In vivo Influences of Auxin-Like and Fusilade® Herbicides on the Essential Oils, Protein and Growth of Marjoram (*Origanum majorana* L.)

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Abstract: Marjoram (*Origanum majorana* L.) plants were treated with different concentrations of auxin-like herbicides: 2,4-D and 2,4,5-T: 2 & 4 g/ L/16m^2 and Fusilade® herbicide: 4.5 ml/L/16m^2. The plants were sprayed two times once at vegetative stage and the other at flowering stage. Our results throughout two stages of plant growth showed that: all growth parameters (plant height (cm), number leaves/plant, number of lateral branches/plant, number of flowers/plant, fresh and dry weights of plant leaves g/plant) increased in all treated plant samples with herbicides as compared with non-treated control. Estimation of protein with SDS-PAGE showed that, there is a significant variation in the protein profile in all herbicides treatments, increase in the number of polypeptide bands and total amount of protein as compared with non treated control. SDS-PAGE detected a presence of number of new polypeptide bands in all herbicides treated samples forming a defence strategy to herbicide stress as compared with control. Analysis of essential (volatile) oils by Gas chromatography (GC) revealed the presence of 15 components was identified. The essential oil was found to be rich in *B. phyllandrene* (63.98% - 69.8%) when treated with 4 & 2 g/L, 2,4-D and 4.5 ml/L Fusilde® as respectively, while Linalool as important component increased to (70.62%-72.39%) if treated with 4 & 2 g/L 2,4,5-T as respectively. Results obtained showed that later vegetative stage is characterized by the highest contents of bioactive compounds and therefore it could be considered as the best stage for harvesting marjoram plants. Finally we concluded that the concentration of 2 g/L/16m^2 of two auxin-like herbicides (2,4-D and 2,4,5-T) and 4.5 ml/L/16m^2 of Fusilade® herbicide were more effective in removal of weeds growing with of *Origanum majorana* L. and have an enhancement effects on growth and yield of marjoram plants.

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1-Introduction

Origanum majorana L. Syn. *Majorana hertensis moench* (Marjoram) plant is an evergreen herbaceous plant belonging to the family Lamiaceae, it is also known as sweet marjoram **(Pimple *et al.*, 2012).** The genus *Origanum* houses around 900 different species and many species are extensively used for the flavoring of beverages, food products and in perfumery owing to their spicy fragrance **(Filippo-Dantuono *et al*., 2000).**

The essential oil yield of some species can change with age, growth cycle, climatic conditions, soil type and cropping pattern (**Hamrouni *et al.*, 2009).** Culturing conditions can affect the quality of essential oil (**Viljoen *et al*., 2005).** Moreover, the age of the plant has a significant effect on its essential oil composition (**Bayder and Bayder**, **2005**).

Vàgi *et al.* (2005) showed that the content of essential oil and extracts of *Origanum* species may change depending on the differences in cultivation, origin, vegetative stage and growing season of the plants.

Many phytochemical studies have been conducted so far to investigate the chemical composition of the essential oil of sweet marjoram. Results obtained by **Banchio *et al.* (2008)** showed that the oils exist in two forms: one with terpinene-4-ol and sabinene hydrate as major components.

Bovey and Meyer (1981) reported that 2,4-D and 2,4,5-T affected the protein content of the wheat. It is generally accepted that 2,4-D and IAA share a common signaling pathway in protein biosyntheis (**Taiz and Zeiger, 2002**). A number of investigations had showed the side effects of the herbicides on the hereditary material of different cells. Other investigations were carried out to indicate the relation between mitotic changes in nucleic acid and protein content as a result of treatment with pesticides and herbicides **(Ebad *et al.*, 1993; Soliman and Ghoneam, 2004)**.

The Fusilade herbicide (fluazifop-p-butyl) is related to aryloxyphenoxypropionate (AOPP), which is a class of graminicides, a grass-selective herbicides acting specifically on inhibiting the enzyme Acetyl CoA carboxylase in susceptible grass species **(Burton**

***et al.*, 1989)**. Fusilade is a worldwide well-known selective grass herbicide for post-emergence application in numerous broad-leaved crops **(Keul *et al.*, 1990)**. Applying Fusilade in soybean plants at 0.25 and 0.5 Kg/ ha effectively controlled several aggressive grasses e.g. (*Echinochloa crus-galli*), (*Cynodon dactylon*) and (*Digitaria ciliaris*), particularly when applied at the early stage of growth **(Tiwari *et al.*, 1997)**.

The aim of this study was to investigate the influences of auxin like herbicides: 2,4-Dichlorophenoxy acetic acid (2,4-D) and 2,4,5-Trichlorophenoxy acetic acid (2,4,5-T) in addition to Fusilade® (R-2-[4-(5-trifluoromethyl-2-pyridyloxy) phenoxy] propionic acid) herbicides on the essential oils, protein and growth of field growing marjoram (*Origanum majorana* L.) during two stage of growth (vegetative and flowering stages).

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-D and 2,4,5-T were purchased from Phytotechnology Lab.-Egypt, while Fusilade was purchased from Syngenta-Egypt.

The experiment was carried out in Agricultural farm in Al Salhya Al Gadida Sharkia- Egypt. The marjoram (*Origanum majorana* L.) seeds were sown in outdoor nursery with sandy soil in land square 16 m^2 at 5th February, 2006. After 10 days from sowing, *Origanum majorana* seeds were germinated. There was no removal of post-emergent grasses in all samples. Weed species are either broadleaf or grassy type. During treatment, four separate sets of land squares of $16m^2$ were used, one for each herbicide: 2,4-D , 2,4,5-T (2 and 4 g/L/$16m^2$) and Fusilade® herbicide: (4.5ml/L/$16m^2$) in addition to non treated control. The plants were sprayed two times once at vegetative stage (4 g/L 2,4-D & 2,4,5-T and 4.5 ml/L Fusilade®) and the other at flowering stage (2 g/L 2,4-D & 2,4,5-T and 4.5 ml/L Fusilade®) treatments.

1- Growth measurements:

Growth measurements involving the following parameters: plant height (cm), number leaves/plant, number of lateral branches/plant, number of flowers/plant, fresh and dry weights of plant leaves g/plant. All growth parameters were measured in all treated plant samples with herbicides as well as non-treated control.

The obtained data of growth measurements, phytochemical analysis were statistically analyzed using the (t-test) of probability to determine standard error (± SE) according to **Schuster and Lochow (1979).**

2-Electrophoretic detection of protein by sodium dodecyle sulphate, polyacrylamide gel electrophoresis (SDS-PAGE):

The method of **Laemmili (1970)** with slight modifications was adopted to use in the present study. The modification, was reduced TEMED from 30 µl to 25 µl and also APS was reduced from 1.5 ml to 1.3 ml. Protein was determined using SDS-PAGE in the National Centre of Agricultural Research, Giza, Egypt.

Approximately 1g freeze dry leaves of herbicide treated and control marjoram (*Origanum majorana* L.) were ground in a mortar and pestle in liquid nitrogen. Crushing continued until the sample completely homogenized. The crushed samples were transferred to 1 ml Eppendorf tube brought to 200 µl with extraction buffer (50 m M tris-HCl buffer, pH 6.8, glycerol 10 % w/v, ascorbic acid 0.1%, cycteine hydrochloride 0.1w/v). Centrifugation, 18,000 rpm for about 30 min, was carried out to remove debris.

The protein content in supernatant was estimated according to the method of **Bradford (1976)** by using bovine serum albumin as a standard protein. Protein content was adjusted to 2 mg / ml per sample. Sodium dodecyl sulphate (SDS) was added to the sample at a rate of 4 mg SDS/1 mg protein, then 50 µl 2-mercaptoethanol were applied to each 950 ml of the sample, then the mixture was heated at 100º C in water bath for 3-5 min.

3-Estimation of Essential oil by Gas Chromatography (GC):

The percentages of essential oil were determined in the fresh herb using 100 g samples for each treatment per plant. Distillation of the essential oil was as described in the **British Pharmacopoeia (1963)**.

The essential oil obtained froth the fresh herb was analyzed using: DsChrom 6200 Gas Chromatography equipped with a flame ionization detector; for separation of essential oil constituents.

Flow rates of gases were nitrogen at 1 ml/min, hydrogen at 30 ml/min and 330 ml/min for air. Detector and injector temperatures were 300°C and 250°C, respectively. The obtained chromatogram and report of GC analysis for each sample were analyzed to calculate the percentage of main components of essential oil.

3-Results

1- Growth parameters of marjoram (*Origanum majorana* L.) treated with different concentrations of herbicides.

Data of the effect of various herbicides on the vegetative and flowering growth parameters of marjoram are presented in Tables 1 and 2 respectively. Data in Table (1 and 2) and Photos (1 and 2) showed that, there was significant differences in all growth parameters (plant height (cm), number leaves/plant, number of lateral branches/plant, number of

flowers/plant, fresh and dry weights of plant leaves g/plant) measured in all treated plant samples with herbicides as compared with non-treated control.

At herbicides concentrations 2 & 4 g/L 2,4-D and 2,4,5-T and 4.5 ml/L Fusilade®, all growth parameters measured were increased with reduction of numbers of weeds as compared with non-treated control. The highest rate of marjoram growth treated with different herbicides was recorded at 2 g/L 2,4-D and 2,4,5-T and 4.5 ml/L (v-vegetative) Fusilade® in both vegetative and flowering growth stages, while at flowering stage the conc. of 4.5 ml/L (f-floweing) Fusilade® slightly reduced all growth parameters.

2- Protein of marjoram (*Origanum majorana* L.) plant treated with different concentrations of herbicides and detected by SDS-PAGE:

Protein was separated into polypeptide bands with SDS-PAGE method which depends on their molecular weights. A polypeptide band was considered polymorphic when absent in at least one breed. This method detected scoreable polymorphism in protein banding patterns of marjoram (*Origanum majorana* L.) from 6 survived herbicides treatments during two stages: vegetative; 2,4-D, 2,4,5-T (4 g/L) and Fusilade® 4.5 ml/L(v), and flowering: 2,4-D, 2,4,5-T (2 g/L) and Fusilade® 4.5 ml/L (f) in addition to non treated control.

The results obtained from Table (3) and Photo (3) showed that, there are a significant variations in the protein profile, number of polypeptide bands and total amount of protein in each band appeared in all treated marjoram (*Origanum majorana* L.) samples with herbicides as compared with non treated control. The number of polypeptide bands increased in herbicide conc. of 2,4-D (2 g/L) & 2,4,5-T (4g/L and 2g/L) which recorded 12, 15 & 14 respectively, while it decreased in herbicide conc. of 2,4-D (4 g/L) & Fusilade® (4.5.ml/L(v) & (f) which recorded 8, 6 & 6 respectively, as compared with the number of polypeptide bands in control (11). The Lowest number of polypeptide bands (6 bands)was recorded Fusilade® conc. of (4.5.ml/L (v&f) , while the highest number of polypeptide bands (15 bands) was recorded in 2,4,5-T (4g/L).

SDS-PAGE detected a presence of number of new polypeptide bands in all herbicides treated samples forming a defense strategy to herbicide stress as compare with control. Molecular weights (kD) of new polypeptide bands showed in Table (3) and Photo (3) during two growth stages of marjoram were: vegetative: 2,4-D (4 g/L): 112, 38, 28 & 23kD & 2,4,5-T (4g/L) :85, 58, 38, 30, 26, 23, 20, 18 & 12kD and Fusilade® (4.5.ml/L(v): 60, 37, 32 &18kD. Flowering: 2,4-D (2 g/L) : 85, 58, 29, 23, 18 & 12kD, 2,4,5-T (2 g/L) :97, 59, 53, 40, 34, 30, 23 & 12kD, and Fusilade® (4.5 ml/L (f):109, 50 & 30kD.

In control marjoram plants there are two polypeptide bands of molecular weights 77 and 21kd will disappeared completely from all treated marjoram plant with herbicides.

The total amounts of protein (%) in each polypeptide band were also estimated by SDS-PAGE method and were detected in Table (4). All polymorphic SDS-PAGE products were confirmed by repeating the reaction. The total amount of protein (%) of all marjoram (*Origanum majorana* L.) plants treated with different concentrations of herbicides during vegetative and flowering (ranged from 98.38 to 100.01 %) stages was increased when compared with non treated control (87.07%).

3- Essential (volatile) oil of marjoram (*Origanum majorana* L.) treated with different concentrations of herbicides and detected by GC:

The variation in the content and composition of *Origanum majorana* L. essential oil (EO) has been studied. Plant material has been harvested at two phenological stages (vegetative and flowering), after irrigation by different concentration of 2,4-D, 2,4,5-T and Fusilade®. Analysis of essential oils by Gas chromatography (GC) revealed the presence of 15 components were identified (Tables 6 and 7). The essential oil was found to be rich in β-phyllandrene (63.98% - 69.8%) when treated with 2&4 g/L 2,4-D and 4.5 ml/L Fusilade® as respectively, while Linalool as important component increased to (70.62%-72.39%) if treated with 2&4 g/L 2,4,5-T as respectively. Results obtained showed that later vegetative stage is characterized by the highest contents of bioactive compounds and therefore it could be considered as the best stage for harvesting marjoram plants.

Essential oils productivity and their component (%):

Data of the effect of various herbicides types (2,4-D, 2,4,5-T, Fusilade®) on EO contents of marjoram plants which were used one for each herbicide concentration; 2,4-D, 2,4,5-T; 4 and 2g/L/16 m^2 and Fusilade® herbicide: 4.5 ml/L/16m^2 in addition to non treated control are presented in Tables (5 and 6).

1-Essential oil percentage (%):

Data in Table (5) revealed that the maximum essential oil percentage at 4 and 2 g/L/16m^2 from 2,4,5-T were 0.39%, 0.35% as respectively. Moreover, Fusilade® at 4.5 g/L/16m^2 and 2,4-D at 2 and 4 g/L/16m^2 recorded 0.34, 0.30 and 0.33 as respectively. All concentration from different auxin-like herbicides exhibited the highest essential oil percentage by 8~10 fold as compared to control (0.04%).

On the other hand there were significant difference between different treatments in essential oil yield (ml/plant), which related with the effect of

various herbicides concentration on growth criteria as leaf number, shoot length, number of lateral branch as in Table (1).

2- Essential oil components:

Data from Table (6) indicated that, variation of essential oil composition produced in plants related to different auxin-like herbicides (2,4-D and 2,4,5-T) at 2 and 4 g/L/16m^2 concentration and Fusilade® herbicide at 4.5 gm/L/16m^2. There were large differences in certain terpenes composition in the different treatments. The main components was β-phyllandrene in the marjoram EO, treatment with 2,4-D where record 68.173% and 63.982% at 2 and 4 g/L/16m^2 Fusilade®, β-phyllandrene record 69.806%. Also in the same treatments other characteristic compounds were determined; sabinene, β-myrcene, limonene, linalool, and β-caryophyllene in amount ranging from 2.420% to 12.503% as compared with control. Meanwhile; α-terpine, α-phyllandrndrene, thyanol, α-terpineal, α-therjene, thugun-4-ol and some of unidentified EO in a low amount (less than 2.2%).

Although, untreated plants (control) have high number of EO than treated plants but mostly with low content (0.04%) and linalyl acetate recorded high amount (72.657%).

On the other hand, plants treated with 2,4,5-T herbicide with 2 and 4 gm/L/16m^2 concentrations, had higher amount of linalool EO (70.617% & 72.392% respectively) followed by linalyl acetate (10.751% & 8.89% respectively).

Table (1): Growth parameters during vegetative stage of *Origanum majorana* L. treated with different concentration of herbicides in addition to non-treated control.

Herbicides concentration	Plant height (cm)	No. of Leaves/plant	No. of Lateral branches/plant	Fresh weight (g/plant)	Dry weight (g/plant)
4g/L (2,4-D)	27.0±0.19	88±1.05	10±0.11	17.3±0.16	2.4±0.02
4g/L (2,4,5-T)	26.5±0.36	86±1.03	9±0.09	17.8±0.18	2.2±0.02
4.5ml/L (v) (Fusilade®)	26.8±0.37	75±0.98	9±0.09	15.2±0.14	1.7±0.02
Control	25.1±0.22	70±0.81	7±0.04	14.0±0.17	1.9±0.02

Each listed value is a mean of five replicates ±SE.

Table (2): Growth parameters during flowering stage of *Origanum majorana* L. treated with different concentration of herbicides in addition to non-treated control.

Herbicides concentration	Plant height (cm)	No. of Leaves/ plant	No. of Lateral branches/plant	No of Flowers/ plant	Fresh weight (g/plant)	Dry weight (g/plant)
2g/L (2,4-D)	34.6±0.36	135±1.6	13±0.19	70±0.36	32.0±0.48	3.2±0.06
2g/L (2,4,5-T)	36.0±0.35	145±1.7	12±0.18	75±0.98	31.0±0.55	3.1±0.05
4.5ml/L (f) (Fusilade®)	31.7±0.33	93±1.3	9±0.18	48±0.78	22.7±0.31	2.5±0.04
Control	35.0±0.37	115±1.5	8±0.17	70±0.98	22.0±0.33	2.9±0.03

Each listed value is a mean of five replicates ±SE.

Photo (1): Morphological appearances of marjoram (*Origanum majorana* L.) treated with different concentrations of 2,4-D (B & C) and 2,4,5-T (D & E) herbicides during vegetative (4g/L) and flowering stages (2g/L) in addition to non treated control (A) respectively.

(A) Control

(B) 4g/L 2,4-D

(C) 2g/L 2,4-D

(D) 4g/L 2,4,5-T

(E) 2g/L 2,4,5-T

Photo(2): Morphological appearances of marjoram (*Origanum majorana* L.) treated with different of 4.5 ml/L Fusilade® herbicides during vegetative (B) and flowering stages (C) in addition to non treated control (A) respectively.

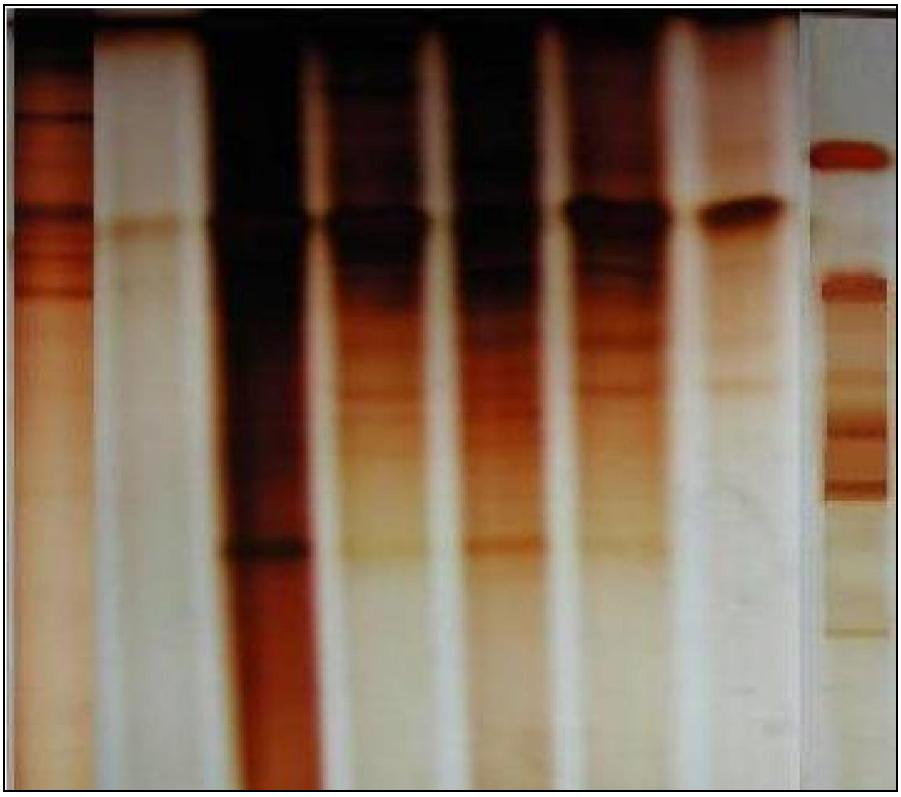
(A) control

(B) 4.5ml/L (v)

(C) 4.5ml/L (f)

Photo(3): SDS-PAGE of protein of marjoram (*Origanum majorana* L.) treated with different concentrations of herbicides during two stages: vegetative; 2,4-D, 2,4,5-T (4 g/L) and Fusilade® 4.5 ml/L(v) (lane 2,4,6 respectively) and flowering: 2,4-D, 2,4,5-T (2 g/L) and Fusilade® 4.5 ml/L (f) (lane 3,5,7 respectively) in addition to non treated control (lane8). Lane (1) represents the protein marker.

Control	Fusilade	2,4,5-T	2,4-D	Marker
4.5ml/L(f).4.5ml/L(v)	2g/L…	. 4g/L	2g/ L …	..4g/L



Table(3): Molecular weights (kD) and amount (%) detected by SDS-PAGE of protein of marjoram (*Origanum majorana* L.) treated with different concentrations of herbicides during 2 stages: vegetative; 2,4-D, 2,4,5-T (4 g/L) and Fusilade® 4.5 ml/L(v) (lane 2,4,6 respectively) and flowering: 2,4-D, 2,4,5-T (2 g/L) and Fusilade® 4.5 ml/L (f) (lane 3,5,7 respectively), in addition to non treated control (lane8). Lane (1) represents the protein marker.

M.WT of protein bands (kD)	Marker Lane 1		Lane 2	Lane 3	Lane 4	Lane 5	Lane 6	Lane 7	Control Lane 8
	M.WT	% Amt	% Amt	% Amt	% Amt	% Amt	% Amt	% Amt	% Amt
117				9.50	8.35	6.30			17.94
116	116	5.08							
112			13.94						
109								21.02	
97						5.75			
85				3.82	3.94				
77									7.35
66	66	6.52							
60							19.06		
59						7.44			
58				7.73	8.02				
57									
53						0.45			
50								16.62	
45	45	16.03							
40						5.16			
38			3.10		7.19				
37							8.37		
35	35	19.12							
34						8.90			
32							10.31		
30					12.19	11.76		7.45	
29				14.00					
28			8.67						
26					1.92				
25	25	7.13							
23			7.15	5.22	4.01	3.45			
21									22.83
20					2.46				
19								18.93	4.24
18	18	14.53		8.01	12.90		15.07		
17				6.94	1.99	19.58	26.73		3.31
16			27.85	4.28	8.37	3.59		9.35	7.53
15						8.73			1.80
14	14	31.58	10.14	6.78	7.52				4.47
13					3.69	3.19			10.88
12				3.01	7.52	5.42			
11			14.72	12.58					5.48
10			12.82	17.46	11.80	8.66	17.93	26.64	1.06

Table (4): General pattern of total amount of protein (%) of marjoram (*Origanum majorana* L.) treated with different concentrations of herbicides during 2 stages: vegetative; 2,4-D, 2,4,5-T (4 g/L) and Fusilade® 4.5 ml/L(v) (lane 2,4,6 respectively) and flowering: 2,4-D, 2,4,5-T (2 g/L) and Fusilade® 4.5 ml/L (f) (lane 3,5,7 respectively). in addition to non treated control (lane8).

Herbicides conc.	4g/L 2,4-D Lane 2	2g/L 2,4-D Lane 3	4g/L 2,4,5-T Lane 4	2g/L 2,4,5-T Lane 5	4.5ml/ l(v) Fusilade® Lane 6	4.5ml/L(f) Fusilade® Lane 7	Control Lane 8
No. of protein bands	8	12	15	14	6	6	11
Total amount of protein (%)	98.39	99.70	98.97	98.38	98.96	100.01	87.07

Table (5): Essential (volatile) oil contents (%) and yield (ml/plant) of marjoram (*Origanum majorana* L.) treated with different concentrations of herbicides and detected by GC:

Herbicides conc. / Analytical content of E.O.	Control	4g/L 2,4-D	2g/L 2,4-D	4g/L 2,4,5-T	2g/L 2,4,5-T	4.5ml/L(f) Fusilade®
Essential oil (%)	0.04%	0.30%	0.33%	0.35%	0.39%	0.34%
Essential oil yield (ml/plant)	0.098	0.057	0.106	0.067	0.067	0.116

Table (6): Essential (volatile) oil components and their contents and yields (% and ml/plant) of marjoram (*Origanum majorana* L.) treated with different concentrations of herbicides and detected by GC:

Herbicidesconc. / Analytical content of EO	Control		4g/L 2,4-D		2g/ l 2,4-D		4g/L 2,4,5-T		2g/L 2,4,5-T		4.5ml/L(f) Fusilade®	
EO components	**EO (%)**	**EO yield ml/plant**	**EO (%)**	**EO yield ml/plant**	**EO (%)**	**EO yield ml/plant**	**EO (%)**	**EO yield ml/plant**	**EO (%)**	**EO yield ml/plant**	**EO (%)**	**EO yield ml/plant**
1- α-Therjene	0.102	0.025	0.157	0.030	0.178	0.057	0.041	0.008	0.221	0.075	0.175	0.030
2- α-Pinene	0.202	0.049	0.932	0.177	0.474	0.152	0.191	0.037	1.135	0.386	0.266	0.046
3- Sabinene	1.293	0.310	0.216	0.041	5.121	1.639	1.157	0.221	0.231	0.079	2.037	0.350
4- β-myrcene	0.261	0.063	2.211	0.420	0.417	0.133	0.185	0.035	0.116	0.039	1.595	0.277
5- α-Terpine	0.178	0.043	1.679	0.319	1.621	0.519	3.108	0.594	3.423	1.164	1.416	0.244
6- α-Phyllandrene	4.319	1.037	1.871	0.356	1.306	0.416	0.159	0.030	0.258	0.088	1.508	0.260
7- β-Phyllandrene	0.969	0.233	68.173	12.953	63.982	20.474	0.314	0.061	0.749	0.255	69.806	12.007
8- Limonene	0.153	0.037	9.025	1.715	7.985	2.554	1.946	0.372	1.796	0.611	12.503	2.151
9- Unidentified	1.185	0.284	0.76	0.147	0.356	0.114	1.899	0.36 3	1.435	0.488	0.492	0.085
10- Linalool	1.513	0.363	3.307	0.628	4.051	1.296	70.617	13.488	72.392	24.613	3.088	0.531
11- Linalyl acetate	72.657	17.438	0.643	0.122	0.919	0.294	10.751	2.053	8.89	3.024	0.987	0.170
12-Unidentified	8.972	2.153	1.622	0.308	1.071	0.343	1.238	0.237	0.568	0.193	0.811	0.140
13- Terpine-4-ol	0.591	0.142	0.446	0.085	1.055	0.338	1.884	0.361	2.790	0.494	0.441	0.076
14- Thuyanol	1.794	0.431	1.094	0.208	1.737	0.556	0.941	0.181	0.473	0.161	0.820	0.141
15- Unidenified	0.953	0.229	1.011	0.211	0.745	0.238	1.077	0.206	0.698	0.237	0.405	0.071
16- α-Terpineal	0.611	0.147	0.454	0.086	0.441	0.141	0.727	0.139	0.415	0.141	0.352	0.061
17- Thugun-4-ol	0.796	0.991	0.315	0.060	0.666	0.213	1.614	0.308	0.821	0.279	2.214	0.381
18- β-Caryophyllene	1.44	0.346	2.420	0.460	3.418	1.094	2.154	0.411	1.781	0.610	1.087	0.187
19- Unidentified	0.337	0.081	3.783	0.694	4.465	1.429	-	-	1.129	0.384	-	-
20- Unidentified	0.937	0.225	-	-	-	-	-	-	0.674	0.229	-	-
21- Unidentified	0.738	0.177	-	-	-	-	-	-	-	-	-	-

4-Disscution

1- Growth parameters of marjoram (*Origanum majorana* L.) treated with different concentrations of herbicides.

At herbicide concentrations 2 & 4 g/L 2,4-D and 2,4,5-T and 4.5 ml/L Fusilade®, all growth parameters measured were increased with reduction of numbers of weeds as compared with non-treated control. The highest rate of marjoram growth treated with different herbicides was recorded at 2 g/L 2,4-D and 2,4,5-T and 4.5 ml/L Fusilade® in both vegetative and flowering growth stages (Tables 1 & 2 and Photos 1 & 2). Also **Cheema *et al.* (1997)** showed that, the combined Fusilade® with the lower and moderate concentrations of rice straw extract increasing the yield (15.10 - 55.90%) and the number of pods/plant, number of seeds/ pod, weight of seed/ pod and weight of 100 seeds as compared with other treatments and control **.** In the same regards **Saygideger and Okkay (2008)** found that, the most stimulatory effect 2,4-D on growth rate, protein and pigment ratio of *Chlorella vulgaris* and *Spirulina platensis* cells was observed at 9.10^{-4} mM concentrations of 2,4-D. The results show that low concentrations of 2,4-D have hormonal effect due to being a synthetic auxin.

The effects of herbicides were known by the type and rates of its application, health and stage of plant growth, and other environmental variables. It has been established that there is morphological changes and disturbances in cell division due to impact of both the herbicides and their combinations **(Qasem, 2007 and Kumar *et al.*, 2011).**

Data in Table (2) and Photo (2) showed that, all growth parameters measured of marjoram plants were slightly reduced at 4.5 ml/L (f) Fusilade®. In this context, **Ahmed and Rashad (1996)** indicated that application of the Fusilade herbicide (400 ml/ fed.) caused a slight decrease of growth potential in soybean plants. Applying the Fusilade alone at (40 g/ fed.) slightly decreased the yield/ plant and its

components e.g. number of pod/ plant, number of seeds/ pod, weight of seeds/ pod and weight of 100 seeds as compared with control **(Cheema *et al.*, 1997). Aksoy and Dane (2007)** results agree with our results, which indicate that shoot growth and lateral root growth was reduced in Fusilade treated groups.

Application of herbicides was accompanied with removal of most of post-emergent young short annual grasses especially at highest concs. of herbicides used (Photo1 and 2). In the same context **Tiwari *et al.* (1997)** indicated that foliar application of Fusilade at 0.25 and 0.5 kg/ ha, after soybean sowing effectively controlled weeds of *Echinochloa crus-galli*, *Cynodon dactylon* and *Digitaria ciliaris*. Similar results were found by **Tesar (1995), Yasin *et al.* (1995) and Avav (2000)** for controlling weeds (e.g. *Imperata cylindrica*) infested soybean, chickpea, lentil and different other forage crops by using Fusilade at rate of 0.35-0.5 kg/ha.

Chopra and Singh (1978) reported that treatment with 2,4-Dinitroasetic acid reduced plant development in *Guizotia*. Plant injury generally progresses from the lower, older foliage to the top. Individual leaves show the greatest injury (chlorosis) along their tips and margins or along the veins **(Nixon, 2003).**

2- Protein of marjoram (*Origanum majorana* L.) treated with different concentrations of herbicides and detected by SDS-PAGE:

The results obtained from Table (3) and Photo (3) showed that, the number of polypeptide bands increased in herbicide conc. of 2,4-D (2 g/L) & 2,4,5-T (4g/L and 2g/L) which recorded 12, 15 & 14 respectively, while it decreased in herbicide conc. of 2,4-D (4 g/L) & Fusilade® (4.5.ml/L(v) & (f) which recorded 8, 6 & 6 respectively, as compared with the number of polypeptide bands in control (11).The Lowest number of polypeptide bands (6 bands)was recorded Fusilade® conc. of (4.5.ml/L(v) & (f) , while the highest number of polypeptide bands (15 bands) was recorded in 2,4,5-T (4g/L). In the same respect **Kumar (2012)** results indicated that both herbicides (2,4-D and Isoproturon (IPU) were able to reduce the carbohydrate and protein content gradually from lower to higher concentration of herbicides. It was observed the effect of herbicides in reduction of band intensity on electrophoretic protein band analysis. The electrophoretic protein band analysis showed the low molecular weight protein bands. Some investigators have reported unacceptable injury in winter wheat on application of 2,4-D **(Soltani *et al.*, 2006)**.

The herbicide effects were based on the differences in the protein bands. The variations in the protein patterns of herbicides treated plants might be assumed to result from changes in the gene expression. Thus, the herbicide treatments applied in the present work are presumed to affect the protein patterns at one or more of the above mentioned points. The differences in quality between varieties are correlated with allelic variations in the composition of seed storage proteins **(Bonfil *et al.*, 1997).** 2,4-D may effect on nitrate reductase activity and nitrogen content in wheat and its relationship to grain protein. Environmental conditions do not usually have a qualitative effect on the expression of the seed storage protein **(Bonfil *et al.*, 1997).**

SDS-PAGE detected a presence of number of new polypeptide bands in all herbicides treated samples forming from a biosynthesis of a new gene to make a defense strategy to herbicide stress as compare with control. It might be due to almost all proteins are soluble in SDS and the resulting protein SDS-complexes are of high negative charges. In same respect Recently, a detailed analysis of the transcriptome revealed that IAA and NAA induce mainly similar genes, clustered in one group, whereas 2,4-D, in addition to the common genes induced by IAA and NAA, also induces a subset of genes that cluster in a unique group **(Pufky *et al.*, 2003).**

In control marjoram plants there are two polypeptide bands of molecular weights 77 and 21kD will disappeared completely from all treated marjoram plant with herbicides which may related to protein turnover to adapt to herbicide stress. The disappearance of electrophoretic bands could be attributed to the loss of the genetic materials due to fragmentation and laggards found in the present study **(Ghareeb, 1998; and Al-Muraikhi, 2000).** 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)**. Also **Hassan (2000)** reported that absence of some bands to the deletion of their corresponding genes.

The total amount of protein (%) (Table 4) of all marjoram (*Origanum majorana* L.) plants treated with different concentrations of herbicides during vegetative and flowering (ranged from 98.38 to 100.01 %) stages was increased when compared with non treated control (87.07%). In the same regards **Chai and Chung (1975)** results showed that, protein content of *Chlorella ellipsoidea* increased with the treatment of 2,4-D at 22 mgl^{-1} concentration. However, the content was decreased in the effect of 2,4-D at 88 mgl^{-1} concentration**. Grist *et al.* (1992)** found that, the herbicides treatments caused changes in band intensity and these changes in band intensity could be explained on the basis of induction of gene mutation at the regulatory system which modulates or enhances transcription rate of a particular structural gene**. Müller and Hoffmann (2005)** results showed

that, the uptake of the herbicide 2,4-dichlorophenoxyacetate (2,4-D) by *Delftia acidovorans* MC1 proceeds mainly by an active, energy-driven process. Several putative proteins seemed to be involved in the uptake of 2,4-D by this strain.

3- Essential oil of marjoram (*Origanum majorana* L.) treated with different concentrations of herbicides and detected by GC:

The results presented in Tables (5 and 6) showed that there were an increases in the essential oil percentage had stably increase in marjoram leaves with all herbicides treatment (2,4-D and 2,4,5-T and Fusilade®) at vegetative stage for developing a defence strategy to herbicide stress. Results obtained showed that later vegetative stage is characterized by the highest contents of bioactive compounds and therefore it could be considered as the best stage for harvesting marjoram plants. These results coincided with those of **Sellami *et al.* (2009)** showed that later vegetative stage is characterized by the highest contents of essential oil and therefore it could be considered as the best stage for harvesting marjoram plants. Moreover **Banchio *et al*. (2008)** showed the reason of increased in EO yield was associated with a significantly larger number of peltate glandular trichroms (The main site of EO synthesis), and the increase in total EO yield per plant in response to different herbicides with different concentration was not due to increased biomass**.**

It has been demonstrated that the content of essential oil and extracts of medicinal plants like *Origanum* species containing antimicrobial, antioxidant and other biological activities may change based on the differences in cultivation, origin, vegetative stage and growing seasons of the plants **(Milos *et al.*, 2000)**. Comparing our results to bibliographic data, it seems that physiological stage could affect significantly the yield and composition of essential oils; this was the case of *Mentha piperita* **(Rohloff *et al.*, 2005).**

The results presented in Table (6) showed that there were large differences in EO composition in the different treatments, mostly was composed of monoterpenes; β-phyllandrene by large amount in 2,4-D and fusillade treated plants, while, linalool EO by large amount in 2,4,5-T through two its concentration treatment as compared to untreated which recorded high amount in linolyl acetate only. Also **Politeo *et al*. (2006)** stated the composition of marjoram volatile compounds and found that, 4-terpinenol, γ-terpinene, α-terpinene, sabinene, β-phellandrene and p-cymene were the most important volatiles in marjoram essential oil. However, it was shown that chemical composition of marjoram is rather variable and depends on geographical origin, plant vegetation phase and handling procedures of harvested herb. In the first chemotype, terpinene-4-ol together with cis-sabinene hydrate is responsible for the characteristic flavour and fragrance of marjoram oil **(Vági *et al.*, 2005)**.

Also Table (5 and 6) showed that, although, untreated plants (control) have high number of EO than treated plants but mostly with low content (0.04%) and linalyl acetate recorded high amount (72.657%). On the other hand, plants treated with 2,4,5-T herbicide with 2&4 g/L/16m^2 concentrations, had higher amount of linalool EO (70.617% & 72.392% respectively) followed by linalyl acetate (10.751% & 8.89% respectively). While **Baratta *et al.* (1998); Edris *et al.* (2003)** showed that the marjoram essential oil contains mainly terpinen-4-ol (>20%) and (+)-cis-sabinene hydrate (3–18. In addition to these compounds, a- and c-terpinene and terpinolene were the other major components (no-unless there is more than 20% present); thymol and carvacrol were determined in smaller amounts.

The Fusilade® effects on the enzyme Acetyl CoA carboxylase, which is responsible for lipid biosynthesis **(Bradely *et al.*, 2001).** The study concerning the biosynthesis of essential oil compounds in marjoram by **Hallahan and Croteau (1989)** dealt with the isolation and characterization of a soluble enzyme (sabinene hydrate synthase) preparation that catalyses the reaction from GPP to sabinene hydrate.

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