

## ***In vitro* Cultivation of Marjoram (*Origanum majorana* L.) under Influence of 2,4-D (2,4-Dichlorophenoxy Acetic Acid) as Herbicide.**

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**Abstract:** Callus induction of marjoram (*Origanum majorana* L., family Lamiaceae) was done by hypocotyl and seed explants which cultured on MS medium supplemented with auxin (2,4-D or NAA) at 1, 2 or 4 mg/L in combination with cytokinins (Kn or 6 BAP) at 0.2, 0.4, 0.5 or 1 mg/L. For seedling culture, the sterilized seeds were germinated on the same basal MS medium free of phytohormones but supplemented with different concentrations of 2,4-D as herbicide (6, 11, 22.5, 23.5 and 45 mg/L). Comparison of different phytohormonal combinations in terms of callus production revealed that: the 2,4-D + Kn (2+0.5 mg/L) and NAA + 6BAP (4+0.4 mg/L) were the most responsive for callus induction. A lower callus formation was obtained on medium supplemented with 2,4-D + Kn (1+1 mg/L) and NAA + 6BAP (1+1 and 1+0.5mg/L). The seed and hypocotyl calli retained high proliferation rate for two subcultures, afterwards in the 3<sup>rd</sup> and 4<sup>th</sup> subcultures the calli grew slower, turned brown and didn't survive in subsequent subcultures. The highest growth rates and fresh weights of aseptic marjoram seedlings were observed on medium contained 6 and 11mg/L 2,4-D, while the lower growth rate was obtained on medium supplemented with 22.5 mg/L 2,4-D. In case of higher conc. of 2,4-D (23.7 and 45 mg/L) no growth was recorded and seedlings dead finally. From the profile of SDS-PAGE, there is no distinct variation on the proteinous bands of the treated marjoram seedlings with 6 and 11mg/L 2,4-D, in addition to control of non-treated seedlings, assuming the absence of inducing effect on gene expression. However the density of proteinous bands was increased upon 2,4-D (6 and 11mg/L) treatment as compared with the control assuming the induction of hyper amount of induced expressed protein to resist herbicide treatment. From the profile of antifungal activity, a slight fungistatic activity was observed by the aqueous extract of marjoram seedlings treated by 2,4-D at concentration of 6mg/L and 11 mg/L, comparing to untreated control.

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**Key words:** Marjoram (*Origanum majorana* L.), 2,4-D, herbicide, *in vitro*, callus, seedling, growth, protein, SDS-PAGE, antifungal.

### **1. Introduction:**

Marjoram (*Origanum majorana* L.) family Lamiaceae, is used as a spice and for treatment of gastrointestinal disturbances, cough and bronchial diseases. Marjoram is used in mouthwashes for oral hygiene and also applied topically to relieve symptoms of the common cold, such as nasal congestion (Bruneton, 1999). A number of the marjoram essential oil components exhibit significant antimicrobial properties when tested separately (Lambert *et al.*, 2001). Several studies reported that methanolic extracts of marjoram had high antioxidant capacity (Hossain *et al.*, 2011).

There are a number of post-emergent grasses (weeds) growing with marjoram plant which reduce its growth and yield. Weed species are either broadleaf or grassy type. Herbicides are used to control weeds within agricultural areas, for example, 2,4-dichlorophenoxy acetic acid (2,4-D) is a systemic auxin-like selective herbicide, belonging to phenoxy acetic group and is recommended to control broadleaf weeds in cereal crops (Tomlin, 2006).

*In vitro* cell cultures have been developed as promising alternative tool for agricultural processes in producing valuable phytochemicals (Langhansova *et al.*, 2005; Srivastava and Srivastava, 2007). The advantage of this method is that it can ultimately provide a continuous, reliable source of natural products. Tissue culture appears to be a good alternative to conventional propagation, requiring less physical space, with high multiplication rate, without incidence of pests and diseases during cultivation, and enabling higher control of the variables involved. Thus, in the *in vitro* environment, with the required stimuli and appropriate conditions, different cell types express different behaviors, possibly leading to cell multiplication and differentiation into a specific tissue, characterized by a form and a function, which may lead to the regeneration of a new individual (Bhojwani and Razdan, 1996). Throughout the history of tissue culture, various kinds of culture media have been developed. However, the MS (Murashige & Skoog) medium (Murashige and Skoog, 1962) is the most widely used for the regeneration of dicots, and

therefore it has a great importance in the applications of tissue culture in agriculture.

Among biochemical techniques SDS-PAGE is widely used for its simplicity and effectiveness for describing the genetic structure of crop germplasm (Siddiqui and Naz, 2009). Egli *et al.* (1985) reported that many herbicides interfere with the protein synthesis, for instance, atrazine or diuron inhibits the synthesis of protein in *Solanum nigrum* cell suspension. A extreme higher concentration ( $10^{-3}$ M) of 2,4-D or 2,4,5-T (auxin-like herbicides) leads to the death of bean and sunflower plants or decrease growth in maize plants by occurrence of abnormalities in the nucleic and protein synthesis (Shaddad *et al.*, 1990).

In this regards our aims are to establish of aseptic cultures (callus and seedling) of marjoram plant and investigate the effects of different concentrations of 2,4-D as herbicide on growth, protein and antifungal activity of these cultures *in vitro*.

## 2. Material and Methods:

The experimental plant used in this investigation was marjoram (*Origanum majorana* L.) family Lamiaceae. Pure strains of seeds of were obtained from National Center of Agricultural Research. Ministry of Agriculture, Giza, Egypt. 2,4-Dichlorophenoxy acetic acid (2,4-D-Auxin like herbicide), phytohormones and MS medium were purchased from Phytotechnology Lab. Egypt. *In vitro* cultivation of marjoram was done in Plant Tissue Culture Lab. in Botany Department, Faculty of Science, Zagazig University, Egypt.

### I- Callus and seedling cultures of *Origanum majorana* L.

#### 1- Surface sterilization of seeds:

Seeds of *O. majorana* were washed with running water and then surface sterilized by immersing in 70% ethanol for 30-50 sec., followed by soaking in sodium hypochloride (6-14% active chlorine) for 10 min. Then the seeds were washed 3 times with sterilized distilled water.

#### 2- Germination of seeds:

The callus induction medium was MS medium containing vitamins (Murashige and Skoage, 1962). The pH of the medium was adjusted to  $5.8 \pm 0.1$  using 1 M NaOH and 1 M HCl. For preparation of solid media, 8 g/L Bacto-agar was added to the media with stirring and heating. The media were then dispersed in 100 ml screw capped glass jars; 30 ml per each jar and autoclaved at  $121^{\circ}\text{C}$  and 15 psi for 20 min.

The sterilized seeds were distributed on the surface of a sterile basal MS medium containing 3% sucrose and 0.8% agar, and then incubated at  $25^{\circ}\text{C}$  in the dark. All manipulations were carried out under aseptic conditions using a laminar flow cabinet with horizontal flow of sterilized air.

### 3-Callus induction:

Pieces of hypocotyl explants were excised from germinated seedlings in addition to sterilized seeds as explants were placed on the surface of sterilized callus initiation MS medium supplemented with different combination of phytohormones (auxin and cytokinin) as in Table (1). All culture incubated at  $25^{\circ}\text{C}$  under 10 hr photoperiod. The light intensity cooled white light was 1700 lux at the shelf surface.

**Table (1):** Phytohormones concentrations (mg/L): Group A: 2,4-D (2,4-dichlorophenoxy acetic acid) + kinetin (kn) & Group B: NAA (Naphthalene Acetic Acid) + 6 BAP (6-Benzyl Amino Purine).

Phytohormones concentrations mg/L			
Group A		Group B	
2,4-D	Kn	NAA	6 BAP
2.0	1.0	4.0	0.4
2.0	0.5	2.0	0.2
1.0	1.0	1.0	1.0
1.0	0.5	1.0	0.5

### 4-Callus maintenance:

Calli of *O. majorana* was initiated and maintained on MS medium supplemented with different combinations of the phytohormones as mentioned before. All cultures were incubated at  $25 \pm 1^{\circ}\text{C}$  in 10 hr photoperiod till sufficient amount of calli were produced. Induced calli were transferred to fresh media of the same composition as the induction media every 2-3 weeks for the earlier subcultures and every 2 weeks afterwards. One of the best growth callus of *O. majorana* was probably transferred to new MS medium free of phytohormones and supplemented with 2,4-D herbicide (6, 11, 22.5, 23.5 and 45 mg/L).

### 5-Aseptic seedling culture:

The sterilized seeds were distributed on the surface of a sterile basal MS medium containing 3% sucrose and 0.8% agar and supplemented only with different concentrations of 2,4-D as herbicide (6, 11, 22.5, 23.5 and 45 mg/L) then incubated at  $25 \pm 1^{\circ}\text{C}$  in the dark. The seeds germinated after 7 days, and then were leaved to continuous growth on the same basal MS medium free of phytohormones. Sterilized seedlings of *O. majorana* were incubated at  $25 \pm 1^{\circ}\text{C}$  in 10 hr photoperiod.

### II- Electrophoretic detection of protein of aseptic marjoram seedlings by SDS-PAGE Analysis:

The homogeneity and molecular structure of the purified protein of marjoram aseptic seedlings was checked by denaturing poly acrylamide gel electrophoresis according to the protocol of Laemmli (1970). After running, the gel was immersed overnight on coomassie blue staining solution, with gently shaking at 50 rpm. After staining, the gel was washed

by distilled water and immersed in de-staining solution till appearance of the protein bands. The gel was photographed by digital camera. The molecular biomass of the appeared protein bands was calculated from the inference of authentic protein marker. Broadway, pre-stained marker. Standard curve of protein marker was plotted based on the electrophoretic mobility (Rf) of proteins against their  $\log_{10}$  molecular weights.

### III- Antimicrobial activity for the aqueous extract of aseptic marjoram seedlings:

The antimicrobial activity of aqueous extract of aseptic marjoram seedlings were evaluated against *Penicillium citrinum* as stock cultures in Mycology lab., Faculty of Science, Zagazig University. After seeding of the solid medium by the microbial suspension (10 ml/ 250 medium), pouring to sterile plates, the cultures were incubated overnight for pre-germination, then 500 $\mu$ l of each tested compound was pipetted to the wells of the plate cultures. Blanks of dissolving solvent were made. The cultures were incubated for 4 days at 30 °C then the diameter of the inhibition growth zone, around each well, was measured. The antimicrobial activity was expressed by the diameter of inhibitory zone, comparing to griseofulvin as standard antifungal agents (Barry, 1980).

### 3.Results:

#### 1- Callus culture of marjoram (*Origanum majorana* L.):

For induction of marjoram callus, hypocotyl and seed explants were cultured on MS medium supplemented with auxin (2,4-D or NAA) at 1, 2 or 4 mg/L in combination with different concentrations of cytokinins (Kn or 6 BAP) at 0.2, 0.4, 0.5 or 1 mg/L. The seed and hypocotyl explants were responsive for callus induction and produced calli on all used combinations of phytohormones with variable response. Callus induction was discerned 2-3 weeks after explants inoculation and sufficient amount of calli were obtained 3-5 weeks later.

Comparison of different phytohormonal combinations in terms of callus production revealed that: the 2,4-D + Kn (2+0.5 mg/L) and NAA + 6BAP (4+0.4 mg/L) were the most responsive for callus induction. While the other phytohormonal combinations were the least responsive for callus induction (Table, 2).

The results obtained revealed that auxins play an important role in callus induction and different types of auxins have various effects. Concentration of 1 mg/L NAA produced compact calli with little root or hairy root formation as small white lumps on the surface of the culture (Photo 1-b, G-h). Whereas, all concentrations of 2,4-D induced the formation of

yellow friable calli (Photo 1-a, A-d and Photo 1-b, e&f).

Data in Table (2) and (Photo 1 a&b) showed that, the highest callus formation was observed on medium contained: 2,4-D + Kn (2+0.5 mg/L) and NAA + 6BAP (4+0.4 mg/L). A lower callus formation was obtained on medium supplemented with 2,4-D + Kn (1+1mg/L) and NAA + 6BAP (1+1mg/L). On the other hand, decreasing 2,4-D and NAA concentration to 1 mg/L significantly delayed and decreased callus formation.

Even though the seed and hypocotyl explants respond similarly to different concentrations of phytohormones used, there were visible differences in the calli formed from them. The seed calli were very friable and dark yellow in color, whereas hypocotyl calli were friable and pale yellow in color.

Combinations of 2,4-D with Kn and NAA with 6BAP at different concentrations in the culture media were tested for their growth promoting activities in seed and hypocotyl derived calli of *Origanum majorana* grown on that media for four subcultures. The seed and hypocotyl calli retained high proliferation rate for two subcultures. Afterwards, in the 3<sup>rd</sup> and 4<sup>th</sup> subcultures the calli grew slower, turned brown and didn't survive in subsequent subcultures or herbicides treatment irrespective of the phytohormones concentrations.

#### 2- Aseptic seedling culture of marjoram (*Origanum majorana* L.)

Different growth parameters were recorded for aseptic seedlings of *Origanum majorana* (Table, 3) growing on MS medium free of phytohormones but supplemented with different concentrations of 2,4-D (6, 11, 22.5, 23.7 and 45 mg/L) as herbicide and incubated in 10 hr photoperiod.

Data in Table (3) and (Photo 2) showed that, the highest growth rate was observed on medium contained 6 and 11mg/L 2,4-D, while the lower growth rate was obtained on medium supplemented with 22.5 mg/L 2,4-D. In case of higher conc. of 2,4-D (23.7 and 45 mg/L) no growth was recorded and seedlings dead finally.

Data in Table (3) and (Photo 2) showed that, the highest fresh weight was observed on medium contained 6 and 11mg/L 2,4-D, while the lower fresh weight was obtained on medium supplemented with 22.5 mg/L 2,4-D. In case of higher conc. of 2,4-D (23.7 and 45 mg/L) no fresh weight was detected.

#### 3- Electrophoretic detection of protein of aseptic marjoram seedlings by SDS-PAGE Analysis

The intracellular protein profile was traced on SDS-PAGE, after extraction by grinding in potassium phosphate buffer containing EDTA. From the profile of SDS-PAGE (Photo 3), there is no distinct variation on the proteinous bands of the treated marjoram seedlings with 6 and 11mg/L 2,4-D, and the control of non-

treated seedlings, assuming the absence of inducing effect on gene expression. However the density of proteinous bands (Tabel 4) was increased upon 6 and 11mg/L 2,4-D treatment assuming the induction of hyper amount of induced expressed protein to resist herbicide treatment.

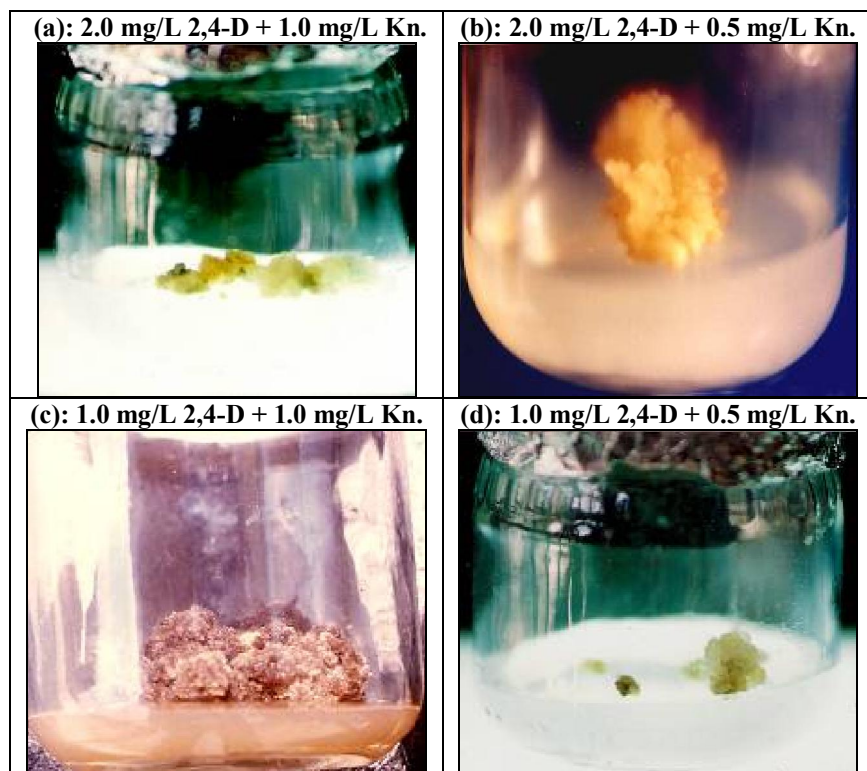
#### 4-Antimicrobial activity for the aqueous extract of aseptic marjoram seedlings

The antimicrobial activity of the plant extract was assessed against the fungus *Penicillium citrinum*, under experimental conditions as described in Materials and Methods. From the profile of antifungal activity (Photo 4), a slight fungistatic activity was observed by the

aqueous extracts of marjoram seedlings treated by 2,4-D at concentration 6mg/L and 11 mg/L, comparing to the untreated control. However, the negative effect of the plant extract was obviously appeared on the sporulation of the fungus, as revealed from the colony color deformation, assuming the interference with normal metabolic traits for pigmentation and conidiogenesis. It was obvious from Photo (4) that the inhibition zone diameter was wider in herbicide treated marjoram seedlings than that of non treated control, which recorded 7cm for zone (3) of 11mg/L 2,4-D and 5cm for zone (2) of 6 mg/L 2,4-D while in zone (1) control recorded 3cm.

**Table (2): Growth parameter of induction of *Origanum majorana* L. callus on MS medium supplemented with different concentrations of phytohormones in 10 hr photoperiod.**

Phytohormones concentrations mg/L		Explants	Date of callus appearance	Color of callus	Consistency	Growth rate
2,4-D	Kn					
2.0	1.0	Hypocotyle or seed	9 <sup>th</sup> week	Pale yellow	Friple	**
2.0	0.5	Hypocotyle or seed	5 <sup>th</sup> week	Dark yellow	Heterogeneous	****
1.0	1.0	Hypocotyle or seed	2 <sup>nd</sup> week	Brown	Heterogeneous	*
1.0	0.5	Hypocotyle or seed	3 <sup>rd</sup> week	Pale yellow	Friple	**
<b>NAA</b>	<b>6 BAP</b>					
4.0	0.4	Hypocotyle or seed	5 <sup>th</sup> week	Pale yellow	Heterogeneous	****
2.0	0.2	Hypocotyle or seed	6 <sup>rd</sup> week	Pale yellow	Friple	***
1.0	1.0	Hypocotyle or seed	3 <sup>rd</sup> week	Brownish	compact	*
1.0	0.5	Hypocotyle or seed	3 <sup>rd</sup> week	Brownish	compact	*



**Photo (1-a): Induction of (*Origanum majorana* L.) callus on MS medium supplemented with different concentration of phytohormones (a,b,c, and d)**

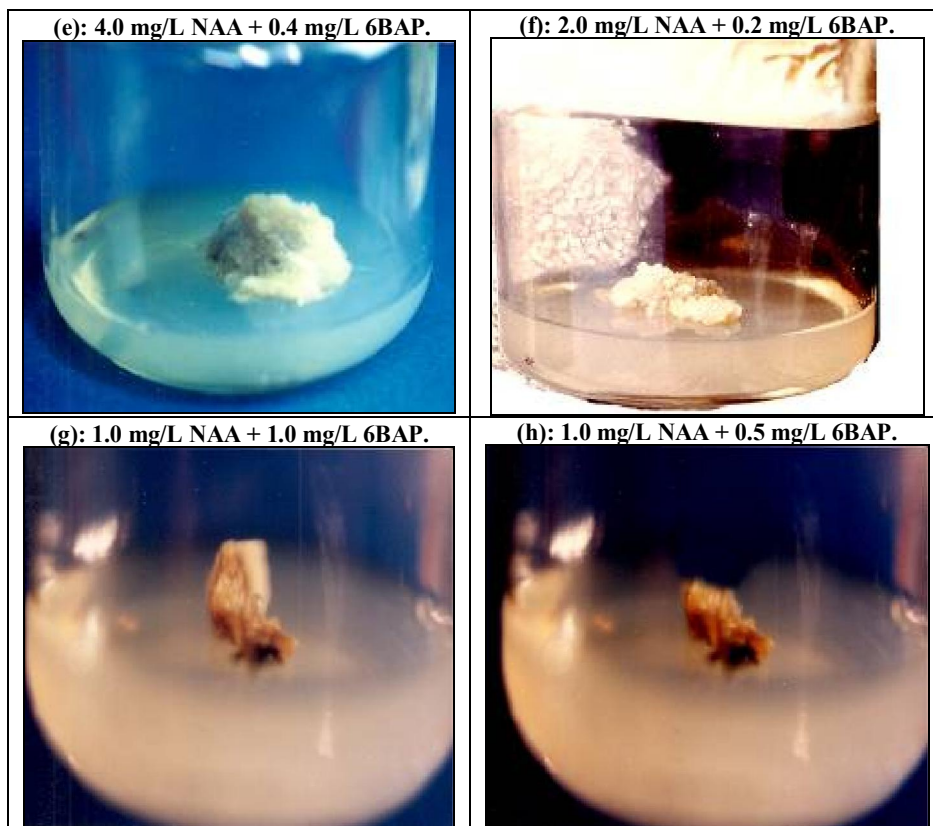


Photo (1-b): Induction of (*Origanum majorana* L.) callus on MS medium supplemented with different concentration of phytohormones (e, f, g and h)

Table (3): Growth parameter of seedlings of *Origanum majorana* L. growing on MS medium free of phytohormones but treated with different concentrations of 2,4-D (6, 11, 22.5, 23.5 and 45 mg/L) as herbicide in 10 hr photoperiod ( $\pm$ SE).

2,4-D concentration	Date of seedling appearance	Color of seedling	Growth rate	Fresh weight rate (g)
6 mg/L	2 <sup>nd</sup> week	Green	Growth without vitality	3.8 $\pm$ 0.44
11 mg/L	2 <sup>nd</sup> week	Green	Elongate by vitality	4.03 $\pm$ 0.35
22.5 mg/L	2 <sup>nd</sup> week	Pale green	Seedling dead after growth	1.5 $\pm$ 0.08
23.7 mg/L	2 <sup>nd</sup> week	Burnt leaf	No growth	No weight detected
45 mg/L	2 <sup>nd</sup> week	Burnt leaf	No growth	No weight detected

Each listed value is a mean of five replicates  $\pm$ SE.

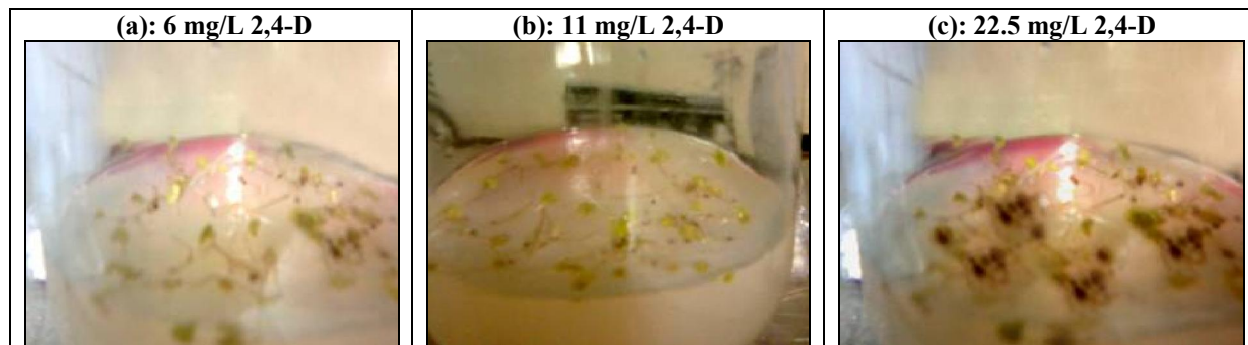
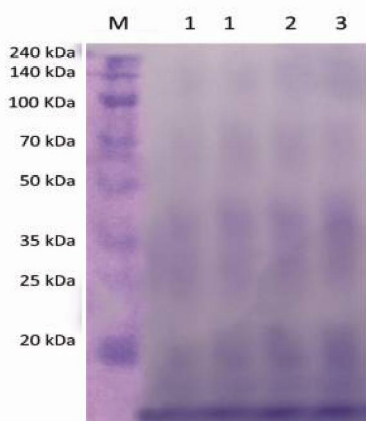


Photo (2): Induction of (*Origanum majorana* L.) seedlings on MS medium free of phytohormones but treated with different concentration of 2,4-D (6, 11 and 22.5 mg/L) as herbicide in 10 hr photoperiod

**Table (4): Molecular weights (kD) and density (+ve) of the intracellular protein of aseptic seedlings of marjoram (*Origanum majorana* L.) treated with zero (lane 1-control), 6 mg/L 2,4-D (lane 2) and 11 mg/L (lane 3) of 2,4-D herbicide in addition to protein marker as detected by SDS-PAGE.**

Marker		Control Lane 1		6mg/L 2,4-D Lane 2		11mg/L 2,4-D Lane 3	
M.WT of protein bands (kD)	Density of protein bands	M.WT of protein bands (kD)	Density of protein bands	M.WT of protein bands (kD)	Density of protein bands	M.WT of protein bands (kD)	Density of protein bands
240	+++++	-	-	-	-	-	-
140	+++++	145	+	145	++	145	++
100	+++++	86	+	86	+++	86	+++
70	+++++	65	+	65	+++	65	+++
50	+++++	-	-	-	-	-	-
35	+++++	30	++	30	++++	30	++++
25	+++++	-	-	-	-	-	-
20	+++++	15	++	15	+++++	15	+++++
No. of bands	8	5		5		5	

Marker      Control      2,4-D 6 mg/L      11mg/L



**Photo (3): SDS-PAGE of the intracellular protein of aseptic seedlings of marjoram (*Origanum majorana* L.) treated with zero (lane 1-control), 6 mg/L 2,4-D (lane 2) and 11 mg/L (lane 3) of 2,4-D herbicide. M, marker (Broad way, Dual pre-stained protein marker (INTRON, Biotechnology).**



**Photo (4): Inhibition zones (2 and 3) of the antifungal activity of aqueous extract of marjoram seedlings treated by 2,4-D herbicide at concentration of 6mg/L and 11mg/L respectively, comparing to untreated control (1), which were assessed against *Penicillium citrinum*.**

#### 4. Discussion:

##### 1- Callus culture of marjoram (*Origanum majorana* L.):

The results in Table (2) and (Photo 1 a and b) showed that, the highest callus formation was observed on MS medium contained: 2,4-D + Kn (2+0.5 mg/L) and NAA + 6BAP (4+0.4 mg/L). While A lower callus formation was obtained on medium supplemented with 2,4-D+Kn (1+1 mg/L) and NAA + 6BAP (1+1 mg/L). These results was agreed with that of **Arafeh (1999)** who reported that, MS medium was more appropriate than B5 medium for establishment of *Origanum vulgare*, a member of Lamiaceae. Also **El-Gengaihi et al. (2006)** stated that MS-medium showed to be the best type of nutrient medium on both calli production and/or calli differentiation from leaf explants of *Origanum* spp. Furthermore, the maximum value of calli production was recorded with *O. vulgare* L. as compared with the other *Origanum* species.

On the other hand, decreasing 2,4-D and NAA concentration to 1 mg/L significantly delayed and decreased callus formation. In the same respect **Nogueira et al. (2007)** results demonstrated that there was no formation of callus in leaf explants maintained in the absence of 2,4-D, and that the addition of BAP had no influence in the callogenetic process. For callus induction and proliferation, the results suggested the use of MS medium, supplemented with 1.0 mgL<sup>-1</sup> 2,4-D. The plant hormone, auxin, influences plant behavior from embryogenesis to senescence. Over the past decade, the mechanism of action of auxin has been revealed by a combination of biochemical and genetic methodologies (**Dharmasiri et al., 2005; Woodward and Bartel, 2005**).

Combinations of 2,4-D with Kn and NAA with 6BAP at different concentrations in the culture media were tested for their growth promoting activities in seed and hypocotyl derived calli of *Origanum majorana* grown on that media for four subcultures.

The results revealed that, the seed and hypocotyl explants were more responsive for callus induction. This may suggest that levels of endogenous phytohormones or their sensitivity might vary between organs. Explants usually require auxins and cytokinins in their culture medium (**Gang et al., 2003**). In a similar manner 2,4-D is the most effective synthetic auxin for promoting callus with inhibiting shoot formation (**Ali and Hasnain, 2007**).

The seed and hypocotyl calli retained high proliferation rate for two subcultures. Afterwards, in the 3<sup>rd</sup> and 4<sup>th</sup> subcultures the calli grew slower, turned brown and didn't survive in subsequent subcultures or herbicides treatment irrespective of the phytohormones concentrations. Such retardation may be related to the oxidation of phenolic compounds (**Chen and Wang, 1995; Ji et al., 1998**). In the same line **Arnaldos et al., (2001)** found that, oxidized phenolic substances generally induced a suppressive effect *in vitro* proliferation due to their inhibitory effects on some essential enzyme activity.

## 2- Aseptic seedling culture of marjoram (*Origanum majorana* L.)

Data in Table (3) and (Photo 2) showed that, the highest growth rate was observed on medium contained 2,4-D (6 and 11mg/L), while the lower growth rate was obtained on medium supplemented with 2,4-D (22.5 mg/L). In case of higher conc. of 2,4-D (23.7 and 45 mg/L) no growth was recorded and seedlings dead finally. *In vitro* meristem culture may be used to produce pathogen-free plants from a systematically infected individual (**Fedotina and Krilova, 1976; Jacoli, 1978**). *In vitro* propagation represents an alternative technique that allows a great number of plants (clones) to be obtained from healthy and high-quality starting material (**Bima, 1997**).

Data in Table (3) and (Photo 2) showed that, the highest fresh weights were observed on medium contained 2,4-D (6 and 11mg/L) and while the lower fresh weight was obtained on medium supplemented with 2,4-D (22.5 mg/L). In case of higher conc. of 2,4-D (23.7 and 45 mg/L) no fresh weight was detected. In the same respect **Goleniowski et al. (2003)** showed that, oregano (*Origanum vulgare x applii*) cultivation has been subject to progressive yield loss as a consequence of continuous vegetative propagation. A method of reducing damage to the shoot tip during sterilization procedures for micropropagation is proposed. Single shoot cuttings are less sensitive to disinfecting substances than green tips. Meristems taken from young plantlets that grew *in vitro* produced less oxidation during the culture than those dissected immediately after disinfecting from plants grown in the field. Treatment with 0.28mM BA and 0.53mM NAA gave greatest efficiency. After 60d 100% of rooted

plantlets could be formed per explant under optimum conditions.

## 3- Electrophoretic detection of protein of aseptic marjoram seedlings by SDS-PAGE Analysis

The results of protein detection in (Photo 3) showed that, there is no distinct variation on the proteinous bands of the treated marjoram seedlings with 2,4-D and the control of non-treated seedlings, assuming the absence of inducing effect on gene expression. In contrast of our findings **Pellet and Saghier (1971)** found that, the application of 2,4-D (auxin-like herbicide) at jointing stage of wheat and barley showed rapid reduction in sugar and proteins. Also the herbicide EL-107 or isoxaben at higher concentration ( $10^{-4}$ M) inhibited the protein synthesis, glucose biosynthesis, cytotoxic and cell wall biosynthesis in *Acer pseudoplatanus* L. (**Lefebvre et al., 1987**). The herbicide: N-(2,6-Diethylphenyl)-N-nutoxymethyl 2-chloro-acetamide also inhibits the protein synthesis in the rice plant (**Omokawa et al., 1988**). **Hassan (2000)** declares that the electrophoretic analysis (SDS-PAGE) of the protein provides information concerning structural genes and their regulatory system that control the biosynthetic pathway of that protein. However in our previous findings (**Hussein et al., 2013**) using field growing *Origanum majorana* treated with 2,4-D as herbicide showed a significant variation in number of proteinous bands and total amount of protein in each band in addition to appearance of new bands.

However the density of protein bands (Table 4) was increased upon 2,4-D treatment assuming the induction of hyper amount of induced expressed protein to resist herbicide treatment. This leads to the production of faint or over expressed protein bands (**Barakat and Hassan, 1997**). Qualitative and quantitative effects are defined when one band disappears or new one appears and when a noticeable change in the band intensity is observed respectively (**Bonfil et al., 1997**). The recorded changes in band intensity could be attributed to the cytological abnormalities induced by herbicides (**Shehata et al., 2000**). The increase in band intensity could be interpreted on the base of gene duplication which is a result of cytological abnormalities (**Soliman and Ghoneam, 2004**).

## 4- Antimicrobial activity for the aqueous extract of aseptic marjoram seedlings

It was obvious from Photo (4) that the inhibition zone -against *Penicillium citrinum*- diameter was wider in aqueous extract of herbicide treated marjoram (*Origanum majorana*) seedlings with 2,4-D (6 and 11mg/L), than that of non treated control, which revealed that the aqueous extract of marjoram seedlings have an antifungal effects and the aqueous extract of herbicide treated marjoram seedlings was more

effective than of non treated control. Our results are agreed with those of **Deans and Svoboda (1990)**, whom stated that marjoram essential oils, stored in glandular hairs, are used as fungicides or insecticides in pharmaceutical and industrial products. Several studies demonstrated that the content of essential oils detected *in vitro* was higher than that detected in greenhouse-grown plants as was the case in the current study. **Arafeh (1999)** reported that the amount of essential oils extracted from *in vitro*-grown *Origanum vulgare* was six folds more than that in greenhouse-grown plants.

The essential oil of *O. majorana* is known for its strong antimicrobial activity, so it could be used by food industries as natural preservatives. A number of the marjoram essential oil components exhibit significant antimicrobial properties when tested separately (**Lambert et al., 2001**). The antifungal and antibacterial activity exhibited by *Origanum* essential oil was previously demonstrated (**Chun et al., 2005; Souza, et al., 2007**). The antifungal effects of aqueous extract of marjoram seedlings is may related to its essential oil which also increased in herbicide treated marjoram plant than that of non treated control (**Hussein et al., 2013**).

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