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Propagation of *Ammi visnaga* (L.) Under the Influence of Inorganic of Some Chromium Metabolites Khellin, Visnaginby *in Vitro* Culture Technique

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The experiment was carried out. To know the effect of adding KNO_3, KH_2PO_4, and $CaCl_2$ to the callus of the plant *Ammi visnaga* that produces metabolic compounds khellin and visnagin. The seeds were planted on Woody plant medium (WPM) supplemented with 2,4-Dichlorophenoxyacetic acid at levels 0, 1.5, 2.0, 2.5 and 3.0 mg L^{-1} mixed with Kinetin 6-furfurylo- amino-purine a level of 2.5 mg L^{-1}. After germination of the seeds the shoot tips were taken and grown on WPM medium supplemented with 2,4-D at 0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0 and 5.5 mg L^{-1} mixed with kinetin at a level. 2.5 mg L^{-1}. After obtaining the callus, it was grown on WPM medium supplied with KNO_3 at a level of 0.0, 0.20, 0.30 and 0.40 g L^{-1} and KH_2PO_4 at a level of 0.0, 0.20, 0.25 and 0.30 g L^{-1} and $CaCl_2.2H_2O$ at a level of 0.0, 0.20, 0.30 and 0.40 g L^{-1}.The results showed that the level was 3.0 mg L^{-1} of 2,4-D and Kinetin at 2.5 mg L^{-1} gave the highest germination rate. The level of 3.0 mg L^{-1} 2,4-D with 2.5 mg L^{-1} Kinetin achieved the highest percentage of callus formation 95.80%. The highest level of visnagin and khellin was obtained when adding KNO_3 at a level of 0.30 g L^{-1}, KH_2PO_4 at a level of 0.30 g L^{-1} and $CaCl_2$ at a level of 0.20 g L^{-1}. The addition of element salts affected the callus content of khellin and vinsangen compounds.

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Introduction

There is a great reliance in the world on the healing properties of plants, as well as the great progress that has occurred in the production of medicines manufactured from them, which have been used since ancient times to treat many diseases (Ali *et al.*, 2012; Hashim *et al.*, 2014). *Ammi visnaga* (L.) LAM is a local Syrian variety that grows wild in the Mediterranean region, especially in Palestine and the Arab Republic of Egypt. Its cultivation has spread in East Asia, India, North America, and Pakistan, and it is one of the most important medicinal plants (Hashim *et al*., 2014).

This species belongs to the Apiaceae family and is an annual herbaceous plant with a height of about 100 cm. It is also known as the medical khala the plant khalla contains a number of chemical compounds of therapeutic importance in treating many diseases when used directly or indirectly in the form of juices, solutions, powders, and compresses, or indirectly by using its active compounds in pure form (Khalil *et al.*, 2020). It has been widely used in ancient medicine after extracting vinsagin, vinegar, and vinegar to treat the urinary tract and stones. It dilates the heart vessels, is a diuretic, soothes renal colic, and dilates the ureters, thus helping to remove stones from the kidneys (Al-Mayah, 2013). It is also used to expel kidney stones by dilating the ureter, reducing the pain of renal colic, and as a bronchodilator because it contains Furanochromon compounds such as Khellin and Visnagin, as these two compounds have a distinct effect in expanding the trachea. And anti-asthma (Msomi and Simelane, 2019; Ahmed *et al.,* 2021). Plant tissue culture technology is one of the most important techniques used in the rapid propagation of some rare varieties or varieties that are characterized by desirable characteristics, such as containing some active compounds that can be extracted from the plant or by inducing callus tissue. And growing it on the plant part containing these compounds and estimating their concentration (Cruz-Cruz *et al.,* 2013). The mineral composition of the nutrient medium plays a major and fundamental role in the growth of the plant part grown in it, and each planted part of the plant has requirements for mineral salts that differ from the other part (Greenway *et al.*, 2012). Salts (KNO_3, KH_2PO_4, $CaCl_2$) are considered among the salts that most affect the growth of the plant part in the laboratory because of their effect on cell growth and the growth of the plant part in general (Kovalchuk *et al.*, 2017). Hunková *et al.* (2020) showed that cultivating lateral shoots *Amelanchier alnifolia* Var cusickii plants on MS medium prepared with different concentrations of KH_2PO_4, $CaCl_2$ and MgSo4 salts led to an increase in the number and length of growing branches four weeks after planting. Given the importance of this plant from a medical and therapeutic point of view, and knowing the possibility of causing callus by the effect of growth regulators Kin and 2,4-D, as well as knowing the effect of salts (KNO_3, KH_2PO_4, $CaCl_2$) on callus growth and its content of the two compounds (khellin, visnagin), this research was conducted**.**

Materials and Methods

The current study was conducted for the period from 3/1/2022 until 28/12/2022 in the Plant Cell and Tissue Culture Laboratory of the College of Agriculture, University of Tikrit, Iraq, to establish tissue cultures from the growing tips of seedlings of the local khella plant and quantitative and qualitative detection of some metabolic compounds (khellin, visnagin) from the Khalla baladi plant in vitro by chromatographic analysis (HPLC) of callus and laboratory analyzes were conducted at the Center for Pharmaceutical Control and Research, Ministry of Health. The study included the following:

1- Planting khella seeds on Woody plant medium (WPM) prepared with different levels of 2,4-Dichlorophenoxyacetic acid (2,4-D) and Kinetin 6-furfurylo- amino-purine (Kin) to germinate seeds and obtain seedlings for callus induction.

2- Cultivating seedling parts (shoot tips) on WPM medium prepared with different levels of 2,4-D and Kin to induce callus.

3- Cultivation of callus with different levels of KNO_3, KH_2PO_4 and $CaCl_2$ salts.

4- Quantitative and qualitative estimation of metabolic compounds (khellin, visnagin) using an HPLC device.

Preparing the food medium

The WPM nutrient medium (Lloyd and McCown 1980) from the German company Dephyte Biotechnology was used. To prepare one liter of the nutrient medium, 600 ml of distilled water was placed in a glass beaker and 2.383 g of the WPM nutrient medium was added to it, after which 30 gm L^{-1} of sucrose was added and was placed On a hot plate magnetic stirrer and adjust the pH to 5.7 by adding drops of one standard solution of sodium hydroxide (NaOH) or hydrochloric acid (HCL), then increase the final volume to 1000 ml using a cylinder and add 7 gm l^{-1} of agar-agar after dissolving it. Completely on a hot plate magnetic stirrer, distribute it into the planting containers at an amount of 20 ml for each tube. It was covered with appropriate covers made of heat-resistant Polypropylene, and sterilized with an autoclave device at a pressure of 1.04 kg. cm^2 and a temperature of 121°C for 20 minutes. After that, the tubes were taken out of the incubator and left to cool and solidify and kept in a sterile, dark place. Thus, they were ready for cultivation. Growth regulators were added during the preparation of the medium and according to the experiments carried out.

Surface sterilization of seeds

Used seeds of the *Ammi visnaga* variety, Syrian Local, obtained from agricultural equipment offices in the capital, Baghdad. I washed the seeds with water and liquid soap to remove the dust and dirt stuck in them for two hours, and then I soaked the seeds in GA_3 at a level of 100 mg L^{-1} for 8 hours. They were then transferred to the Laminar air flow cabinet planting table, after which the seeds to be sterilized were immersed in a solution of commercial trace elements with a level of 4% of NaOCl, the active ingredient with a level of 6 % (volume: volume) for 15 minutes with continuous stirring, then they were washed with sterile distilled water three times for 5 minutes. Minutes each time to ensure that the harmful effect of the sterilizer is removed from the seed surface. The sterilized seeds were then planted in cultivation containers in semi-solid WPM medium devoid of growth regulators, but the germination rate was very low, so the experiment was repeated and the seeds were planted on WPM medium supplemented with 2,4-D in level 0, 1.5, 2.0, 2.5, and 3.0. mg L^{-1} mixed with Kin, which was added at a fixed level of 2.5 mg L^{-1}, with five seeds per container. The seeds were incubated in complete darkness for 48 hours, after which they were incubated in 16 hours of light and 8 hours of darkness, at a temperature of 25 °C ± 2, and after obtaining the growing tips of the seedlings were cut three weeks after planting at a length of 5 mm and taken for callus induction experiments.

Callus induction experiments

An experiment was conducted to find out the interaction effect between 2,4-D and kinetin. In the formation of callus tissue from the shoot tips of the khella plant and its growth, ten levels of 2,4-Dichlorophenoxyacetic acid (2,4-D) were used, and its levels were 0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, and 5.5 mg L^{-1} mixed with a fixed level of Kinetin N6-Furturyle amino-purine at a level of 2.5 mg L^{-1}. The shoot tips with a length of 5 mm were used in this experiment to determine the extent of the shoot tips response to callus induction and the optimal level for the emergence and growth of callus tissue. It was planted the shoot tips were grown on the solid nutrient medium WPM at a rate of 10 replicates for each treatment. Then the crops were transferred to the growth room under the same seed incubation conditions and were re-cultivated on the same media four weeks after cultivation to determine the effect of the interactions between 2,4-D and Kin to determine the optimal level. To carry out subsequent experiments, the following measurements were taken.

1- **Percentage of callus formation 8 weeks after planting**: It was calculated according to the following equation:

Response rate = Number of plant parts that make up the callus / Total number of plant parts × 100

2- **Fresh weight of callus**: Fresh weight was measured using a sensitive balance.

3- **Dry weight of the callus**: The dry weight was measured after it was dried in an oven at 65 °C until the weight was stable.

4- The diameter of the calamity (mm) in this study using the Vernier Caliker.

5- **Dry matter percentage**: The dry matter percentage of the callus tissue was calculated according to the equation used by Sakthivelu *et al*. (2008). It was calculated according to the following equation:

Percentage of dry matter = Dry weight at the end of the experiment / Wet weight at the end of the experiment × 100

6- **Callus size**: Observations were recorded about the size of the callus formed, and marks were given to estimate the size of the callus according to the method used by Al-Noah (2009) after making some modifications to it and according to the study that was conducted, as follows:

Estimation of callus size (0 = no callus formed, *very little callus less than 0.2 cm, **little callus 0.2 - 0.4 cm, ***moderate callus 0.5 - 0.7 cm, ****large callus from 1.0 - 3.0 cm).

Perpetuation of callus tissue

After determining the best level of growth regulators used in callus emergence and induction experiments, the induced callus was transferred from the shoot tips to WPM medium supplemented with a level of 3.0 mg L^{-1} of 2,4-D with a fixed level of Kin 2.5 mg L^{-1}. This gave the highest percentage of callus formation and the highest fresh and dry weight of callus. The

replanting process was repeated every four weeks until the amount required for subsequent experiments was reached.

Addition of inorganic salts (KNO_3, KH_2PO_4 and $CaCl_2$)

A fixed weight of 150 mg of callus was taken and grown on a WPM nutrient medium supplemented with a level of 3.0 mg L^{-1} of 2,4-D, with the addition of Kin at a level of 2.5 mg L^{-1} (as the best treatment in callus induction experiments), to which different levels of Inorganic salts, each experiment separately, with all experiments participating in a single comparison treatment free of any addition of inorganic salts. The first experiment involved adding different levels of potassium nitrate KNO_3 (0.0, 0.20, 0.30, and 0.40 g L^{-1}), while calcium chloride was added. Aqueous $CaCl_2.2H_2O$ was added at levels of 0.0, 0.20, 0.30, and 0.40 g L^{-1}, while potassium phosphate KH_2PO_4 was added at levels of 0.0, 0.20, 0.25, and 0.30 g L^{-1} at a rate of ten replicates for each level. The crops were incubated in the development room at a temperature of 25 °C ± 2, with 16 hours of light and 8 hours of darkness. After eight weeks of cultivation and maintenance, the fresh and dry weight of the callus was taken, and 3 homogeneous samples were separated from every ten replicates to conduct the extraction process for the metabolic compounds.

Determination of Khellin and Visnagin compounds in callus

Simultaneous determination by HPLC of both Khellin and Visnagin: in *Ammi visnaga* L. extract, where the main compounds were separated on FLC (fast liquid chromatography) under ideal conditions:

Column: zorbaxc-18.3 micrometer size (50×0.2 mm)

Mobile phase: water: methanol: tetrahydrofuran (50:45:5, v/v/v) at a flow rate of 1.5 ml/min at a wavelength of 245 nm and an injection volume of 20 µl:

Standard Concentration of Sample = Sample Area / Standard Area × Standard concentration × dilution Factor

Separation equipment on a Shimadzu 10AV-LC liquid chromatograph equipped with a Shimadzu LC-10A dual conduction pump model. The removed peaks were monitored by a UV-V is 10 A-SPD spectrophotometer.

Statistical analysis

A completely randomized design was used in the analysis of the data, which consisted of 10 replicates, and a program was used (SAS, 2001) to analyze the data, and a serious correlation according to the least significant difference test (LSD) and a probability level of 5% (Al-Rawi and Khalaf Allah, 2000).

Results and Discussion

Effect of adding different concentrations of 2,4-D mixed with Kin at a level of 2.5 mg L^{-1} on the percentage of germination of plant seeds of *Ammi visnaga*

Table 1 shows that the germination rate of seeds was affected when grown on WPM medium supplied with different levels of 2,4-D (0, 1.5, 2.0, 2.5, and 3.0 mg L^{-1}) and mixed with Kin at a

level of 2.5 mg L^{-1} different responses were shown, as it was noted that the best germination rate of seeds, which reached 100 %, was when adding 2,4-D at a level of 3.0 mg L^{-1} and Kin at a level of 2.5 mg L^{-1}, as it led to giving the highest germination rate with the formation of a total vegetative and rooting are good and no callus was formed at all, The control treatment gave the lowest germination rate, which reached 9 % Figure 1. The reason may be due to the increase in seed germination when adding 2,4-D at a level of 3.0 mg L^{-1} and Kin at a level of 2.5 mg L^{-1}. This addition achieved an internal balance in auxins and cytokinins, which encouraged increased cell division, or the role of cytokanins in activating the H^+/K^+ exchange system, which causes cell wall relaxation and a decrease in cellular osmotic capacity, which leads to improved water absorption and increased germination rate (Bhatla and Lal, 2023). Or it may be due to the role of cytokinin in cell division, activating seed dormancy formed by abscisic acid, and inhibiting the greening of the cotyledons (Guan *et al*., 2014; Wang *et al.,* 2011).

Table 1. Shows the effect of adding different levels of 2,4-D and Kin at a level of 2.5 mg L^{-1} on the percentage of seed germination of *Ammi visnaga*

2,4-D (mg L^{-1})	Kin (mg L^{-1})	germination rate (%)
0	0	9
0	2.5	66.7
1.5		66.9
2.0		71
2.5		82
3.0		100

Figure 1. Seedlings of the local Khalla plant, grown on WPW medium containing the growth regulator 2,4-D and Kin at a level of 3.0 mg L^{-1}, 2.5 mg L^{-1} at respectively after 25 days of planting

Effect of adding different concentrations of 2,4-D mixed with Kin at a level of 2.5 mg L^{-1} on the induction of callus from the shoot tips of *Ammi visnaga*

The data in Table 2 showed that the WPM nutrient medium supplemented with a level of 3.0 mg L^{-1} of 2,4-D and 2.5 mg L^{-1} of Kin gave the best response rate to callus induction and the highest fresh and dry weight of callus induced from the shoot tips as well. It gave the best diameter, dry matter percentage, and callus volume of 95.80 %, 4.61 gm, 67.07 mg, 2.91 mm, and 23.63 %, ****, respectively. Compared with a level of 5.5 of 2,4-D, which gave the lowest values in terms of the percentage of callus induction, the fresh and dry weight of the callus, the diameter of the callus mass, the percentage of dry matter of the callus, and its size 62.25 %, 1.76 g, 36.70 mg, 0.97 mm, and 11.90 %, **, respectively. The results showed that the level of auxin has an effect in increasing the weight of the induced callus, as it was found that there was an increase in the average weight of the induced callus with an increase in the level of auxin added to the nutrient media, reaching the optimal concentration of it and in the presence of a constant level of cytokinin, which gave the highest weight to the callus. After that, the response to an increase in the rate of callus decreased. Weight with increasing level. Increasing the level of 2,4-D auxin and keeping the level of Kin the same may have caused the rate of fresh and dry weight of callus to go up. This is likely because auxins help cells divide and grow (Hartmann *et al*., 2002). In addition to their other effects, it appears on the middle lamina of the cells, which helps in the expansion of the cell wall until the optimum level of auxin is reached (Ikeuchi *et al*., 2013). The balance between cytokinin and auxin is necessary in the formation of callus, as cytokinin, in its presence with auxin, acts as a key to initiating cell division. The adenine present in the cytokinin may be the part that plays the main role in the process of cell division, and adding cytokinins to nutrient media may lead to important effects in the process. Cell division and increase, especially the increase in meristematic cells, and this in itself leads to an increase in the size of the various tissues of the plant organs, whether these parts are connected to the mother plant or separated from it and grown in different sterile nutrient media (Ioio *et al.*, 2007).

Table 2. The effect of adding different levels of 2,4-D and a fixed level of 2.5 mg L^{-1} of Kin on the growth and multiplication of callus tissue induced from the shoot tips of the *Ammi visnaga*

Growth hormones (mg L^{-1}) 2,4-D	Callus induction percentage (%)	Fresh weight of callus (g)	Dry weight (mg)	Callus mass diameter (mm)	Dry matter percentage of callus	Callus size
0.0	68.90	2.00	37.73	0.80	13.13	**
1.5	87.10	3.23	52.10	2.16	21.26	***
2.0	84.64	2.93	43.56	2.01	18.57	**
2.5	85.10	3.01	47.20	1.80	19.48	***
3.0	95.80	4.61	67.07	2.91	23.63	****
3.5	74.00	2.87	42.41	1.25	16.74	***

4.0	73.20	2.54	40.29	1.18	15.49	**
4.5	72.10	2.20	38.38	1.07	14.07	**
5.0	66.00	1.96	37.73	1.01	13.81	**
5.5	62.25	1.76	36.70	0.97	11.90	**
LSD ≤ 0.05	13.61	1.11	15.36	0.44	5.46	—

Estimation of callus size (0 = no callus formed, *very little callus less than 0.2 cm, **little callus 0.2 - 0.4 cm, ***moderate callus 0.5 - 0.7 cm, ****large callus from 1.0 - 3.0 cm).

Increasing the concentration leads to an imbalance in the optimal balance, which leads to a decrease in the response rate. This may be due to a decrease in the callus weight rate. When the concentration of 2,4-D in the growth medium increases, as mentioned above, auxin plays a role in stimulating the production of ethylene gas, which in turn reduces the rate of plant cell division (Trigiano and Gray, 2000). As for giving callus from the comparison treatment, it may be due to the potential energy of the cells and the tips of the branches containing a percentage of internal auxin, as they are the sites of auxin production (Al- Hasany, 2021; Al- Qasam and Ghanim, 2023; Al-Hadidi, 2002).

Effect of adding different levels of KNO_3 on the growth of callus of *Ammi visnaga* plant and its production of some metabolic compounds

Table 3 shows that adding different levels of KNO_3 to the WPM nutrient medium led to significant differences between the studied traits, as the medium prepared with a level of 0.20 g L^{-1} of KNO_3 gave the best rate of fresh and dry weight of callus, which amounted to 2.942 g, 0.862 mg over straight. Which did not differ significantly from the two treatments of adding 0.30 and 0.40 g L^{-1} of KNO_3, which gave a similar rate in fresh and dry weight. The comparison treatment was the least effective and gave the lowest rates in terms of fresh and dry weight of callus 0.906 g and 0.144 mg respectively. The same table also shows that there was a significant increase in the compounds Visnagin and Khellin, and that the highest response occurred at the level of 0.30 g L^{-1} of KNO_3 added to the medium, as it gave 4.214 and 3.440 ug mL^{-1} dry weight of callus, respectively. This differed significantly from the treatments 0.20 and 0.40 g L^{-1}, which gave 2.013, 2.636, 1.936, and 1.650 ug mL^{-1} dry weight of calcium, respectively, and did not differ significantly between them, while the comparison treatment recorded the lowest response of 0.883 and 0.826 ug mL^{-1} dry weight of calcium.

Table 3. Effect of adding different levels of KNO_3 on the growth of callus tissue induced from the growing shoot of *Ammi visnaga* and its production of some metabolic compounds

Concentration KNO_3 (g L^{-1})	Wet weight (g)	Dry weight (mg)	Secondary metabolite	
			Visnagin (ug mL^{-1})	Khellin (ug mL^{-1})

0.0	0.906	0.144	0.826	0.883
0.20	2.942	0.862	2.636	2.013
0.30	1.610	0.411	3.440	4.214
0.40	1.808	0.571	1.650	1.936
LSD ≤ 0.05	0.656	0.403	1.495	1.109

Effect of adding KH_2PO_4 on the growth of callus of *Ammi visnaga* plant. And its production of some metabolic compounds

Noted from Table 4 that adding different levels of KH2PO4 led to a significant increase in the average fresh and dry weight of the callus, given the WPM medium prepared with 0.25 g L^{-1} of KH_2PO_4. The highest rate for the fresh and dry weights reached 1.726 g and 0.770 mg, respectively, which did not differ significantly from the treatment of adding 0.20 g in the rate of the fresh and dry weights and the treatment of adding 0.30 g of the rate of the dry weight only, The control treatment gave the lowest average for the two fresh weights. And dry reached 0.906 g and 0.144 mg, respectively. The amount of visnagin and khellin in the callus tissue gradually went up as the level of KH2PO4 salts went up compared to the control treatment. The level of 0.30 g L^{-1} of KH2PO4 salts had the most significant effect on stimulating callus production of visnagin and khellin compounds, reaching 3.466 and 3.636 µg mL^{-1} dry weight of callus, respectively. This was significantly higher than the other levels, except for the treatment of adding 0.25 g L^{-1} KH2PO4, which gave a response of 3.034 and 2.540 µg mL^{-1} dry weight of callus from visnagin and khellin, respectively. The lowest response was recorded to the compounds visnagin and khellin when treated as a comparison.

Table 4. Effect of adding different levels of KH_2PO_4 on the growth of callus tissue induced from the growing top of medicinal plant *Ammi visnaga* produces some metabolic compounds

Concentration KH_2PO_4 (g L^{-1})	**Wet weight (g)**	**Dry weight (mg)**	**Secondary metabolite**	
			Visnagin (µg ml^{-1})	**Khellin (µg ml^{-1})**
0.0	0.906	0.144	0.826	0.883
0.20	1.492	0.571	1.697	1.463
0.25	1.726	0.770	2.540	3.034
0.30	1.233	0.478	3.636	3.466
LSD ≤ 0.05	0.442	0.416	1.675	1.792

The effect of adding different levels of $CaCl_2$ on the growth of callus of the *Ammi visnaga* and its production of metabolic compounds

The results in Table 5 showed that the highest average fresh weight of callus tissue occurred in the treatment of adding 0.40 g L^{-1} of $CaCl_2$ to the WPM medium, which outperformed the rest of the treatments as it gave an average of 2.957 g, while the highest average dry weight was obtained from the treatment Adding 0.30 and 0.40 g L^{-1} of $CaCl_2$ to the WPM medium gave a rate of (0.647 and 0.925 mg), respectively, while the control treatment gave the lowest rate of fresh and dry weight of 0.906 g and 0.144 mg, respectively. It is also noted from the results of the same table that there are significant differences in the level of the studied secondary compounds when different concentrations of $CaCl_2$ are added to the nutrient medium, as the level of 0.20 gm L^{-1} gave the highest amount of Visnagin and Khellin, amounting to 3.783 and 0.883 µg mL^{-1} dry weight of calcium, respectively. The control treatment gave the lowest value of Visnagin and Khellin compounds, reaching 0.883 and 0.826 µg mL^{-1} dry weight of callus, respectively.The increase in the fresh and dry weight of callus and the level of metabolic compounds that were estimated by increasing different levels of salts in the nutrient medium and shown in Tables (2, 3, 4) may be due to the fact that these elements are considered essential for the growth and reproduction of plant cells (Sudheer *et al.*, 2022). As well as its involvement in most of the vital activities of the plant cell, such as building chlorophyll and including phosphorus as a structural component of nucleic acids, phospholipids, and ATP (Adelberg *et al*., 2010). The increase obtained in the studied traits may be due to achieving an ideal balance between the nutrients that were added to the nutrient medium and what the plant part needs for growth and metabolic processes within the cell.

Table 5. Effect of adding different levels of $CaCl_2$ on the growth of callus tissue induced from the growing top of medicinal plant *Ammi visnaga* produces some metabolic compounds

Concentration $CaCl_2$ (g L^{-1})	Wet weight (g)	Dry weight (mg)	Secondary metabolite	
			Visnagin (µg ml^{-1})	Khellin (µg ml^{-1})
0.0	0.906	0.144	0.826	0.883
0.20	1.385	0.524	3.880	3.783
0.30	2.134	0.647	2.416	2.180
0.40	2.957	0.925	1.296	1.160
LSD ≤ 0.05	0.676	0.367	1.780	0.899

Conclusions

This study concludes that adding auxin 2,4-D at a level of 3.0 mg $L^{-1}$and Kin at a level of 2.5 mg L^{-1} gave the highest germination rate for the seeds of the local Khalla plant, reaching 100%, and the best percentage of callus formation from the growing apex, reaching 100%. 95.80%. Also, the addition of KNO_3 at a level of 0.30 mg L^{-1}, the addition of KH_2PO_4 at a level of 0.30 mg L^{-1}, and the addition of $CaCl_2$ at a level of 0.20 mg L^{-1} achieved the highest level of compounds Visnagin and Khellin from callus induced from the growing apex of the Khalla plant.

Conflicts of interest

The authors declare that there is no conflict of interest regarding the publication of this manuscript.

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