Evaluation of some active constituents in callus of *Bougainvillea spectabilis*

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Abstract

Callus produced from internodes cultured in MS contain 0.5 mg L^{-1} 2,4-D and 0.2 mg L^{-1} BA after 14 days treated by cold shock 0 °C at (5, 10, 15) minutes, or at different concentrations of glutamine at (10,15,20) mg L^{-1} after that evaluate secondary metabolites (gallic acid, rutin, catechin) using HPLC. The result showed that treatment Callus with 15 mg L^{-1} glutamine gave the biggest FW 15.60 g, protein ratio 23.68 % and the highest content from gallic acid, rutin, and catechin 74.56, 33.65, and 31.58 µg gm^{-1} respectively, while FW and protein, 14.21 g and 20.82 % respectively, when exposed callus 0°c for 10 minute and this treatment gave the highest content from gallic acid 68.90 µg gm^{-1}, rutin 31.55 µg gm^{-1}, and catechin 25.49 µg gm^{-1}.

Keywords: Cold shock, Glutamine, Callus, Gallic acid, Catechin, Rutin, *Bougainvillea*.

Introduction

The *Nyctaginaceae* family is distributed in tropical and subtropical regions worldwide, consisting of approximately 31 genera and more than 400 species, including herbs, shrubs, subshrubs, vines, and trees. [14] *Bougainvillea spectabilis* Wild infernal is one of the most species of the genus Bougainvillea and is considered one of the most important ornamental Landscape plants and flowering plants native to South America – Brazil [1]. The bougainvillea plant is characterized by its bright bracts colors therefore, it can be grown as a shrub in gardens, climber to make fences and cover entrances, and grown in pots as stunted (dwarf) plants [34], It withstands high temperatures, fluctuating climatic conditions, drought, and strong winds, as well as its tolerance to high levels of salinity [20]. It is also characterized by reducing environmental pollution through its alternate ovate hairy leaves [33], Bougainvillea has yellowish-white (creamy) flowers with no decorative value surrounded by dense and abundant red-pink bracts that appear in most months of the year and remain on the plant for long time if managed correctly (especially in places with sufficient lighting and high temperature) [20]. Tissue culture technique is used to propagate plants of bougainvillea due to the difficulty of propagating vegetatively (cutting) or sexually (seeds) because of their low seed formation rate [38], furthermore callus culture initiation for the production of secondary materials, Through phytochemical analysis, it was discovered that the bougainvillea plant contains plant components that include alkaloids, flavonoids, glycosides, saponins, steroids, terpenoids, and tannins present in the extract of its leaves, stems, and bracts [16], [35] isolated and characterized caffeic acid and quercetin from stems and leaves respectively [8] [9], [19] while isolated material quercetin and isorhamnetin from flowers with white bracts, moreover pinitol extracted from stem bark of the *B. spectabilis* in the last few years there has been a heighten

Pharmacological studies revealed of B. *spectabilis* which has Antiviral activity [5], the extracts of leaf showed very Anti-hyperglycemic activity [6] and anti-bacterial [31].

Auxin and cytokinin are often used to stimulate callus formation as they promote cell growth by inducing cell division and elongation, 2,4-D a growth regulator, is considered one of the auxins used to stimulate callus induction either alone or in combination with cytokinin, especially BA, for extracting active substances within the laboratory [36].

Plant Heat shock proteins play a crucial role in providing tolerance to each biotic or abiotic stress. additionally, enhance membrane stability and detoxify-reactive-oxygen-species (ROS) by positively regulating the antioxidant enzyme system [37] under heat stress making plants adjust their gene-expression [26] the increased production of secondary metabolites in response to heat stress is a mechanism employed by plants to defend themselves against heat stress [11].

Glutamine (Gln) is one of the amino acids formed $C_5H_{10}N_2O_3$ is the first amino acid synthesized in nitrogen assimilation in plants, Gln synthetase (GS) converting glutamate (Glu) and ammonia (NH_4^+) at source by 1 ATP, Gln is introduced in the building and structure of protein, nucleic acid (RNA & DNA) and vitamin B through the enzymes synthesized by protein [22].

Explain [12] that leaf of *Stevia rebaudiana* growing on the MS with 0.5 mg L^{-1} BA and 2 mg L^{-1} 2,4-D supplemented by 50 mg L^{-1} glutamine gave highest callus induction % 88.09±2.6 and were weight 35.77±0.6 mg, 138.01±0.8 mg after 20 and 45 days respectively, Studied [21] effect of different concentrations 0.1, 0.3, 0.5,1.0 µM 2,4-D from cultivation leaf of *Bougainvillea* cv. Bhabha on MS basal medium gave a better response of callus formation at 81 % after 21 days, [27] obtained the highest rate of callus induction in varieties of *Oryza sativa*, reaching 85.3% with a fresh weight of 67.5 mg, by growing on MS medium supplemented with 2.0 mg L^{-1} 2,4-D and 500 mg L^{-1} proline and glutamine after 30 days from cultured, [7] achieve the highest percentage of Callus formation at all 100 % after cultivate leaves of *Byrsonima verbascifolia* on all treatments Supplemented combination 2,4-D plus BA comparison 2,4-D or BA alone and was biggest fresh weight 1.66 g when the concentration 4.52 µM 2,4-D + 4.44 µM BA after 45 days from cultivation, [4] obtain from cultivated internode of *bougainvillea* cv. Bhabha on MS media with 2,4-D (6 mg L^{-1}) the highest callus induction at 88.75 % with the lowest days for callusing 10.50 days, was fresh weight 328.94 (mg), [30] Observed that treatment leaf explants of *Bougainvillea glabra* Chois with 2.0 mg L^{-1} BAP plus 1.0 mg L^{-1} NAA, 2.7 mg L^{-1} BAP plus 0.3 mg L^{-1} 2,4-D on MS medium gave best callus induction rito 66%, 100% respectively,

[39] exposed two genotypes of ginseng plant *Withania somnifera* to low temperature (4°C) for 7 days and discovered the presence of compounds Biologically active, including withanolide A in the roots and withaferin A in the leaves in two genotypes, [23] note that growing callus of *Spilanthes acmella* cv. Murri on MS medium supplemented with 2.0 mg L^{-1} BA and 2.4 -D with the addition of 300 mg L^{-1} of glutamine and an increased amount of secondary compounds Scopoletin and Spilanthol when 350 mg L^{-1} of glutamine addition to the medium, reaching 415.78 and 89.399 µg.g^{-1}, respectively, compared to control treatment which gave 152.07 and 50.270 µg.g^{-1}, after 4 weeks from planting, [13] obtained the highest percentage of callus

initiation 90.9%, when planting the shoot tip of the palm *Phoenix dactylifera* cv. Ghorm Ghazal on MS medium supplemented with 200 mg L^{-1} glutamine acid with 100 mg L^{-1} 2,4-D, 3.0 mg L^{-1} 2ip, 3.0 g L^{-1} AC, and 0.1 mg L^{-1} folic acid after 6 weeks from culture, [17] obtained the batter percentage of callus induction %100from culture node of *Bougainvillea buttiana* (Miss Manila) on MS Basal Medium contain 2.0 mg L^{-1} BA and 0.1 mg L^{-1} NAA, [2] noted higher callus formation 80% through planting leaf of *Bougainvillea glabra* on MS in addition 0.5 mg L^{-1} BA and 0.5 mg L^{-1} 2,4-D and with highest Fresh Weight 1.45 gm from treatment 0.5 mg L^{-1} BA and 1.0 mg L^{-1} 2,4-D after 30 days from culture, [25] found that callus frequency 100 % were obtain from a culture nodale segment of *Bougainvillea glabra* on woody plant medium supplemented with 5 µM 2,4-D plus 0.5 µM BAP otherwise biggest fresh weight (FW) 5.23±0.16 g get from treatment 7.5 µM 2,4-D plus 1.5 µM BAP after 4 weeks, [18] exposure callus of flax *(Linum usitatissimum)* to cold shock 0 C ° for 10 minutes grown on MS supplemented with 0.25 mg L^{-1} and 0.4 mg L^{-1} BA, gave the highest protein percentage 27.51 %, biggest wet weight 14.493 g and 37.88 mg g^{-1} SDG after 4 weeks from culture.

The aim of this study was to show the effect of Glutamine and cold shock in callus of *Bougainvillea spectabilis grow*ing in MS supplemented with BA and 2,4-D to estimate some phenolic compounds in callus by HPLC.

Materials and Methods

In this study used internodes of the *Bougainvillea spectabilis* plant and was carried out in the Tissue culture Laboratory of the Department of Horticulture and Landscape Design/ College of Agriculture and Forestry, University of Mosul- Iraq.

The sterilization

Explants with of 3 cm length put under running water for 30 minutes after removing all Leaves and Thorns, then were flood in an antioxidant solution containing 150 mg L^{-1} Citric acid and 100 mg L^{-1} ascorbic acid, for forty-five minutes, after that surface sterilized with (NaOCl) 4% for 20 minutes and washed 3 times for 300 seconds each once in Sterile Distilled Water to removed materials sterilizer then planted horizontally with a length of 1 cm.

Establishment of callus culture

The explant cultured in MS medium [24] (Murashige and Skoog) supplemented with 3% sucrose, 0.6% agar-agar, and different concentration of PGRs (0.0, 0.25, 0.5,1.00, 2.00) mg L^{-1} 2,4-D alone or combination with (0.1, 0.2, 0.3) mg L^{-1} BA with best treatment from 2,4-D. The pH adjusted to 5.75±0.1 with 1N then sterilized in Autoclave at 121 °c for 20 minutes, all cultures transferred from the culture room to the incubation room under environments at a temperature of 25±2 °C with 8 hours of dark and 16 hours of light daily by 2000 lux fluorescent Light.

Exposure callus to cold shock

The callus originating from better treatments (0.5 mg L^{-1} 2,4-D and 0.2 mg L^{-1} BA) after 14 days from initiation expose to cold shock (0 °C) treatments as follows 5,10,15 minutes by using an ice bath with control treatment (without exposure), then transferred to water bath with 24°C which was a laboratory temperature [18], with 10 replicates for each treatment and two-explants for each replicate, the data were recorded after 14 days from exposing.

Treatment callus with glutamine:

The callus produced from the previous synthesis was re-culture on MS medium supplemented with the same treatment (0.5 mg L^{-1} 2,4-D and 0.2 mg L^{-1} BA) with the addition of glutamine at concentrations of 10,15,20 mg L^{-1} and control treatment, then transferred to the incubation room and the data were recorded after 14 days of treatment. Each treatment was repeated 10 times, and each relicate contained two explants.

Determination of protein in bougainvillea plant callus:

The Bougainvillea plant callus was dried in an electric oven then was ground, and 0.25 g of powdered callus collected from the five replicates for each experimental unit was taken and placed in special bottles in which the samples were digested by adding 10 ml of concentrated sulfuric acid, left for 72 hours and placed The samples were placed on a heat source and with the help of 2 ml hydrogen peroxide to carry out the digestion process, until the color of the solution became clear white. The volume was added to 50 ml of distilled water for each sample according to the method. After completing the digestion process, the nitrogen in the sample was estimated using a Microkaldal device [32] the protein percentage was calculated as follows:

Protein ratio = Nitrogen ratio × 6.25 [29]

Diagnosis and quantification of phenolic compounds using HPLC

Using a high - performance-liquid-chromatograph (SYKAM HPLC) model for the quantification of individual phenolic compounds (gallic acid, catechin, and rutin) with the following specifications: Column type, length, and internal diameter (C18-OSD), 25 cm and 4.6 mm respectively, the detection was done using a UV detector and the chromatographs were analyzed (Curve) by Chemstation Software. The injection volume was 100 Microliters and the mobile phase was: methanol: water: formic 10:20:70 v/v. After conducting the tests, the retention time and curve area of the measured samples were compared with the standard samples of the compounds, and the separation process was conducted. Under the following reaction conditions: the flow speed of the mobile phase is 1.2 ml/ min, the volume of the injected sample is 100 microlitres for each one separately, 30°C the column temperature, and 280 nm ultraviolet radiation is used. Then the retention time and area of the curve of the samples are compared with the standard sample and the concentration of the compounds is determined [28].

Statistical analysis

This study used a completely randomized design (CRD) using an SAS system with each treatment consisting of ten replicates, each replicate containing two explants. The results were compared using the Duncan multiple range test at a $p<0.05$ probability level [3].

Results

Table (1) shows the effect of 2,4-D Dichlorophenoxy acetic acid on callus initiation from internode on MS medium, and treatments 0.25,0.5,1.0 mg l^{-1} gave response ratio 100% for callus formation and the biggest fresh weight 2.53 g when medium supplemented 0.5 mg l^{-1} 2,4-D, after 30 days (Fig-4-c). table (2) explains the effect combination between 2,4-D and BA, which refers that callus initiation was 100% on all treatments except the control treatment, and the biggest fresh weight (FW) was 4.46 g after 14 days (Fig-5-c).

Table 1. Effect of 2,4-D on callus initiation from internode of *Bougainvillea spectabilis* cultivated on MS medium after 30 days

2,4-D mg L^{-1}	Initiation of callus%	Callus Weight g	Callus Size
Control	% 30 b	0.075 c	+
0.25	% 100 a	1.20 b	+
0.5	% 100 a	2.53 a	++
1.00	% 100 a	1.53 b	++
2.00	% 50 b	0.39 c	+

*Numbers with similar letters within the same row do not differ significantly among themselves according to Duncan's polynomial test at 5% probability level.

Table 2. Effect of 2,4-D and BA on callus initiation from internodes of *Bougainvillea spectabilis* cultivated on MS medium after 14 days

2,4-D mg L^{-1}	BA mg L^{-1}	Initiation of callus %	Callus Weight g	Callus Size
0.5	0.0	% 0 b	0.0 b	-
	0.1	% 100 a	3.98 a	++
	0.2	% 100 a	4.46 a	++++
	0.3	% 100 a	4.19 a	+++

*Numbers with similar letters within the same row do not differ significantly among themselves according to Duncan's polynomial test at 5% probability level.

Table (3) declares effect of cold shock on callus after exposure grown on MS medium supplemented with 0.5 mg l^{-1} 2,4-D and 0.2 mg l^{-1}, result refers that exposure callus to 0 °C For 10 minutes gave the biggest freash weight 14.21 gm (Fig 6-c) and highest protein 20.82 % and this treatment gave a highest galic acid content 68.90 µg gm^{-1} and highest area under the peak 98521.32 (Fig 2-c), which closed for standard (Fig 1-a).

Table 3. Effect of Cold Shock 0 °C on Callus of *Bougainvillea spectabilis* grown on MS medium supplemented with 0.5 mg L^{-1} 2,4-D and 0.2 mg L^{-1} BA after 28 days from culture

Treatment		Retention Time (min)	Area under the peak	Gallic acid µg gm^{-1}	Callus Weight	Protein %
Control Untreated		6.45	89854.00	41.25	3.99 c	17.15
Exposure 0°C	5 minutes	6.46	95214.45	55.65	8.65 b	19.6
	10 minutes	6.41	98521.32	68.90	14.21 a	20.82
	15 minutes	6.48	97852.02	58.99	11.07 ab	20.21
Galic acid Standard sample		6.45	1854.08	100	--------	-------

*Numbers with similar letters within the same row do not differ significantly among themselves according to Duncan's polynomial test at 5% probability level.

Table (4) shows the effect of cold shock at 0 °C on the content catechin in callus growing in MS medium supplemented with 0.5 mg l^{-1} 2,4-D and 0.2 mg l^{-1} BA after 14 days from exposure, As noted from the table all cold shock treatments were Retention Time over close to a standard sample of Catechin (Fig 1-c) and this treatment gave the highest area under the peak 28564.25 (Fig 2-c) which in turn gave the highest content catechin 25.49 µg gm^{-1}, fresh weight and protein 14.21 g and 20.82 % (Table-3).

Table 4. Effect of Cold Shock 0 °C in Catechin content at *Bougainvillea spectabilis* Callus grown on MS medium supplemented with 0.5 mg L^{-1} 2,4-D and 0.2 mg L^{-1} BA after 28 days from cultured

Treatment		Retention Time (min)	Area under the peak	Catechin µg gm^{-1}
Control Untreated		3.85	25698.08	13.65
Exposure 0° C	5 minutes	3.89	26521.49	16.99
	10 minutes	3.88	28564.25	25.49
	15 minutes	3.85	27541.25	22.58
Catechin Standard sample		3.80	2654.19	100

Data in Table (5) appear the effect of cold shock 0 °C on quantity Rutin in callus growing on MS medium supplemented with 0.5 mg L-^{1} 2,4-D and 0.2 mg l^{-1} after 14 days from exposure, the best quantity of Rutin 31.55 µg gm^{-1} get from callus exposed to 10 minutes with higher area under the peak 65201.46 (Fig 2-c) while retention time 4.06 (min) and this time very close to Standard sample 4.08 (Fig 1-b), and then gave biggest fresh weight, protein percentage 14.21 g, 20.82 % respectively (Table-3).

Table 5. Effect of Cold Shock 0 °C on Rutin content at *Bougainvillea spectabilis callus* grown on MS medium supplemented with 0.5 mg L^{-1} 2,4-D and 0.2 mg L^{-1} BA after 28 days from culture

Treatment		Retention Time (min)	Area under the peak	Rutin µg gm^{-1}
Control (Untreated)		4.08	58521.49	12.99
Exposure 0° C	5 minutes	4.05	62325.14	21.45
	10 minutes	4.06	65201.46	31.55
	15 minutes	4.02	66523.66	22.67
Rutin Standard sample		4.08	3698.21	100

Result in Table (6) demonstrate the effect of different concentrations of Glutamine on Callus formation from internodes of *Bougainvillea spectabillis* cultured on MS medium supplemented with 0.5 mg L^{-1} 2,4-D and 0.2 mg L^{-1} BA, result show that there is yonder increase on fresh weight of callus after treatment with Glutamine at 15 mg L^{-1} and gave best protein percentage 23.68 % with highest weight 15.64 g after 14 days (Fig-7-c).

Table 6. Effect of Glutamine on Callus initiation from internodes of *Bougainvillea spectabilis* cultivated on MS medium supplemented with 0.5 mg L^{-1} 2,4-D and 0.2 mg L^{-1} BA after 28 days from culture

2,4-D + BA mg L^{-1}	Glutamine mg L^{-1}	Callus Weight gm	Protein %
0.5 + 0.2	0	3.99 D	17.15
	10	7.84 C	19.12
	15	15.64 A	23.68
	20	12.484 B	22.93

*Numbers with similar letters within the same row do not differ significantly among themselves according to Duncan's polynomial test at 5% probability level.

Table (7) declares the effect of different concentrations of Glutamine on Galic acid quantity *Bougainvillea spectabilis* Callus cultured on MS medium supplemented with 0.5 mg L^{-1} 2,4-D and 0.2 mg L^{-1} BA after 14 days from re-culture, statement shows that all treatments had retention time very close to stander sample of gallic acid (Fig 1-a), and highest area under the peak 99250.45 (Fig-3-c) with best quantity gallic at 74.56 µg gm^{-1} when treatment with 15 mg L^{-1} Glutamine.

Table 7. Effect of Glutamine on Gallic acid quantity a *Bougainvillea spectabilis* callus cultivated on MS medium supplemented with 0.5 mg L^{-1} 2,4-D and 0.2 mg L^{-1} BA after 28 days from culture

Glutamine mg L^{-1}	Retention Time (min)	Area under the peak	Gallic acid µg gm^{-1}
0.0	6.45	89854.00	41.25
10.0	6.49	90251.65	68.25
15.0	6.42	99250.45	74.56
20.0	6.48	95985.60	60.25
Gallic acid Standard sample	6.45	1854.08	100

Table (8) explain the effect of different concentrations glutamine on catechin content in *Bougainvillea spectabilis* callus cultured on MS medium supplemented with 0.5 mg L^{-1} 2,4-D plus 0.2 mg L^{-1} BA after 14 days from re-culture, the result shows all treatment had retention time over close to stander sample of catechin (Fig-1-c), and highest area under the peak 129621.45 (Fig-3-c) with best content catechin at 31.58 µg gm^{-1} at treatment 15 mg L^{-1} Glutamine.

Table 8. Effect of Glutamine in Catechin content of *Bougainvillea spectabillis* Callus cultivated on MS mediu supplemented with 0.5 mg L^{-1} 2,4-D and 0.2 mg L^{-1} BA after 28 days from culture

Glutamine mg L^{-1}	Retention Time (min)	Area under the peak	Catechin (µg gm^{-1})
0.0	3.85	25698.08	13.65
10.0	3.84	120652.59	23.65
15.0	3.86	129621.45	31.58
20.0	3.83	123214.00	26.99
Catechin Standard sample	3.80	2654.19	100

Table (9) illustrate the effect of different concentrations 10،15 and 20 mg L^{-1} of glutamine on Rutin quantity in *Bougainvillea spectabilis* Callus grown on MS medium supplemented of 0.5 mg L^{-1} 2,4-D with 0.2 mg L^{-1} BA after 14 days from treatment, the result indicate to treatment with 15 mg L^{-1} Glutamine gave best rutin quantity and area under the peak 33.65 µg gm^{-1}, 211254.80 respectively (Fig 3-c) and all treatments which was retention time over close for stander sample of Rutin (Fig 1-b).

Table 9. Effect of Glutamine in Rutin quantity at *Bougainvillea spectabilis* Callus cultivated on MS medium supplemented with 0.5 mg L^{-1} 2,4 and 0.2 mg L^{-1} BA after 28 days from culture

Glutamine mg L^{-1}	Retention Time (min)	Area under the peak	Rutin (µg gm^{-1})
0.0	4.08	58521.49	12.99
10.0	4.05	200321.45	25.99
15.0	4.05	211254.80	33.65
20.0	4.01	205621.45	28.98
Rutin Standard sample	4.08	645.80	100

Discussions

Cold shock explains the increase in weight of callus and the content *B. spectabilis* callus of the phenolic compound (gallic acid, rutin, catechin) as in Tables (3) (4) (5) promote cell division and increasing protein percentage which in turn led to a stimulating in the biosynthesis nucleic acids in the explants (callus). The plants produced heat shock protein to raise their resistance to cold as a heat stress tolerance mechanism [10], and this leads to an increase growth callus and a change in the processes of demolition and construction and then increase in secondary materials in explants. In this study noted the cold shock gave the highest level of gallic acid, rutin, and catechin. While the interpretation of the results from Tables (6) (7) (8) and (9) highlights to the positive effect of glutamine due to its role as one of the amino acid involved in protein synthesis and also plays a crucial role in forming important enzymes for biological processes within plants, thereby promoting cell division and increasing callus mass, and the level of secondary metabolites evidence suggests. when treated with 15.0 mg L^{-1} of glutamine plus 0.2 mg L^{-1} and 0.5 mg L^{-1} 2,4-D gave the biggest weight and best level of total protein 15.64 g and 23.68 % respectively, which in turn led give the best quantity of gallic acid, rutin, and catechin at 74.56, 33.65, 31.58 µg/ gm, this is consistent with stated by [15] that adding glutamine to the MS media with the presence of growth regulators led to a multiple of the growth of callus.

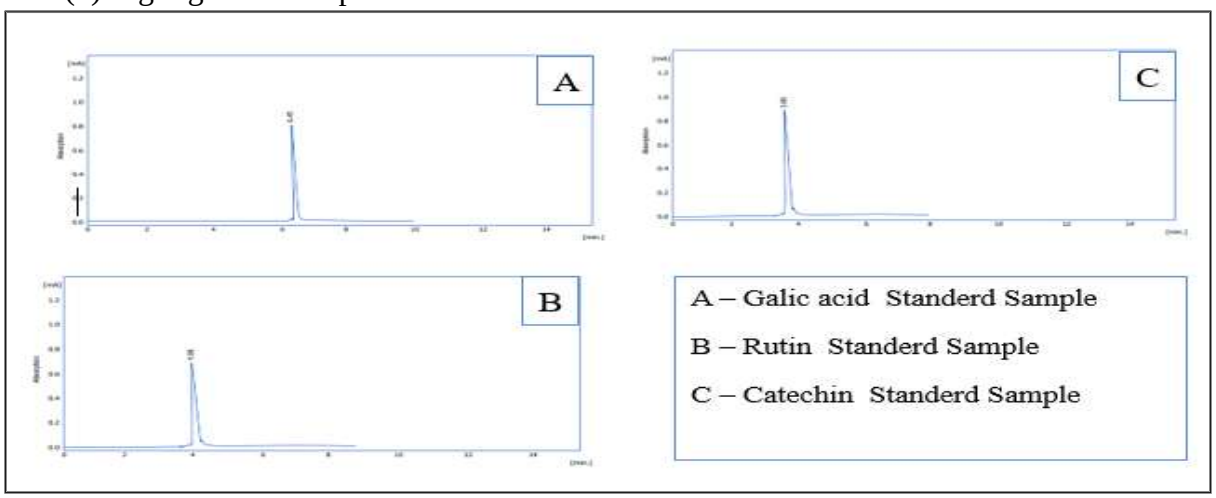

Fig 1. Retention time and area under the peak for the Standard sample of phenolic compound.

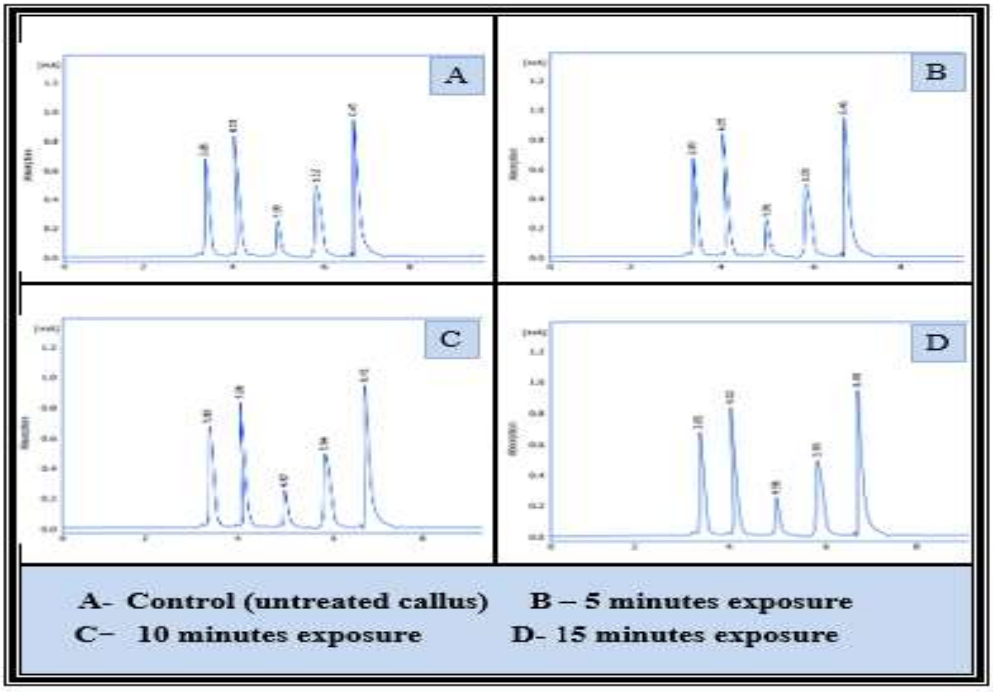

Fig 2. Retention time and area under the peak of phenolic compound in *Bougainvillea spectabilis* callus grown in MS medium supplemented with 0.2 mg L^{-1} BA and 0.5 mg L^{-1} 2,4-D exposed to cold shock treatment.

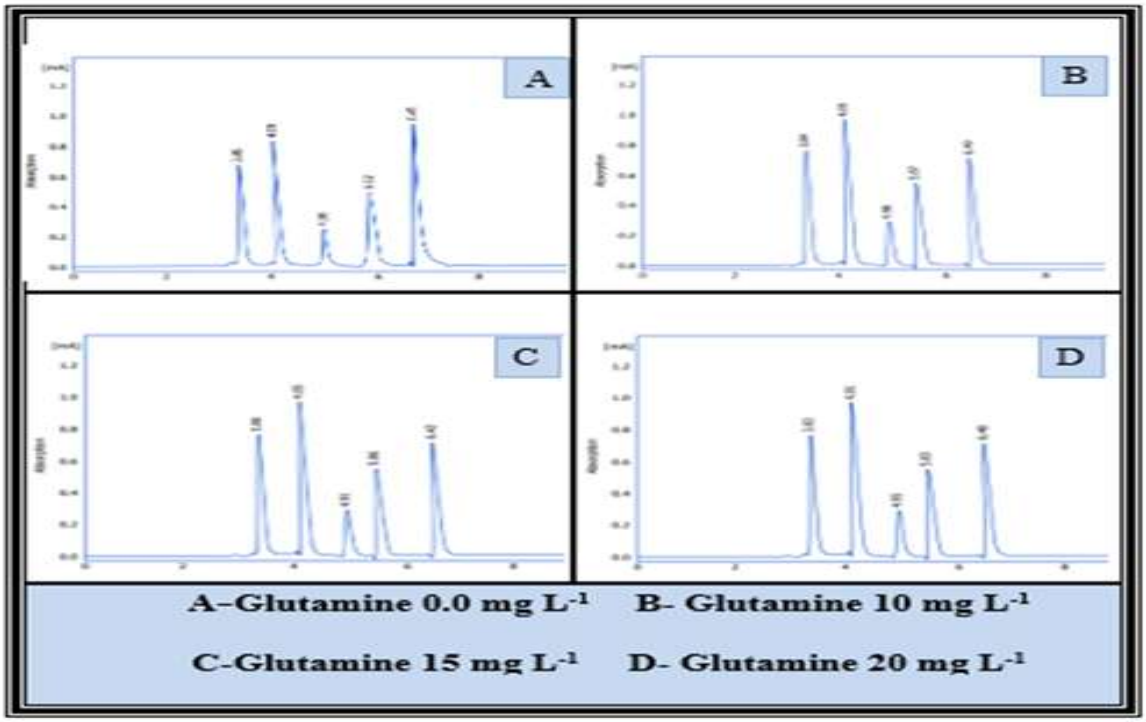

Fig 3. Retention time and area under the peak of phenolic compound in *Bougainvillea spectabilis* callus grown in MS medium supplemented with 0.2 mg L^{-1} BA and 0.5 mg L^{-1} 2,4-D and glutamine.

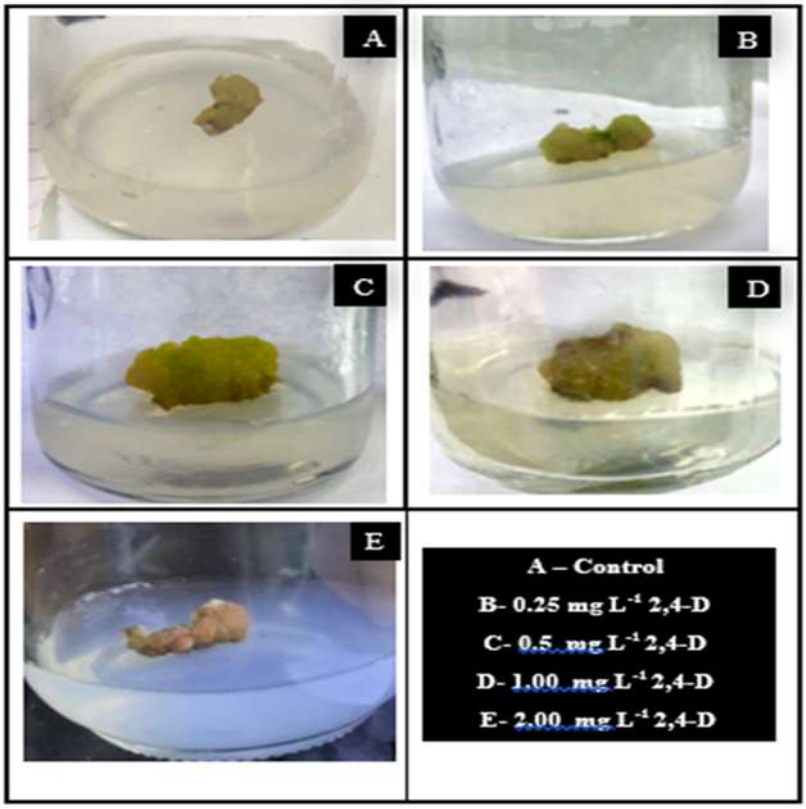

Fig 4. Effect of 2,4-D on callus initiation of *Bougainvillea spectabilis* internodes cultivate on MS medium after 30 days.

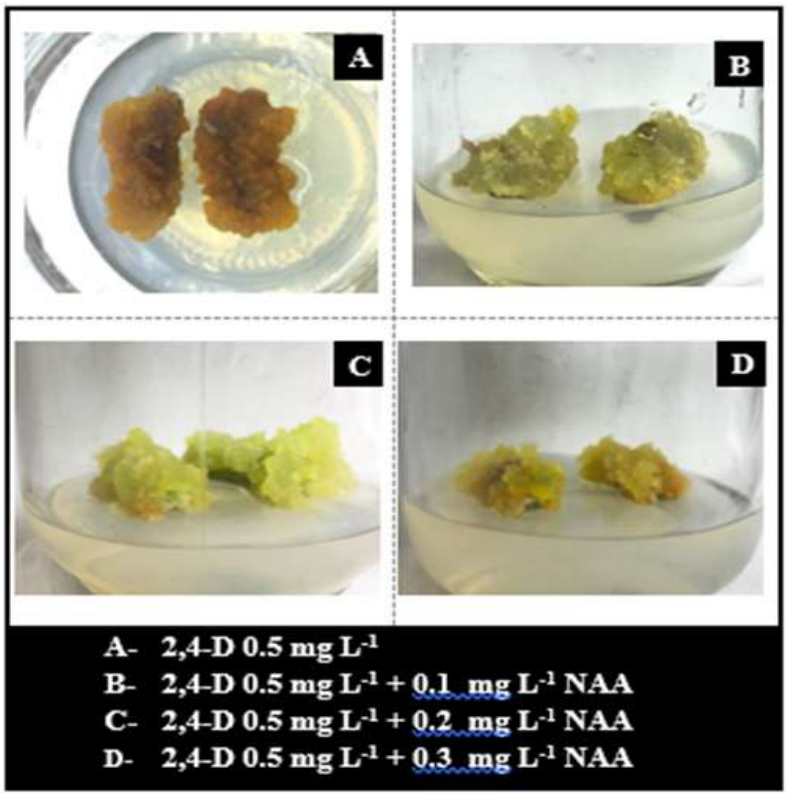

Fig 5. Effect of combination between 2,4-D and BA on callus initiation of *Bougainvillea spectabilis* internodes cultivated on MS medium after 14 days.

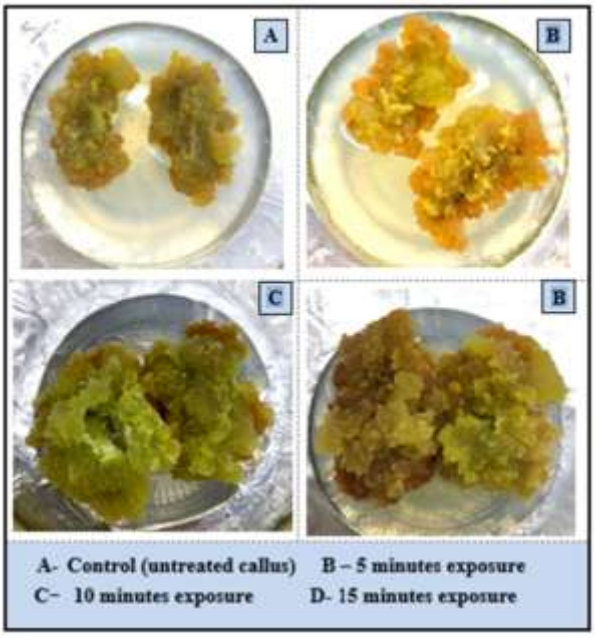

Fig 6. Effect of Cold Shock 0 °C on Callus of *Bougainvillea spectabilis* grown on MS medium supplemented with 0.5 mg L^{-1} 2,4-D and 0.2 mg L^{-1} BA after 28 days from culture.

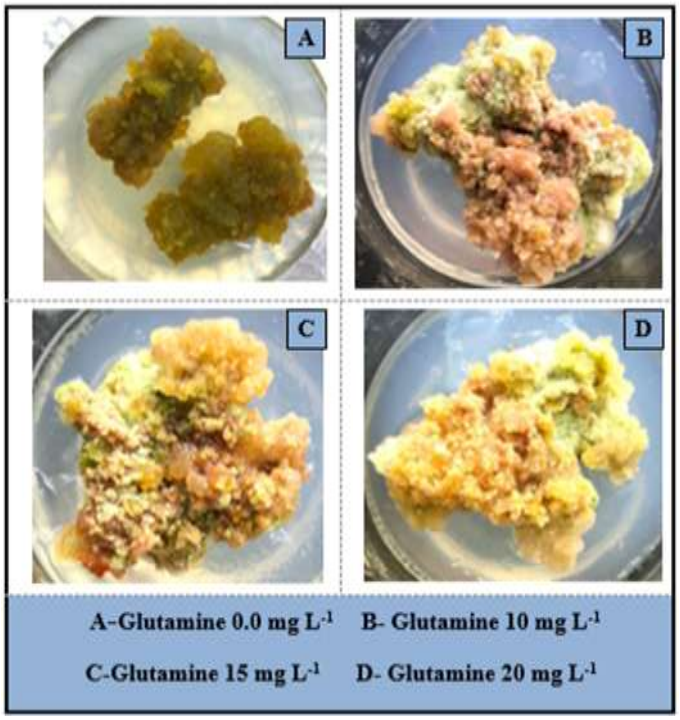

Fig 7. Effect of Glutamine on Callus initiation of *Bougainvillea spectabilis* cultivated on MS medium supplemented with 0.5 mg L^{-1} 2,4-D and 0.2 mg L^{-1} BA after 28 days from culture.

Conclusion

According to the obtained results, the glutamine treatment improves all studied parameters compared with cold shock. Treat callus with Glutamine significantly affects fresh weight, protein rate, and quantity of secondary metabolites in *Bougainvillea spectabilis* of callus. Acknowledgment We are grateful to the Department of Horticulture and Landscape Design – College of Agriculture and Forestry – University of Mosul for carrying out this experiment in tissue culture and plant cell laboratory.

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