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EFFECT OF PLANT GROWTH REGULATORS ON CALLUS INDUCTION FROM SEEDLING EXPLANTS OF *Ammi visnaga* L. AND PHENOLIC COMPOUNDS CONTENT

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ABSTRACT

Ammi visnaga (L.) Lam. Belong to family Umbelliferae. During the present study an efficient in vitro protocol has been standardized viz, callus production from seedling explants. Best callus production from seedling explants was obtained on MS medium supplemented with 0.5mg/l NAA +0.05/1BA. Callus was also obtained when MS medium was fortified with 1mg/1NAA+0.5mg/1BA, 1mg/1 (2, 4D+0.5 mg/1BA and 3mg/l PCIB. But the percent culture response on these concentrations was lesser. The lowest amount of callus was found to be on MS medium containing 1mg/l PCIB 9(1) callus amount.

Callus of explants were grown on half MS media gave the significant highest value (3641) of phenolic content, followed by callus of free-growth regulators MS medium (3509.5). While the lowest value (7) was observed with callus obtained from seedling cultured on MS medium contained 1 mg/1 2, 4-D+ 0.5 mg/1K.

***Conclusively,** the Ammi visnaga callus formation can be obtained from different concentrations of growth regulators. The growth regulators also had a significant impact on the amount of callus produced. The efficiency of callus formation depended on the hormone concentrations and the proportion between NAA and BA. The best results were achieved on the medium containing 1mg/lNAA and 0.05mg/lBA. The callus of Half MS plant gave the significant highest value of phenolic content, followed by callus of Free-growth regulators MS medium.*

Key words: *Ammi visnaga (L.),* NAA, PCIB, callus formation, 2,4-D and phnolic compounds.

INTRODUCTION

Ammi visnaga (L.) Lam. Apiaceae (Umbelliferae) is an indigenous herb on the waste lands of the Nile Delta. According to Hutter and Dale (Huttrer *et al.*, 1951) and Quimby (Quimby, 1953), the Egyptians referred to this plant as Khilla, chellah, or khella and in Europe the plant has often been referred to as the toothpick Herb or Bishop's weed. In 1934 both the decoction and tincture of *A.*

visnaga were admitted into the Egyptian Pharmacopoea (Quimby, 1953). The physiological action of *Ammi visnaga* is due to principally to the furanochromones, khellin and visnagin. Furanochromones are unique secondary metabolities that have been identified only in *Ammi visnaga* (Apiaceae) and *Eranthus hyemalis* (Ranunculaceae) (Gomes, 1956).

Studies of the *Ammi visnaga* plant for the production of khellin and visnagin include the effect of vernalization temperatures and heat hardening (Reda *et al.,* 1977a and Reda *et al.,* 1977) a reversed phase HPLC analysis for the furanochromones, khellin and visnagin, in the fruits of *Ammi visnaga* and in various plant organs at different developmental stages (Franchi *et al.,* 1985; Martelli, *et al.,* 1984); and the localization of furanochromones in the primary rib channel (lacuna) and the endosperm of the fruits (Franchi *et al.,* 1984).

The initiation and development of embryos from somatic tissues in plant culture was recognized in 1958 by Reinert (Reinert, 1958) and Steward (Steward *et al*., 1958). Prior to 1979 somatic embryogenesis was reported in 132 species (Ammirato, 1983), the highest frequency occurring in the Apiaceae and Solanaceae families. Somatic embryogenesis is being used to reduce the propagation time of plants, to select and replicate virus-resistant or horticultural variants, or to produce "artificial" seeds (Evans *et al*., 1981; Redenbaugh *et al*., 1987).

Hence, a better understanding of the complex and often intricate factors involved during biotechnological processes is required for improved efficiency. These factors include the type of plant growth regulators (PGRs), explants and elicitors used, all of which are known to affect the production of bioactive secondary metabolites (Do¨rnenburg and Knorr, 1995 and Collin, 2001). It is also evident that these afore mentioned factors apparently affect the pharmacological activity of regenerated plantlets (Amoo *et al.,* 2012). In addition to a thorough understanding of the biosynthesis and accumulation in different morphogenetic tissues during tissue culture stages, the carryover effects of these factors upon transfer to the ex vitro environment remain crucial for acceptability of in vitro grown plants as an alternative to the wild populations (Martı´nez-Bonfil *et al.,* 2011).

Therefore, the aim of our study was to assess capacity of natural population of seedling *Ammi visnaga* (L.) for callus formation from six old weeks seedling leaves.

MATERIALS AND METHODS

Plant material, sterilization and preparation of explants.

Seeds of (*Ammi visnaga* L.) var. Maurane were collected from Fayoum farms during March 2014 to septemper 2015. The seeds were identified to the representative herbarium specimens in Cairo University.

Sterilization steps:

The seeds were carefully washed with detergent and rinsed with tap water. They were washed with disinfectant agent commercial sodium hypochloride 60% for 20 minutes. The seeds were carefully washed with 3 times distilled water.

Culture media.

These explants were then cultured aseptically on basal solid MS-medium with several treatments and half MS medium. The pH was adjusted to 5.7 with 1 N KOH or 1N HCl before adding gel rite and prior to autoclaving at 121 ˚C (0.1.MPa) for 20 min. The cultures were kept in a growth chamber at 21 ± 1 ˚C, and a photoperiod of 16.h (30 µE m-2s-1, Philips TL 33 light) (Maroufi *et al*, 2012).

Seeds were cultured on media containing half strength MS (half MS) medium.

Callus formation

Six weeks old seedling leaves were cultured for callus formation on media containing different concentrations of auxin1-Naphthaleneacetic acid (NAA) and 2, 4-Dichlorophenoxyacetic acid (2.4-D) (1,2 and 4 mg/L) in combination with benzyl adenine and kinetin and auxin transport inhibitor *p*-chlorophenoxyisobutric acid (PCIB) (1, 3 and 10 mg/l).

The cultivation was done in 300 ml glass jars containing 50 ml of basal MS-medium.

Phytochemical screening of the extract

The total phenol content of *Ammi visnaga* was determined by the Folin–Ciocalteu (FC) method (Singleton & Rossi, 1965) with some modifications made by Nand *et al*. (2012), and expressed as grams of gallic acid equivalents per 100 g plant extract. Distilled water (3.00 ml) was mixed with the test compound (50 µl). Then, 200 µl of FC reagent was added. After 5 min, 500 µl of 20% sodium carbonate solution was added and the solutions were mixed again. The solutions were left at room temperature for 2 h. Then the absorption of the developed blue colour was determined at 765 nm. The standard curve was used to determine the equivalent that expresses total phenol content as grams of gallic acid equivalents per 100 g plant extract. (Figure 1).

Statistical analysis

Thirty explants were cultured per treatment. Each treatment consisted of 40-50 polypropylene jars (5 cm high) with 5 explants in each jar. In the table, we show percentages and means ± SE. The Student *t*-test and the χ2-test were used to evaluate the significance of differences with respect to

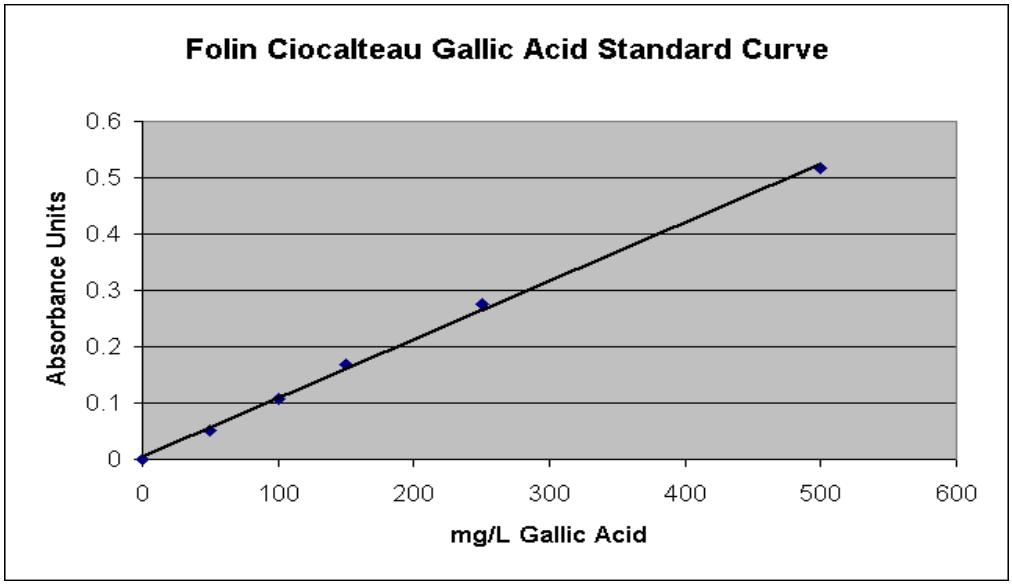

Figure 1. Standard curve of gallic acid.

means and percentages, respectively. The experiments were carried out at least twice and similar results were obtained.

Results were statistically analyzed by a factorial analysis of variance, in completely randomized design according to the procedure by Snedecor and Cochran (1989) and means were compared by multiple range tests.

RESULTS

In our research, the beginning of callus production by the explants on all media was observed after 4 weeks.

The amount of callus depended on the composition of the medium. After 6 weeks, the mean ratings of the amount of callus ranged from 0.5–5.0°. The most intense callus development was observed in the seedling explants grown on the medium containing 1 mg/L NAA +0.05/1BA. The lowest callus amount explants was observed in the seedling explants grown on the medium containing MS media supplemented with1mg/1 PCIB. (Figure 5). All the data illustrated in Table 1 demonstrated the effect of different plant growth regulators used during the experiments.

1-Effect of different conc. of NAA on callus induction

Figure 2 showed that the growth regulators, also had a significant impact on the amount of callus produced. At all concentrations of NAA, the

Table 1. Effect of different growth regulators with different concentrations on callus induction. (Data ±standard error)

Plant growth regulators	Callus Amount (1-5°)	Fresh weight of callus (g)	Dry Weight of callus (g)
1mg/lNAA	2.3±0.7	5.5± .76	0.434±0.04
0.5mg/l NAA +0.05 mg/l BA	1.7±0.3	10.1± 1.59	0.963±0.04
1mg/1NAA+0. 5 mg/1K	1.5±0.5	5.66±.072	0.481±0.07
1mg/1NAA+0.5mg/1BA	2.6± 0.6	6.83±0.44	0.736±0.05
1mg/l NAA +0.05mg/1BA	5± 0.1	10.1± 1.59	0.963±0.04
3mg/1 NAA	2.2±0.5	5.33±0.88	0.521±0.07
1mg/1 (2,4D)	1±0.1	0.0	0.000
1mg/1 2,4D+0. 5 mg/1K	1±0.1	6.33±1.20	0.697±0.11
1mg/1 (2,4D+0.5 mg/1BA	2.8±0.2	7.23±0.29	0.668±0.02
2mg/1(2,4D+0.05 mg/1BA	1.7±0.3	0.0	0.000
2mg/1 (2,4D+0. 5 mg/1K	1±0.1	0.0	0.000
1mg/l PCIB	0.5±0.5	0.0	0.000
3 mg/l PCIB	2.4±0.4	8.6±1.31	0.817±0.08

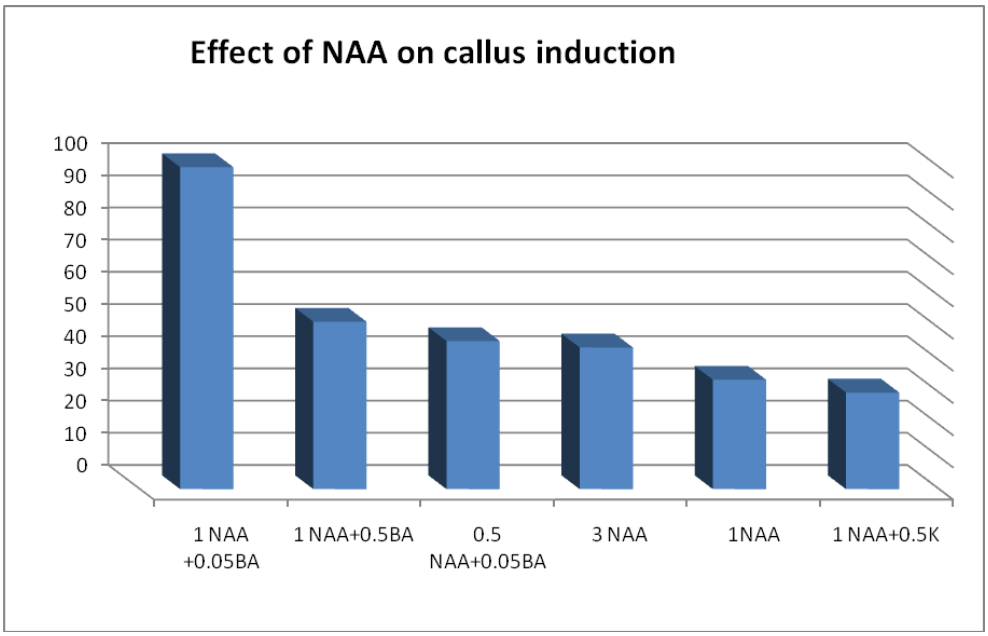

Figure 2. Effect of different conc. of NAA on callus induction.

amount of callus increased with the concentration of 1 mg/L NAA +0.05/1BA which give the highest amount of callus (5).

2-Effect of different conc. of 2, 4-D on callus induction

At the different concentrations of 2,4-D, the amount of callus increased with the concentration of 1mg/1NAA+0.5mg/1BA, which give The highest amount of callus (2.8)**.** While, the lowest amount of callus was obtained from concentration of 1mg/1 2, 4-D, 1mg/1 2,4-D+0. 5 mg/1K and 2mg/1 2,4-D+0. 5 mg/1K as shown in Figure 3.

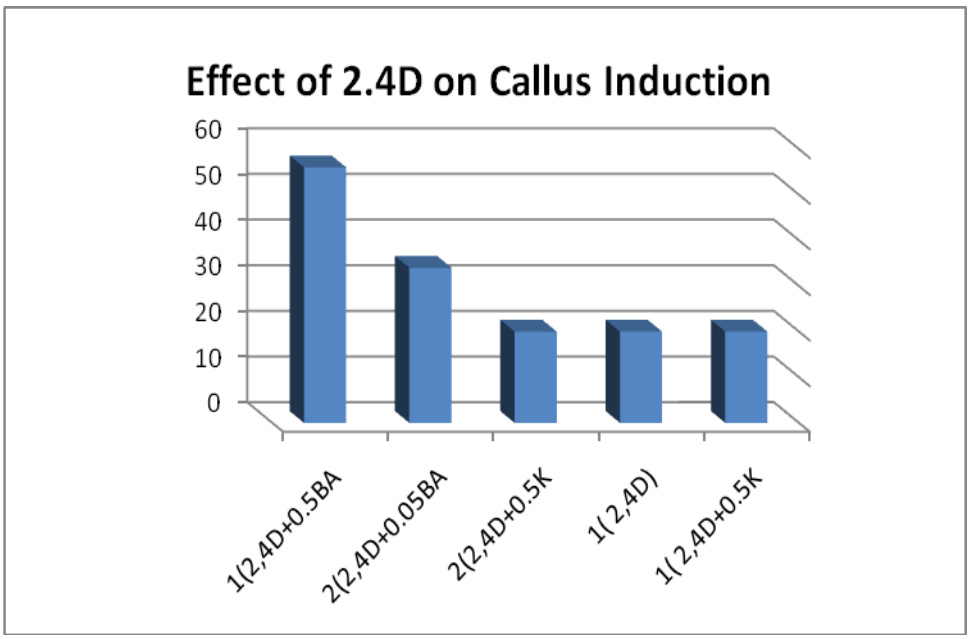

Figure 3. Effect of different conc. of 2,4-D on callus induction

3- Effect of different conc. of PCIB on callus induction

The best results were obtained on medium containing 3mg/l PCIB, 0.5 mg. Callus was produced with (2.4) of the callus amount.

DISCUSSION

During the present study, different growth regulators both auxins and cytokines were used both individually as well as in combination to produce callus from seedling explants. Best callus production from seedling explants was obtained on MS medium supplemented with 0.5mg/l NAA +0.05/1BA.

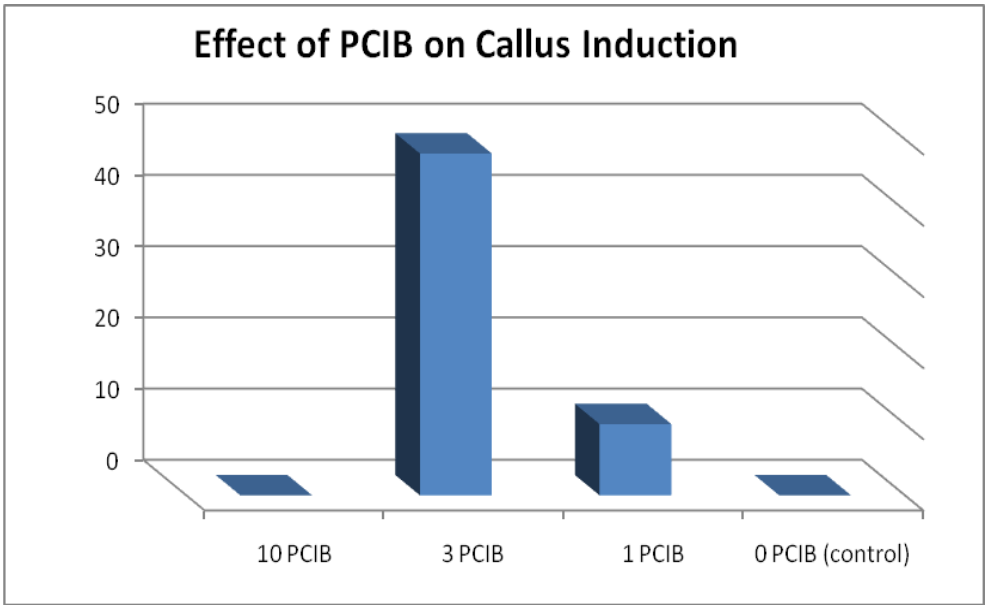

Figure 4. Effect of different conc. of PCIB on callus induction

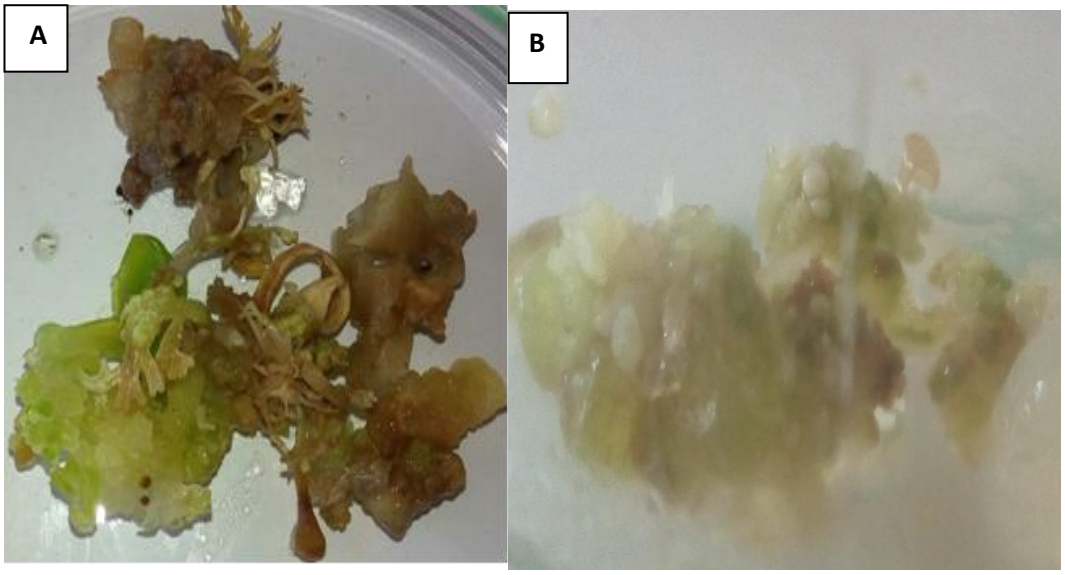

Figure 5 A) MS medium containing 1 mg/L NAA +0.05/1BA.and B) MS medium containing 1mg/1 PCIB.

Callus was also obtained when MS medium was fortified with 1mg/1NAA+0.5 mg/1BA, 1mg/1 (2, 4D+0.5 mg/1BA and 3mg/l PCIB. But

the percent culture response on these concentrations was lesser. Singh *et al.*, (2010) also, obtained callus from nodal explants in *Jatropha curcas* on MS medium augmented with Kn (3 mg/l)+IBA(3 mg/l).12 MS medium fortified with BAP(2 mg/l)+IAA(2 mg/l) showed a 90% response from cotyledon explants. However callus was also obtained on MS medium fortified with BAP(2 mg/l); 2,4-D(0.5 mg/l) and 2,4-D(1 mg/l)+Kn (2 mg/l),but the percent culture response was less in these cases.

Callus was also obtained from hypocotyls explants with 80% response on MS medium fortified with BAP (2 mg/l). Valizadeh and Tabar, (2009) also obtained callus using hypocotyl explants of seedling, but they achieved best callus production on MS medium supplemented with 2,4-D(1 mg/l). Among the three explants best explant was cotyledon explant in terms of percent culture response.

Total phenol contents:

Phenolics or polyphenols have received considerable attention because of their physiological function, including antioxidant, anti-mutagenic and antitumour activities (Othman *et al*., 2007).

The total phenol content of *Ammi visnaga* was determined by Total phenolic content of the CE was assessed using the Folin—Ciocalteu method with the help of Standard curve of gallic acid (Fig.1). Data of total phenol content for *Ammi visnaga* extracted samples used in this investigation showed in (Figure 6).

Callus of explants were grown on half MS media gave the significant highest value (3641) of phenol content, followed by callus of Half MS on Free-growth regulators MS medium (3509.5). While the lowest value (7) was observed with Callus obtained from seedling cultured on MS medium contained 1 mg/1 2, 4-D+ 0.5 mg/1K.

As previously hypothesized (Amoo and Van Staden 2012), the positive influence of cytokinins on the production of phenolics might be related to their indirect role (via the repression of certain macronutrient transporters) on the expressions of genes associated with the biosynthesis of secondary metabolites (Sakakibara *et al*., 2006). The stimulatory role of auxin on secondary metabolite production in different plant species has been reported by other researchers (Zhou *et al*., 2011; Aremu *et al*., 2012b). This stimulatory effect has been partly linked to the ability of auxin or its constituent compounds to induce gene expression geared towards enhanced phenolic compound biosynthesis in plant tissues (Soo´s *et al.,* 2010).

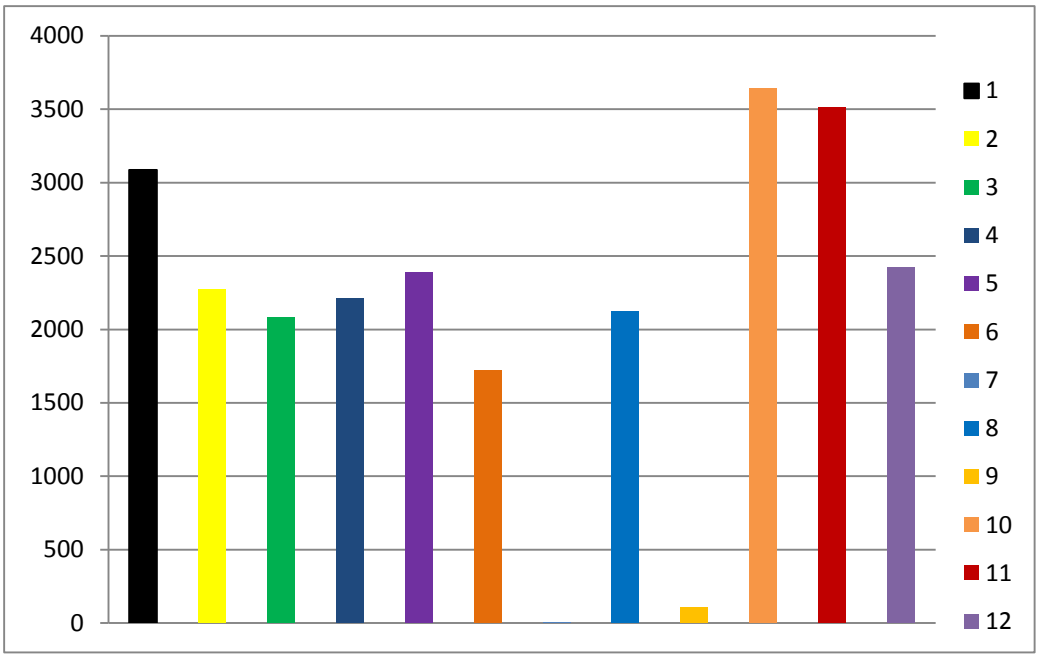

Figure 6. Total phenolic content (GAE mg/100g sample) of *Ammi visnaga* test extract

1. Callus obtained from seedling cultured on MS medium contained 0.5 mg/ 1NAA+0.05 mg/1BA
2. Callus obtained from seedling cultured on MS medium contained 1 mg/1NAA
3. Callus obtained from seedling cultured on MS medium contained 1 mg/1 NAA+0.5 mg/1K
4. Callus obtained from seedling cultured on MS medium contained 1 mg/1 NAA+0.5 mg/1BA
5. Callus obtained from seedling cultured on MS medium contained 1 mg/1 NAA +0.05 mg/1BA
6. Callus obtained from seedling cultured on MS medium contained 3 mg/1 NAA
7. Callus obtained from seedling cultured on MS medium contained 1 mg/1 2,4-D+ 0.5 mg/1K
8. Callus obtained from seedling cultured on MS medium contained 1 mg/ 1 2,4-D+0.5 mg/1BA
9. Callus obtained from seedling cultured on MS medium contained 3 mg/1 PCIB
10. Callus obtained from seedling cultured on MS medium contained Half MS
11. Callus obtained from seedling cultured on MS medium contained Full MS
12. Callus obtained from seedling cultured on MS medium contained Double MS

Conclusively, the *Ammi visnaga* callus formation can be obtained from different concentrations of growth regulators. The growth regulators also had a significant impact on the amount of callus produced. The efficiency of

callus formation depended on the hormone concentrations and the proportion between NAA and BA. The best results were achieved on the medium containing 1MµNAA and 0.05MµBA. The callus of Half MS plant gave the significant highest value of phenolic content, followed by callus of callus of Half MS on Free-growth regulators MS medium.

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دراسة تأثير منظمات النمو النباتية على استحثاث الكالس من بادرات بذرة نبات الخلة البلدى وكذلك تقديركمية المركبات الفينولية

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فى هذه التجربة تم الكشف عن نسبة الكالس وكذلك الوزن الرطب والجاف للكالس، تم الحصول على اعلى نسبة .من الكالس الطازج (١٠جم) من البادرات النابتة على نصف تركيز الملح فقط وكذلك من البيئة المحتوية على وكذلك من البيئة المحتوية على ١ مجم نفثالين أسيتيك أسيد NAA مع ٠,٥مجم بنزيل ادينين حيث بلغت كمية الكالس (٧,٣جم).

- تم الحصول على أعلى نسبة من الكالس الوزن الجاف عن طريق زراعة الشتلات نبتت على البيئة المحتوية على نصف تركيز الاملاح.

- تم تقدير الفينولات فى مستخلص الميثان لانواع الكاس الناتجة على بيئات مختلفة المختلفه من نبات الخلة وقد أوضحت النتائج أن جميع الأجزاء المدروسه تعتبر مصدر جيد للفينولات بإستثناء معاملة البيئة المحتوية على ٠,٥ مجم من هرمون 2,4-D مع ١ مجم من الكاينتين ، - وقد أعطى الكالس الناتج مباشرة على البيئة المحتوية على نصف تركيز الاملاح أفضل نتائج (٣٦٤١) من المحتوى الفينولي، تليها الكالس من تركيز كامل لاملاح MS المتوسطة (٣٥٠٩,٥) بينما سجل أدنى قيمة (٧) لوحظ مع الكالس تم الحصول عليها من البيئة المحتوية على ٠,٥ مجم من 2,4-D مع ١ مجم من الكاينتين .

التوصية: