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INDUCED CALLUS OF FENNEL PLANT (*FOENICULUM VULGARE* MILL.) *IN INVITRO*

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Abstract

This current study is to find an easy and efficient way to induce the callus tissue of the fennel plant in vitro on Murashige and Skooge medium (**MS**) by using different concentrations of growth regulators. The growth regulators of Naphthalene acetic acid (**NAA**) and Benzyl adenine **(BA**) were used in concentrations (0.5, 1, and 1.5 mg/L) for each hormones, to reach the best auto lithic concentration between them. The plant parts (stems, leaves) obtained from plants that derived from seed cultivation were cultured in vitro. The results indicated that stem explants gave a best result compared with the leaf in percentage of callus induction (dry and fresh weight). The interaction between explants types and plant growth regulators in medium showed that (1mg NAA +1mg BA) per litter for stem gave the highest callus induction percentage of dry and fresh weight of callus, as in statistical tables (1 and 2).

Key words: *Foeniculum vulgare* Mill, Seeds, Callus, Naphthalene acetic acid, Benzyl Adenine**.**

Introduction

Plants have long been known to be of great importance not only as a source of food but as a source of access to a wide range of chemicals such as pharmaceutical compounds, insecticides, coatings, perfumes and colors (Dixon, 1985). In recent years, there has been a clear interest in the cultivation of medicinal plants and their investment in the acquisition of therapeutic or pharmacological substances rather than chemically processed substances (Asree *et al*., 2019). Experiments have shown that the active, laboratory-manufactured material does not perform the physiological effect of the same active substance extracted from medicinal plants (Hussein, 2011). The technology of textile agriculture allows access to vehicles with high medical value and their production is fast without relying on the season in which these plants grow without the need to allocate large areas for the purpose of cultivation and use these areas to grow crops economically important (Mohammad, 1990). Fennel (*Foeniculum vulgare* Mill) belongs to the Apiaceae family, and has aromatic, flavoring and medicinal properties; it can be used in alternative medicine as well as in the cosmetic industry, due to its essential oil which is rich in various biologically active ingredients(Souza *et al*., 2014) . Seeds are an efficient means of transmitting pathogens, often introducing them into previously non infected areas. The presence of pathogens can reduce the physiological quality of the seeds, in modern medicine, he described the fennel, is a plant with a good aromatic extract, and is characterized by multiple positive properties. It contains vitamins A, B and C in addition to various minerals, including calcium, phosphorus, iron, sulfur and potassium (Ethan et al., 2003) . In view of the importance of medical Fennel Plant and containing important secondary compounds involved in the pharmaceutical industries for each of their production compared to the demand and the increasing need for these compounds, so it is necessary to employ the technique of cultivation of plant tissues and the use of hormones and the addition of stimuli to the agricultural medium they increase the production of secondary active and propagation of the plant syllabus even Invest his textile farms throughout the year in the production of pharmaceutical concentrates. The aim of this study to stimulate callus from the fennel plant by using different concentrations of growth regulators NAA and BA with

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different parts of plant (leaf and stem).

Materials and Methods

Preparation of solutions and medium:

Dissolve (50mg) of Naphthalene acetic acid (NAA) in (1ml) of absolute ethyl alcohol to ensure complete solubility and complete the volume to 50ml with the distilled water to become our concentration solution 1 mg/L. While the Benzyl adenine (BA) was obtained by dissolving (50mg) of BA in (1ml) hydrochloric acid (1N) to ensure melting and complete the volume to (50ml) with distilled water to become our concentration solution of (1mg/L).

Medium Cultivation of Seeds:

Use the medium (MS) Murashige and Skoog (Murashige and Skooge, 1962), free of growth regulators by experimenting to cultivate seeds in the medium that prepared from MS medium (4.61g) per liter and sugar additive at (30g/L) and then put on the heater for 5 minutes to ensure complete solubility of sugar and all ingredients. The pH was then adjusted to 5.8 with (1N) NaOH or (1N) HCL and then added (7g/L) of agar (agar_agar) and placed the medium on the preheated until boiling. When the solution became transparent, place the (10 ml) from the medium of each of the sterile agricultural tubes and then put the autoclave in a temperature (121°C) and pressure (15 inch sq/Ib) for 15 minutes. Then got out of the autoclave and became ready to planting.

Seed collection and sterilization:

The seeds of the funnel were collected from the local market of the city of Hilla and was educated in the Faculty of Science/Babylon University. Placed a suitable quantity of seeds in the flask and wash with distilled water three times to get rid of the dust and impurities, then transfer to the chamber of the laminar air flow cabinet and refine it with 15% sodium hypochlorite with stirring for 7 minutes. Wash with distilled water for one minute for three times and sterilize as well with 70% ethyl alcohol for 30 seconds and then rinse with distilled water for one minute at three times to remove ethyl alcohol, then put seeds in a petri dish on sterile filter paper to water removal (Awika and Rooney, 2004).

Cultivation of seeds:

After preparing the MS medium and sterilizing the seeds, three seeds were taken and planted in each tube of the container tubes on (10ml) of MS free medium of growth regulators (Fig. 1) the purpose to obtaining plants free of pathogens, then incubated under (1000 Lux) pear 16 hours lighting and 8 hours darkness daily, at a temperature of 25±2ºC.

The process of induction of callus:

Plant parts (leaves and stems) were taken from cultivated plants in sterile conditions after 21 days of cultivation. The above plant parts were planted with a length of 0.5-1 cm on the medium of MS (Figs. 2, 3, 4), the technique used to plant tissues for this purpose was to stimulate the callus from its fennel explants add sugar with concentration 30 mg/l and NAA with concentration (0.5, 1, 1.5) mg/l and BA with concentration (0.5, 1, 1.5) mg/l.

Determination of fresh weight:

The appearance of callus after 15 days of planting the seeds on the medium and after 45 days completed the growth of callus where the weight was calculated for fresh weight of callus in glass tubes and measured the fresh weight by using the sensitive electrical balance after removing the remnants of the medium on callus by washing with distilled water.

Determination of dry weight:

Place fresh callus in Petri dishes, each dish containing 6 pieces of callus tissue for both of NAA and BA combinations and dry weights of the callus were determined after drying in the oven at a temperature of 40ºC for 24 hours.

Statistical analysis:

The results were analyzed according to the complete random design (C.R.D) through a practical experiment and the extraction of the values of the least significant difference between the workers and the interaction between them for the weights of dry and dry according to the selection of the least significant difference (L.S.D) at the level of probability. 0.05 (AL-Rawi and Khalaf Allah, 2000). The present study was conducted in the plant tissue culture laboratory of the department of

Table 1: Shows the fresh weight of the plant parts (stem/ leaves) and the effect of interaction between different concentration BA and NAA.

Plant part (leave)g	Plant part (Stem)g	BA mg/L	NAA mg/L
0.89	1.26	0.5	0.5
0.85	1.16	1	0.5
0.32	0.73	1.5	0.5
0.92	1.64	0.5	1
1.34	2.66	1	1
0.81	1.36	1.5	1
0.77	1.47	0.5	1.5
0.96	1.92	1	1.5
0.53	0.87	1.5	1.5
0.192	0.252	0.05	L.S.D

Table 2: Shows the Dry weight of the plant parts (stem/ leaves) and the effect of interaction between different concentration of BA and NAA.

Dry weight of leave	Dry weight (g) of plant	BA mg/L	NAA mg/L
0.103	0.119	0.5	0.5
0.112	0.172	1	0.5
0.03	0.07	1.5	0.5
0.92	0.159	0.5	1
0.159	0.258	1	1
0.05	0.09	1.5	1
0.113	0.138	0.5	1.5
0.120	0.195	1	1.5
0.04	0.09	1.5	1.5
0.0123	0.0329		L.S.D(0.05)

Fig. 1: Seeds cultivated on the medium (MS)

Fig. 2 : plant parts cultivated on the (MS) medium

Fig.3 : Stems cultivated on the medium (MS)

Fig.4 : Leaves cultivated on the medium (MS)

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Results and Discussion

Effect of the interaction between NAA and BA in the fresh and dry weight of fennel callus

The interaction between the concentrations of the growth regulators NAA and BA has a significant effect on the induction of the callus tissue of the fennel plant to the vegetal parts (leaf, stem). The highest weight of fennel (2.66g) was obtained for the explant of the stem while (1.34g) for leaf Concentration with growth regulators (1mg/L) of NAA with (1 mg/L) of BA (Fig. 5). Followed by the combination (1.5 NAA) + (1 BA) mg/L, with the fresh weight of the callus of the explant of the stem (1.92g) while the fresh weight of the leaf part was (0.96g). The fresh weight of the callus was decreased at (0.5 NAA/L + 1.5 BA) mg/L at (0.73g) for the stem, while the callus weight of the leaf was (0.32g). It differs significantly from all the coefficients of the growth regulators NAA and BA.

In the same vein, dry weights showed different values in the table 2, it was observed that the highest dry weight of the callus stem plant part was (0.288)g and leaf (0.159)g

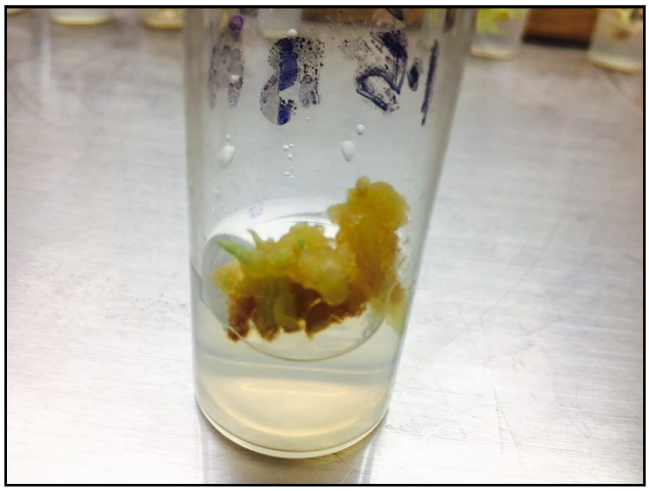

Fig. 5: Interference between concentration of (1mg NAA +1mg BA) per litter for Callus of (*Foeniculum vulgare* Mill.) stem explant on the MS medium.

at the interaction (1 NAA + 1BA) mg/L, which is significantly different from the rest of the treatments, and the lowest dry weight of the callus between the combinations at concentration (NAA 0.5, BA1.5) mg/L was 0.07g, (0.03) for the leaf and significantly different from all the treatments for BA and NAA except for the interference treatment (NAA1.5+1.0BA) mg/L.

The ratio between the growth regulators of oxy-cytokinin added to MS has had an important role in obtaining the best fresh and dry weight of callus in the medium of agriculture due to the physiological balance between Auxin and cytokinein. The increase in the concentration of Auxin and cytokinin at the expense of the other affects callus and reduces its growth (Mino, 1990). (Genteno *et al.,* 1996) also noted the need for hormonal balance between Auxins and cytokines in the induction and growth of the callus which explains the increase in the fresh and dry weight of the induced callus of the vegetative parts of the leaf, stem of funnel. The composition of the callus depends on the genetic and the physiological state of the plant's part of the plant and its hormones inside it, the physiological age of the plant part, the nutrient type, and the conditions of tissue culture (temperature, light, sugar, etc.) have a significant impact on the composition of the callus and the amount of its fresh and dry weight (Garcia *et al*., 2011).

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