while comparison treatment recorded lowest value for the calus volume of 1.100 gm.

Table (1) also showed a significant superiority in wet weight of callus, as the concentration of 1 mg L^{-1} achieved a significant increase of 1.364 g compared to control treatment 0 mg L^{-1}, which gave the lowest value of 0.00 g wet weight, while concentrations 0.5 and 1 did not achieve. 5 and 2 mg L^{-1}, any significant difference for this characteristic.

With regard to percentage of callus-forming parts, we note that concentrations (0.5, 2, 1, 1.5 mg L^{-1}), respectively, gave a significant superiority in this characteristic, reaching (1.00, 0.900, 0.800, 0.700%) respectively, compared to comparison treatment 0 mg L^{-1}, which gave the lowest value for the parts forming callus, as it eliminated 0.300%.

Table (1) effect of different concentrations of 2,4-D and kin on induction of leaf callus of Chinese clove plants propagated by tissue culture.

Kin	2,4-D Mg.L^{-1}	Callus Volume	Wet weight of callus (g)	Percentage of callus-(%) forming parts
0.5 Mg.L^{-1}	0	1.100 a	0.000 b	0.300 b
	0.5	2.300 a	0.623 ab	1.00 a
	1	1.450 a	1.364 a	0.800 a
	1.5	1.550 a	0.583 ab	0.700 a
	2	1.450 a	0.512 a	1.090 a

- Coefficients with similar letters are not significantly different from each other according to Duncan's multinomial test at the 5% probability level.

Callus induction and growth depend on several factors, including type of growth regulator and its quantity present in culture medium, type of plant part, its source, and cultivation conditions. In general, this tissue is used in propagating plants through tissue culture by creating and growing it on media supplied with auxins and cytokanins, in addition to extracting and estimating some active substances and by-products [4] [5]

Through tissue culture, plant cells can be stimulated to form a callus by cultivating distinct plant shoots and by adding growth regulators to the nutrient media under tissue culture conditions so that the cultured living cells can lose their functional dedifferentiation and return to their meristematic state by restoring the ability to divide again and produce a large group of undifferentiated cells. Differentiated callus problem [14] .And the callus formed from these procedures can be re-implanted and its cells can be influenced by several factors, the most important of which are hormonal factors, in order to preserve and multiply it, or stimulate it to differentiate into new cells to form new vegetative organs, organogenesis, or embryogenesis, all way to plants. complete [26].

Process of callus generation and differentiation from plant parts resulting from tissue culture is demonstrated and explains the process of callus formation, as well as the compatibility of the nutrient medium and growth regulators with concentrations that promote cell division in plant parts whose ability to respond varies depending on their source [19] and use and combination of regulators used. 2,4-D with kin, which gave a better volume and weight than callus, and this is explained by the fact that 2,4-D is one of the auxins that encouraged cell expansion and division, and also its role in cellular elongation and expansion, which results due to role of auxin in removing calcium ions attached to the carboxyl group. Supporting cell roots and building nucleic acids [12]**.**

These results are in line with [31**]** when they obtained the largest callus volume on medium prepared with 1.0 and 2.0 mg.L^{-1} of 2,4-D on belladonna plant. Reason for the significant superiority in callus weight and volume may be attributed to a compound kin, which is used in tissue culture to increase the multiplication of cell growth and to build amino acids and its role in development of emerging cells [13]**.**

- **Callus stimulation experiments**

Effect of different concentrations of Jasmonic acid and Sorbitol and their interaction on the wet weight of Chinese clove plant calluses.

Table (2) showed that the different concentrations of jasmonic acid achieved a significant increase in wet weight characteristic of callus, as treatment Ja_2 recorded highest value, amounting to 2.438 g, compared to comparison treatment Ja_1, which gave the lowest value, amounting to 0.797 g.

same table also showed that sorbitol acid had a significant effect on this value. The characteristic: Treatment SOR2 recorded the highest weight, amounting to 2.137 grams, compared to the comparison treatment SOR1, which recorded the lowest value, amounting to 0.767 grams.

Results of Table (2) also showed that interaction had a significant effect on wet weight of callus, as treatment Ja_4SOR_1 achieved the highest wet weight of callus, amounting to 3.038 grams, compared to the comparison treatment Ja_1SOR_1, which recorded the lowest value of 0.000 grams, while treatment Ja_2SOR_2 did not achieve any significant superiority in wet weight of callus.

Table (2) Effect of different concentrations of jasmonic acid and sorbitol and the interaction between them on wet weight of callus of the Chinese clove plant.

Ja / SOR	Ja1	Ja2	Ja3	Ja4	average (SOR)
SOR1	0.000 c	1.595 b	1.837 b	3.038 a	0.767 b
SOR2	0.000 c	2.195 ab	1.231 b	1.720 b	2.137 a
average (Ja)	0.797 b	2.438 a	1.097 b	1.475 a	

- Coefficients with similar letters are not significantly different from each other according to Duncan's multinomial test at the 5% probability level.

Effect of different concentrations of Jasmonic acid and Sorbitol and their interaction on the callus volume of Chinese clove plants

The data shown in Table (3) indicate that there was a significant increase in the volume of callus with addition of jasmonic acid, as Ja_2 gave the highest value for the volume of the callus, reaching 3,650 grams, compared to the comparison treatment, Ja1, which gave lowest value, amounting to 1,400 g . Sobitol acid also outperformed it in this capacity. The SOR_2 treatment achieved the highest value, amounting to 2.875 grams, compared to comparison treatment SOR_1, which achieved the lowest value, amounting to 1,525 g.

Regarding the binary interaction between jasmonic acid and sorbitol, it was found that treatment Ja_3SOR_1 recorded a significant superiority over the rest of treatments by giving it a volume of 3,800 g , while treatments J_1SOR_2, J_1SOR_1, J_3SOR_2, and Ja_4SOR_2 achieved lowest values, amounting to (0.000, 0.100, 2,200, and 2,300 g). While the following coefficients J_2SOR_1, Ja_2SOR_2 and J_4SOR_1 did not reach significance in this capacity.

Table (3) Effect of different concentrations of Jasmonic acid and Sorbitol and the interaction between them on callus volume of Chinese carnation plant.

Ja / SOR	Ja1	Ja2	Ja3	Ja4	averge (SOR)
SOR1	0.100 d	2.700 bc	3.800 a	3.500 ab	1.525 b
SOR2	0.000 d	3.000 abc	2.200 c	2.300 c	2.875 a
(Ja) averge	1.400 c	3.650 a	1.500 c	2.250 b	

- Coefficients with similar letters are not significantly different from each other according to Duncan's multinomial test at the 5% probability level.

Effect of Jasmonic and Sorbitol on wet weight :

The data in Table (4) showed that adding different concentrations of jasmonic acid and sorbitol leads to an increase in wet weight of callus, as it was found that the Ja_2, Ja_4, and Ja_2 treatments,

respectively, recorded the highest values for jasmonic acid, reaching (1.786, 1.748, 1.440 g). Respectively compared to comparison treatment Ja_1, which recorded the lowest value amounting to (0.00 g), sorbitol acid also achieved significant superiority for this characteristic if treatment SOR_2 achieved the highest value amounting to 1.264 g while treatment SOR_2 achieved the lowest value amounting to 1.222 g.

Interaction between the two treatments also achieved significant superiority in the wet weight characteristic, as the following treatments (Ja2SOR2, Ja_3SOR_1, and Ja_4SOR_1) respectively recorded the highest values, reaching (2.204, 1.573, 2.116 g), respectively, while the comparison treatment Ja_1SOR_1 recorded the lowest value, amounting to 0.00. g, while the following treatments (Ja_2SOR_1, Ja_3SOR_2, and J_4SOR_2) did not achieve any significant improvement in wet weight.

Table (4) Effect of Jasmonic and Sorbitol on wet weight

Ja / SOR	Ja1	Ja2	Ja3	Ja4	averge (SOR)
SOR1	0.000 b	1.368 ab	1.573 a	2.116 a	1.264 a
SOR2	0.000 b	2.204 a	1.307 ab	1.379 Ab	1.222 b
(Ja) averge	0.000 b	1.786 a	1.440 a	1.748 a	

- Coefficients with similar letters are not significantly different from each other according to Duncan's multinomial test at the 5% probability level.

Effect of Jasmonic and Sorbitol on dry weight

The data in Table (5) indicated that there was a significant increase in the dry weight characteristic after adding different concentrations of jasmonic acid, as treatments Ja_2, Ja_3, Ja4 gave the highest values, reaching (0.203, 0.112, 0.117 g), respectively, while the comparison treatment gave Ja_1. Lowest value was (0.00 g), and sorbitol acid also gave a significant increase in this characteristic, as treatment SOR_1 gave highest value, amounting to 0.120 g, while treatment SOR_2 gave lowest value, amounting to 0.096 g, for dry weight.

Binary interaction coefficients for the experiment between jasmonic acid and sorbitol also recorded a significant increase in dry weight, as following coefficients (Ja_2SOR_1 and Ja2SOR2) respectively gave the highest values, reaching (0.210, 0.196 g), respectively, in comparison with the comparison treatment Ja_1SOR_1, which gave the lowest value of (0.00 g), while the following treatments did not achieve any significant differences for this trait: Ja_3SOR_1, Ja_4SOR_1, Ja_3SOR_2, Ja_4SOR_2, respectively.

Table (5) Effect of jasmonic and sorbitol on dry weight

Ja / SOR	Ja1	Ja2	Ja3	Ja4	averge (SOR)
SOR1	0.000 b	0.210 a	0.135 ab	0.137 ab	0.120 a
SOR2	0.000 b	0.196 a	0.090 ab	0.097 ab	0.096 b
(Ja) averge	0.000 b	0.203 a	0.112 a	0.117 a	

- Coefficients with similar letters are not significantly different from each other according to Duncan's multinomial test at the 5% probability level.

Callus cultivation is unique as one of tissue culture techniques and is particularly important because of rapid metabolism and growth in callus cells compared to their counterparts in natural plants, which can provide, within a short period, a high rate of production of secondary metabolic

compounds, many of which are of vital importance, in addition to high rate of mutation formation in During the formation of callus tissue, it can result in the formation of new strains that may be highly productive with active compounds [6]

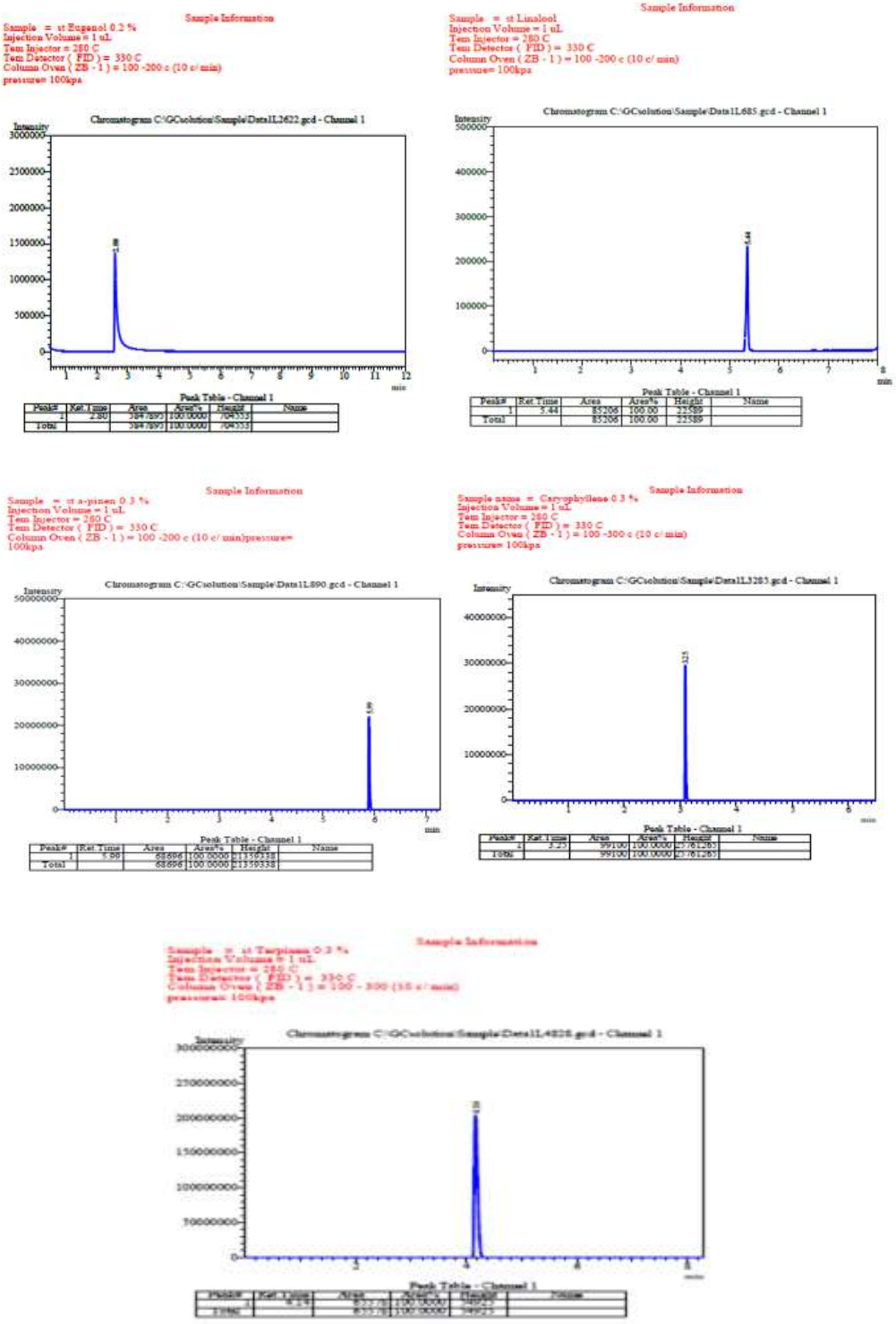
The positive results in wet weight and dry weight of callus were achieved by stimulating the cultivated plant parts to produce secondary compounds when exposed to stress, which is represented by adding some compounds to the nutrient medium, including sugars such as sorbitol, as [25]. Showed that increasing the concentration of sucrose added to medium from 2% To 4%, it led to an increase in the production of the polyphenol compound in callus of shrub rose plant Rosa hybrida, while [10] .Indicated that raising the percentage of sucrose from 2% to 5% in growing medium of the callus of the rosemary plant led to an increase in the concentration of Rosmaric acid.

Active compounds in seeds of coriander plant, increased with the addition of sorbitol, as the percentage of flavonoids in seeds increased by 79.80%, and accumulation of antioxidants increased after analyzing them with a GC-MS device [17]

Reason may be due to jasmonic acid, which is one of modern plant growth regulators that is characterized by its high ability to hinder the growth of plant tissue. Jasmonic acid is one of most important, common and widely used compounds, as laboratory experiments have proven the role of jasmonic in causing many physiological changes within plant tissues accompanied by morphological changes. In the laboratory-grown plant part, in addition to its role in reorganizing production of plant cells for both primary and secondary compounds by stimulating them to express the genes responsible for the biosynthesis of those compounds, jasmonic has been used in many of the genes responsible for the biosynthesis of those compounds. Practical applications of agricultural technology Plant tissues, including the production of some medicinal compounds and the stimulation and initiation of somatic embryos of some economic plants outside the living body [15] [22].

Table (6) Effect of treatment with jasmonic acid and sorbitol on some secondary metabolites of Chinese cloves using a GC device

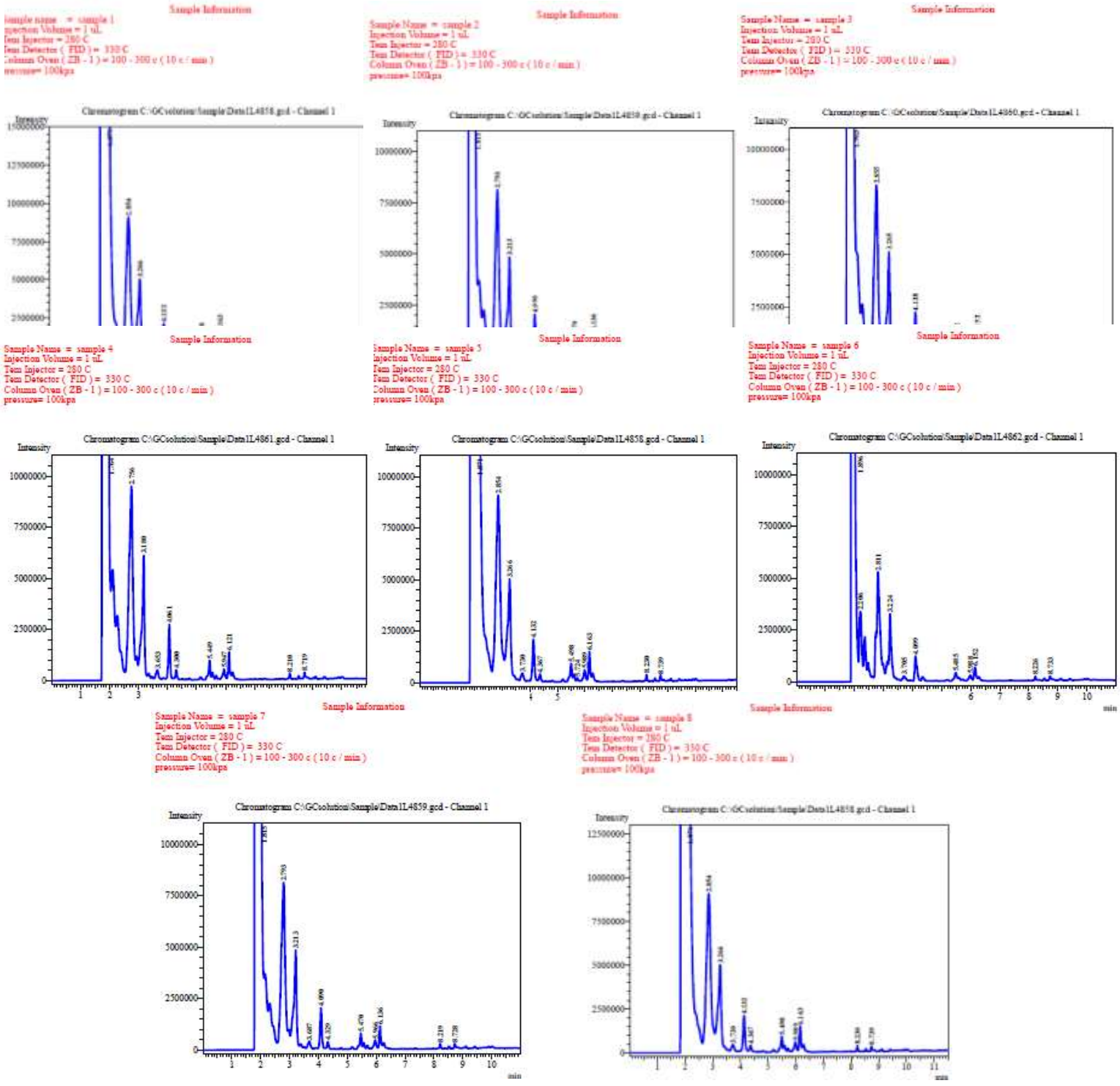
		Secondary Metabolites				
Sorbitol acid	**Jasmonic acid**	Eugenol %	Caryophyllene %	Terpinen %	Linalool %	Pinen %
0	**0**	60.25	25.19	1.33	0.88	1.11
	25	60.88	25.44	1.50	1.02	1.40
	50	61.12	25.90	1.65	1.22	1.69
	75	62.47	26.80	2.13	1.75	2.33
20	**0**	61.52	26.32	1.78	1.35	1.89
	25	61.80	23.60	1.94	1.60	2.10
	50	62.90	27.25	2.33	1.88	2.50
	75	63.33	27.50	2.50	2.00	2.70



Effect of treatment with jasmonic acid and sorbitol on some secondary metabolites of Chinese cloves using a GC device.

Results shown in Table (6) indicate an increase in secondary metabolite compounds with the addition of jasmonic acid and sorbitol after analyzing them with a GC device, as the interaction treatment of 20 mg L-1 sorbitol with 75 mg L-1 jasmonic acid outperformed by giving the highest values for compounds (Eugenol, Caryoph yllene). , Terpinen, Linalool, Pinen) reaching (

63.33, 27.50, 2.50, 2.00, 2.70%) respectively, while the comparison treatment achieved the lowest values for the studied compounds (Eugenol, Caryophyllene, Terpinen, Linalool, Pinen) respectively, reaching their values (60.25, 25.19, 1.33, 0.88, 1.11%), respectively.Increase in the percentage of secondary metabolites upon adding jasmonic may be due to ability of jasmonic acid to stimulate cells, vascular tissue differentiation, and the development of lateral roots. It may also be due to the increase in parenchymal cell division and elongation [30**].** It may be attributed to the role of jasmonic in causing many diseases. Physiological changes within plant tissues are accompanied by morphological changes in laboratory-grown plant part, in addition to their role in reorganizing the production of plant cells for both primary and secondary compounds by stimulating them to express the genes responsible for the biosynthesis of those compounds [15] [22].Discrepancy in proportions of active substances when using sorbitol acid may be due to its role in stimulating some enzymes within the plant and that it is transported over long distances within plant tissues and that sorbitol acid encourages the biosynthesis process and is a source of energy [25] .These results are consistent with the findings of [10] on rosemary plants and [7] on basil plants[18] [21].



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