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SEAWEED EXTRACT ENHANCING GROWTH, FRESH HERB AND ESSENTIAL OIL OF SWEET MARJORAM (*Origanum majorana* L.)

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Abstract

The present study was conducted to investigate the effect of exogenous sprayed with different levels seaweed extract (0.0, 0.25, 0.50, 0.75 and 1.0 ml/L) on morphological characters of vegetative growth (Plant height, number of primary branches/plant and leaves/plant), fresh herb yield /plant, anatomical features of vegetative organs (main stem and leaf) in addition to percentage and components of essential oil in sweet marjoram. Results indicated that all investigated levels of seaweed extract showed no significant influence on plant height of sweet marjoram.On the other hand, increased sprayed level of seaweed extract induced a gradual significant increase in the number of primary branches/plant, developed leaves/plant and fresh herb yield /plant of sweet marjoram. It was realized that sprayed plants with 1 ml seaweed extract/L. achieved noticeable changes in histological characters of vegetative organs (stem and leaf) of sweet marjoram. All Seaweed extract increased main stem diameter and thickness of leaf blade. The increment of stem diameter could be attributed mainly to the increments in all internal tissues; Phloem, xylem tissues in addition to vessel diameter over those of the control as a result of spraying seaweed extract.As well as, application of 1 ml seaweed extract/L. increased leaf blade thickness (midvein and lamina) as a result of prominent increases in thickness of palisade, spongy tissues and vessel diameter. Also, such treatment (1 ml seaweed extract/L.) increased the percentage of essential oil and its major components in fresh herb of sweet marjoram at full blooming stage.

Keywords: Sweet marjoram, Seaweed Extract, Growth, Fresh herb, Anatomy, essential oil.

Introduction

The family Lamiaceae (often called Labiatae, the traditional name) is a large family, included about 200 genera and 3200 species which are widely distributed almost all over the temperate and tropical regions, but centered in the Mediterranean region (Shukla and Misra, 1979; Cronquist, 1981; Jones and Luchsinger, 1987; Pandey, 2004 and Kumar, 2009). Plants of this family characterized by square stem, opposite, decussate leaves with many gland dots and zygomorphic flowers with two distinct lips. Many species of this family are aromatic due to occur monoterpenes, sesquiterpenes and phenylpropanoids in its essential oil (Nahak *et al*., 2011).

Sweet marjoram (*Origanum majorana* L., syn. *Majorana hortensis* Moench, *Majorana majorana* (L.) H. Karst) is one of the most important economic aromatic species of the family Lamiaceae.

It is an annual, sometimes a biennial herb or subshrub, with an erect, branched, square slightly hairy stem. The greyish leaves are opposite, oval and short-stalked. Its white or purplish two-lipped small flowers are arranged in roundish clusters ('knots') in the leaf axils. The fruit consists of four smooth nutlets, which ripen only in warm regions. Different parts of the plant are considerable aromatic.

Sweet marjoram is native to the Mediterranean region but it has been grown as a culinary and medicinal herb since ancient Egyptian times. It was later used by the ancient Greeks and Romans and was introduced to Britain probably during the middle ages. It is now cultivated commercially in many countries. It is not as hardy in Britain as Pot Marjoram (*O. onites*) or Marjoram (*O. vulgare*) (Pl.156). The names *Marjorana* and Marjoram come from the medieval Latin name *marjorana.*

The flowering stems are a medicinal parts which contain 1-2 percent of an aromatic oil with a spicy fragrance containing terpinines and terpineol, plus tannins, bitter compounds, carotenes and vitamin C so, its substances give Sweet Marjoram stomachic, carminative, choleretic, antispasmodic and weak sedative properties. In herbalism it is used mainly for various gastrointestinal disorders and to help digestion. Also, it is an ingredient of ointments and bath preparation used to alleviate rheumatism.

Mostly, however, Sweet Marjoram is used as a culinary herb. Of all the marjorams it has the best flavoring for cooking and is an excellent addition to soups, sauces and meat dishes.

Sweet marjoram considerable an important aromatic and medicinal plants in Egypt. It is recommended to increase the productivity of fresh herb and essential oil per unit area to cover human consumption of sweet marjoram plant.

The quantity of hormones within the plant affected of plant growth and development. So, recently a great attention effort to use natural and safety substances which increase the phytohormones inside the plant to enhance its growth, flowering and fruit setting.

In this connection, seaweed products, either powder or extract, are a new generation of natural organic as well as in plant growth promoting substances; encourage faster germination of seeds, induced vigorous growth, enhance yield and resistant ability of several crops (Dhargalkar and Pereria, 2005).

The seaweed resources increase harvest quantity and quality in agriculture and horticulture. The beneficial effects of seaweed products on the plants are well documents. seaweed products improve seeds germination, seedlings development and increase plant tolerance to environmental stresses (Zhang and Ervin, 2004,2008) and enhance plant growth and yield (Hong *et al*., 2007; Zodape *et al*., 2008; Khan *et al*., 2009; Kumari and Sahoo, 2011 and Craigie, 2011). The influence is explained by content of plant growth promoting substances such as cytokinins, auxin, gibberellins, abscisic acid, ethylene, polyamines and betaines in algal

extract (Crouch and Van Staden, 1993; Stirk *et al*., 2003; Yokoya *et al*., 2010; Blunden *et al*., 2010 and Prasad *et al*., 2010).

Thus, the present investigation is carried out to evaluate the influence of exogenous sprayed with different concentrations of seaweed extract on vegetative growth, fresh herb yield, anatomical structure of vegetative organs and chemical composition of aromatic oil of sweet marjoram plant.

Material and Method

The present investigation was performed in Agriculture Experimental and Research Station, Faculty of Agriculture, Cairo University, Giza, Egypt during two growing seasons of 2018 and 2019 in order to investigate the impact of exogenous application of seaweed extract at 0.25, 0.50, 0.75 and 1.0 ml/L. on vegetative growth, fresh herb yield, anatomical features as well as essential oil (percentage and constituents) of (*Origanum majorana* L.). Seeds of sweet marjoram (in a form of nutlets) were obtained from the Experimental Station of Medicinal Plants, Faculty of Pharmacy, Cairo University, Giza, Egypt. Seaweed extract (CT stimulant-4) was obtained from Meristem Company, Spain.

The Field procedures:

Seeds of sweet marjoram were sown on 7th March, 2018 and replicated on 14th March, 2019. Seeds were planted in plastic trays (40 × 60 cm) filled with mixtures soil (1:1, peat moss and clean sand). After 21 days from sowing date, the emerged uniform seedlings were transplanted to the experimental land of the open field.

The experiment includes five treatments in randomized complete block design with three replicate. The experimental land of each replicate divided into five plots each plot contain only one treatment and every plot divided into six ridges with four meters long, 60 cm apart. Every seedling planted in hills, 20cm between hills.

The treatments were (0.25, 0.50, 0.75 and 1.0 ml /L.) seaweed extract which applied at seven weeks after planting date; i.e., four weeks from transplanting and after 21 days from the first one. The untreated plants were treated with tap water. Tween-20 was applied as spreading agent for tested treatments. Every plot treated with 1.50 and 2.25 liters of seaweed in the first and second application; respectively.

The chemical analysis of seaweed extract according to Meristem Company, Spain is as follows:

Appearance	Liquid	*Ascophyllium nodosum* seaweed extract	15%
Colour	dark brown		
Solubility	100%		
PH	7	Total nitrogen	5.6%
Density	1.2g/ml	Organic nitrogen	0.6 %
		Ureic nitrogen	5%
Magnesium Oxide (MgO) chelated by EDTA	0.2%	Water soluble magnesium (MgO)	0.2
Iron (Fe) chelated by EDTA	1%	Water soluble iron (Fe)	1%
Manganese (Mn) chelated by EDTA	0.5%	Water soluble manganese (Mn)	0.5%
Zinc (Zn) chelated by EDTA	0.5%	Water soluble zinc (Zn)	0.5%

Nitrogen fertilizers in a source of ammonium sulphate (20.5% N), calcium superphosphate (15.5% P_2O_2) and potassium sulphate (48% K_2O_2) were applied based on local recommendation of sweet marjoram plant.

Data Recorded:

1. Vegetative growth: In both seasons data on five plants for each replicate (15 plants from each treatment) were recorded at full blooming stage after 3.5 months from sowing date; i.e., four weeks after the second application of seaweed extract. parameters includes average plant height (cm), number of brunches/ plant, leaves/ plant and fresh weight of shoot(g)/ plant (represent yield of fresh herb / plant).

2. Anatomical studies: These studies were aimed to make a comparative microscopical examination on plant materials which clarify the best prominent reaction to seaweed extract with control. In the second growing season of 2019 at the age of three months from sowing date; i.e. two weeks from the second application of seaweed extract, Specimens were collected from the median portion of the main stem and lamina of its corresponding leaf. Samples were killed and fixed for at least 2 days in F.A.A.(10ml formalin, 5ml glacial acetic acid and 85 ml ethyl alcohol 70%). anatomical processing were carried out according to (Nassar and ElSahhar, 1998). Sections were read to detect histological manifestations of the noticeable responses resulted from spraying with 1.0 ml/L Seaweed extract (the most positive effect concentration in this investigation) compared to control and photomicrographed.

3. Extraction and composition of essential oil: Essential oil was extracted to evaluate response of sweet marjoram herb to foliar spray with seaweed extract on percentage and composition of essential oil. Samples were taken at full blooming stage of the second growing seasons 2019 (the age of 14 weeks from sowing date). Determination of essential oil quantitative was achieved by Hydro-distillation using the technique described by Densy and Simon (1990). The essential oil was extracted by water distillation using a modified Clevenger trap (ASTA, 1968). GC-MS at National Research center, Dokki, Giza, Egypt was used to separate and detect essential oil constituents.

Statistical Analysis:

Since there was homogeneity between the collected data (data of morphological characters and herb yield per plant) in both seasons, a combined analysis was performed. Analysis of data of the two seasons were subjected to conventional methods of analysis of variance according to Snedecor and Cochran (1982). The least significant difference (L.S.D.) at

0.05 level of probability level was calculated for each determined characters under different treatments.

Results and Discussion

I-Growth Parameters:

Data from Table (1) showed that all investigated concentrations of seaweed extract showed no statistical response on plant height of sweet marjoram. on the other hand, all assigned concentrations increased number of primary branches and leaves/plant of sweet marjoram. It is clear that number of primary branches and total number of leaves per plant were gradually increased as the concentration of seaweed extract increased. Worthy to note that the significant increase in such characters was recorded at concentration of 0.5 ml seaweed extract/L. and the maximum increase was obtained at 1 ml seaweed extract /L. (Figure 1), being 64.5% more than the control for number of primary branches/plant and being 46.9% more than the control for total number of leaves/plant.

From the above mentioned results on the influence of exogenous sprayed of seaweed extract on the morphological characters of sweet marjoram growth, it could be stated that all sprayed concentrations of seaweed extract showed an insignificant response in plant height of sweet marjoram plant. The obtained results are in contradiction with those mention by Sivasankari *et al*. (2006) on *Vigna sinensis* plant showed that soaking seeds in aqueous extract of seaweeds (*Sargassum wightii and Caulerpa chemnitzia*) enhanced seedling growth and shoot length. Likewise, Thirumaran *et al*. (2009) on *Abelmoschus esculentus* found that seed germination and shoot length reached its maximum at 20% seaweed liquid with or without chemical fertilizer. Hamed (2012) reported that spraying plants *Phaseolus vulgaris* with seaweed extract at 750 ppm significantly increased plant height. In this respect, Boghdady *et al*. (2016) found that seaweed extract enhanced plant height of chickpea plant. Such contradiction could attributed to the differences in investigated species, method and time of application and / or the concentration used of seaweed extract. On the other hand, the present results indicated that seaweed extract had promoted effect on both characters (number of branches and leaves per plant). The obtain results are in harmony with those mention Hamed (2012) on *Phaseolus vulgaris* and by Boghdady *et al*. (2016) on chickpea, being in harmony with the present investigation.

Table 1: Morphological aspects of vegetative growth and fresh herb yield of sweet marjoram as affected by foliar application with seaweed extract (a combined data of the two seasons 2018 / 2019)

Treatments	Vegetative growth characters			Fresh herb yield / plant (g)
	Plant height (cm)	No. of primary branches / plant	No. of leaves / plant	
Control (tap water)	41.3	7.6 D	1784.2 D	59.16 D
Seaweed extract				
0.25 ml/L.	39.6	8.1 D	1921.7 D	63.28 D
0.50 ml/L.	43.8	9.4 C	2196.2 C	70.14 C
0.75 ml/L.	44.2	10.6 B	2382.7 B	78.55 B
1.00 ml/L.	39.9	12.5 A	2617.9 A	89.72 A
L.S.D. (0.05)	**N.S**	**1.19**	**185.2**	**6.17**

Means having the same letter are not significantly different at 0.05 level.

Fig.1: A photograph showing the form of sweet marjoram plant at full blooming stage as effected by exogenous application with 1ml seaweed extract /L.

A- Un sprayed plant (Control). **B-** Plant sprayed with 1ml seaweed extract /L.

II-Fresh herb Yield /plant:

Concerning the effect of seaweed extract on fresh herb yield on sweet marjoram plant at blooming stage are shown in Table (1).It is clear that, all studied concentrations of seaweed extract increased fresh herb yield of sweet marjoram plant and the significant increase was found at 0.5 ml seaweed extract/L. Worthy to mention that increasing concentration of seaweed extract induced significant increase in fresh herb yield reached its maximum at 1 ml seaweed extract/L., being 51.7% more than that of the control. The aforementioned results concerning the effect of spraying seaweed extract on the fresh herb of sweet marjoram indicated that different concentrations of seaweed extract increased fresh herb of sweet marjoram and increasing concentration of seaweed extract up to 1 ml/L. induced gradual significant increase in fresh herb. In this respect, it could be explained the increase in vegetative growth including fresh herb which represents shoot fresh weight per plant due to foliar application with seaweed extract to the presence of growth phytohormones (IAA, IBA and cytokinins) trace elements, vitamins and amino acids in the components of seaweed extract (Crouch and Van Staden, 1993). The obtained results are in harmony with those obtained by Salama and Yousef (2015) on basil plant.

III-Anatomical studies

(a) Anatomy of the stem:

Measurements and counts of some anatomical aspects in cross section of the main stem of sweet marjoram plants (aged three months from sowing date) as influenced by exogenous application with 1 ml/L. seaweed extract and those of untreated plant are shown in Table (2) and figure (2).

Table 2 : Cross sections through the median portion of the main stem of sweet Marjoram as affected by exogenous application with seaweed extract (Average of six readings)

Histological characters	Treatments		
	Control (µm)	1 ml/L. seaweed extract (µm)	± % to control
Stem diameter	2118.0	2859.5	+ 35.0
Cortex thickness	282.3	249.5	- 11.6
Phloem tissue thickness	56.5	88.6	+ 56.8
Xylem tissue thickness	352.9	662.5	+ 87.7
Vessel diameter	24.8	32.3	+ 30.2
Pith diameter	705.2	824.9	+ 17.0

Results in Table (2) and Figure (2) indicated that sprayed sweet marjoram plants with 1 ml/L. seaweed extract resulted in a prominent increase in diameter of the main stem by 35.0% more than control although a decrement of 11.6 % in the thickness of cortex below the control was observed. It was noticed that the increment which observed in stem diameter due to foliar application with seaweed extract followed by another increase in the most tissues share in the structure of the stem. The phloem and xylem tissue thickness as well as diameter of the pith were increased by 56.8, 87.7 and 17.0% more than control as a result of seaweed extract treatment; respectively. Although, vessel diameter was increased compared to untreated (control) by 30.2% due to foliar spray with 1 ml/L. seaweed extract.

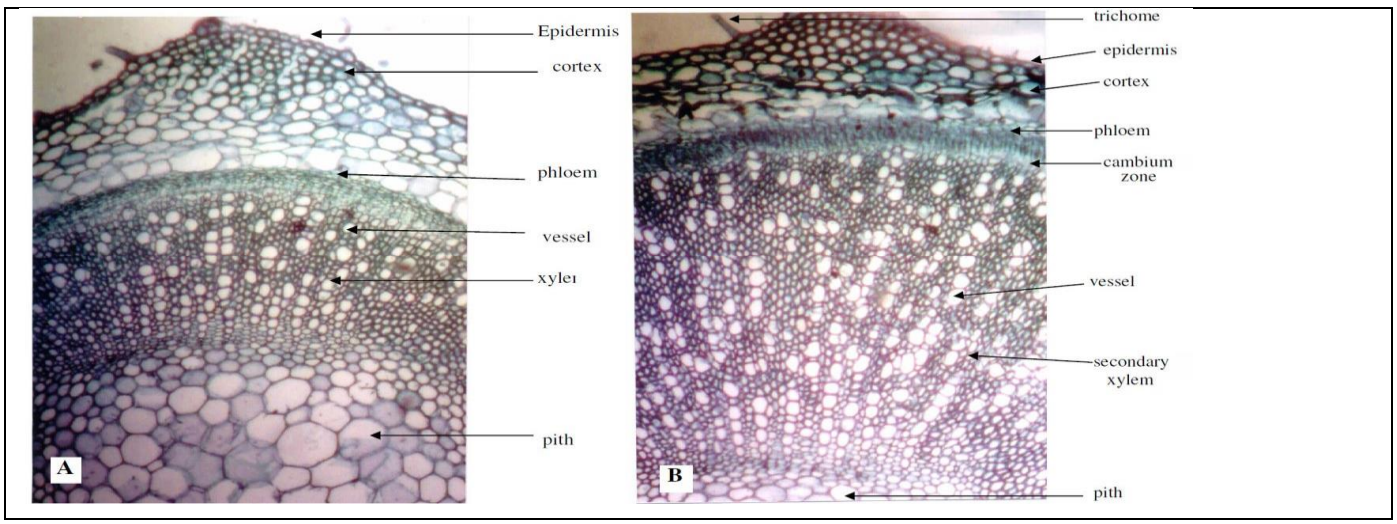


Fig.2: Cross section of sweet marjoram plant stem as affected by foliar application with seaweed extract. (x 125).

A. From untreated plant (control). **B.** From plant treated with 1 ml/L. seaweed extract.

(b) Anatomy of the leaf:

Measurements and counts of certain anatomical aspects in cross sections of sweet marjoram leaf blade aged three months as affected by foliar spray with 1 ml/L. seaweed extract are presented in Table (3). Moreover, microphotographs illustrating these treatments are shown in Figure (3).

It is obvious from Table (3) and Figure (3) that foliar application with 1 ml/L. seaweed extract induced prominent increase in the thickness of midvein and lamina by 26.4 and 31.2% over those of the control; respectively. It is clear that the induced thicker leaves as a result of spraying seaweed extract could be attributed mainly to the prominent increase in palisade and spongy tissue by 68.8% and 7.2 %, respectively more than that of the control. Likewise, there was a slightly increased in the size of the vascular bundle of the midvein due to a slight increase in its length and width by 2.5% and 1.7% respectively, over those of the control as a result of such treatment. Moreover, xylem vessels increased in diameter, being 27.3% over the control, which amounted to more total active conducting area to cope with vigorous growth resulting from spraying treatment with 1 ml/L. seaweed extract.

Table 3: Cross sections of sweet marjoram leaf blade as affected by exogenous application with seaweed extract (Average of the six readings)

Histological characters	Treatments		
	Control (µm)	1 ml/L. seaweed extract (µm)	± % to control
Midvein thickness	212	268	+ 26.4
Lamina thickness	276	362	+ 31.2
Palisade tissue thickness	93	157	+ 68.8
Spongy tissue thickness	152	163	+ 7.2
Dimensions of midvein bundle:			
Length	79	81	+ 2.5
Width	91	92	+ 1.1
Vessel diameter	11	14	+ 27.3

Previous information about the effect of exogenous application seaweed extract on anatomical structures of vegetative organs (stems and leaves) of sweet marjoram plants are not available in the literature. However, Salama and Yousef (2015) using 1.5 ml. seaweed extract/ L. on basil plant as well as Salama *et al*. (2016) using 1 ml seaweed extract/L. on stevia plant induced remarkable changes in stems and leaves anatomical structure due to foliar application with seaweed extract. They found that the increase induced in stem diameters was attributed mainly to the increase in the thickness of vascular bundle tissue as well as in pith tissue compared to control. Likewise, seaweed extract treatment induced thicker leaves due to the increase induced in the thickness of both midvein and lamina. The increment which was observed in the lamina thickness could be attributed mainly to the increase achieved in both palisade and spongy tissues. Likewise, the increase observed in the thickness of midvein region could be attributed mainly to the increase induced in the dimensions of the midvein bundles, these results in line with the present findings. Similar results were also reported by Boghdady *et al*. (2016) using 1 ml. seaweed extract/L. on chickpea plant.

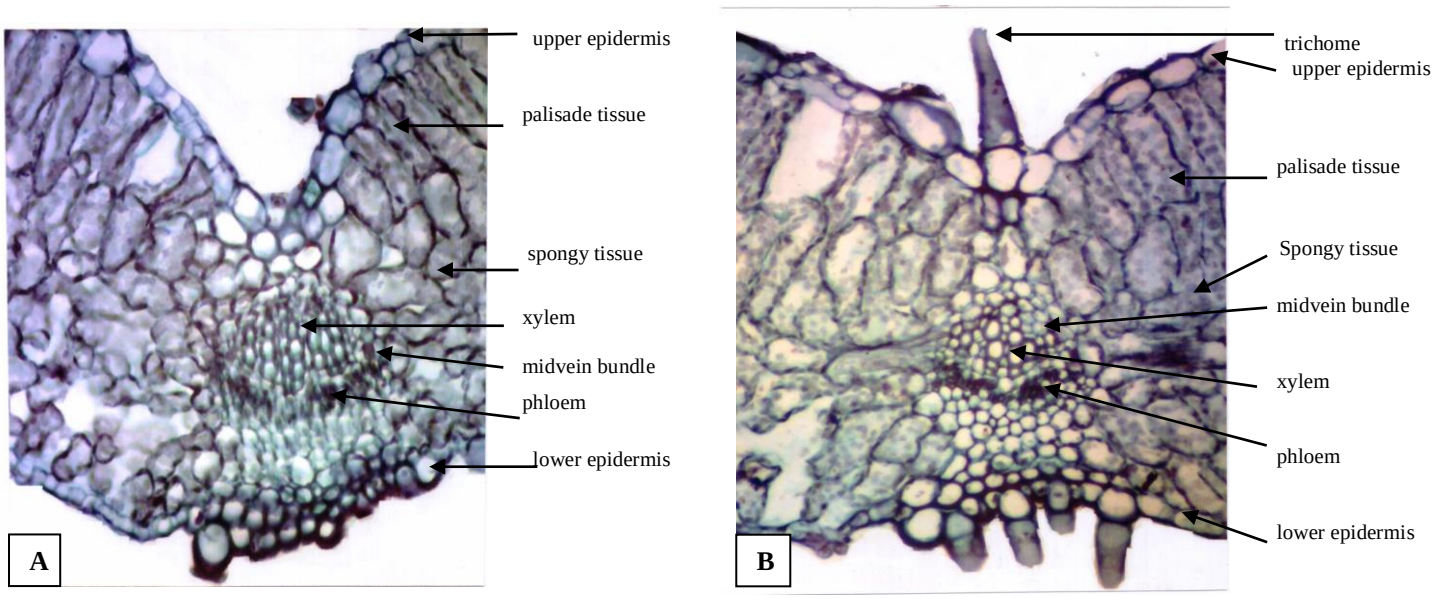


Fig. 3 : Cross sections of sweet marjoram leaf blade as affected by foliar application with seaweed extract. (X 300).
A. From untreated plant (control). **B.** From plant treated with 1 ml/L. seaweed extract.

IV-Essential oil:

The composition and percentage of essential oil of sweet marjoram herb at full blooming stage, as affected by exogenous application with 1ml seaweed extract/L. are shown in Table (4). Likewise, the components of essential oil analyzed by GC-MS are illustrated in Figures (4 and 5). The essential oil of sweet marjoram herb at flowering stage was obtained by means of water-steam distillation. It yielded 0.75 and 0.88% for control and treated plants; respectively (Table, 4).

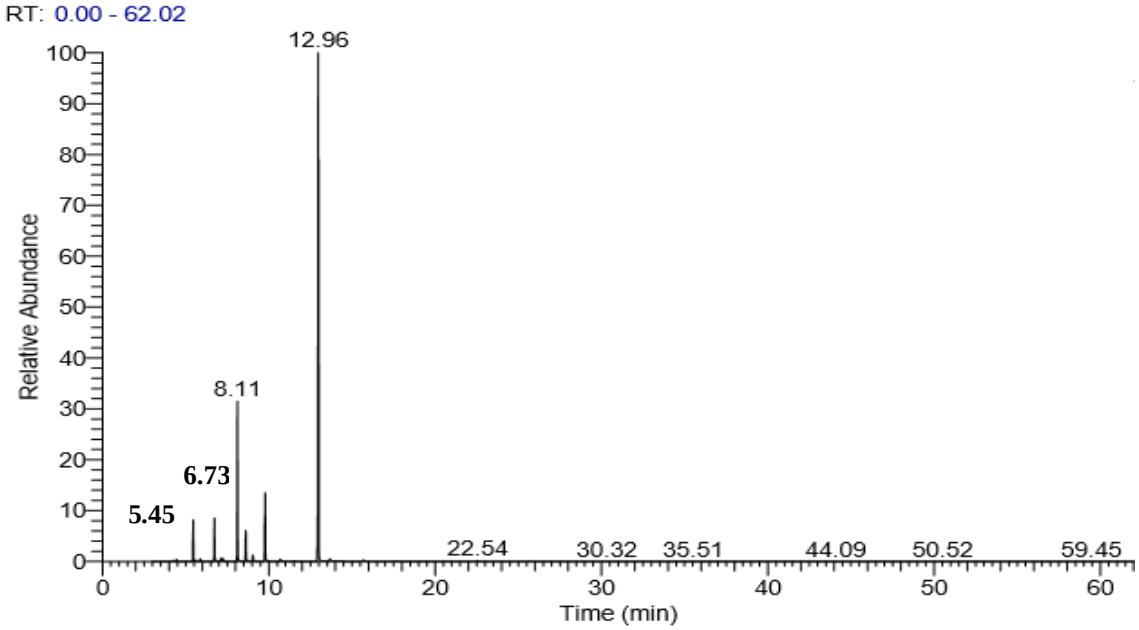
Using GC-MS technique in analyzing essential oil of sweet marjoram herb (Figures 4 and 5) proved the presence of 19 compounds in both essential oils of control and treated plants with 1ml seaweed extract/L. (Table 4).There are 3 main compounds (major ones) in the composition of essential oil namely 4-Terpineol, γ-Terpinene and β-Terpineol. Such three main components comprised 82.16% of the essential oil obtained from herb of untreated plants against 92.85% of the essential oil obtained from herb of plants sprayed with 1ml seaweed extract/L. Worthy to mention that the rest 16 compounds of essential oil comprised 17.37% of essential oil of untreated plants against 7.15% of essential oil of treated plants.

It is clear that the first major component (4-Terpineol) comprised 58.40% of the essential oil obtained from un sprayed plants against 77.80% of the essential oil obtained from plants sprayed with 1ml seaweed extract/L. Whereas, the second main component (γ-Terpinene) recorded 15.48% for the essential oil of untreated plants against 4.75% for the essential oil of treated plants. At the same time, the third main component (β-Terpineol) comprised 8.73% of the essential oil of un sprayed plants against 10.30% of the essential oil of treated plants. The obtained results indicated that treated sweet marjoram plants with 1ml seaweed extract induced favorable changes in both percentage and composition of volatile oil.

The literature survey about the effect of exogenous application with seaweed extract on essential oil(percentage and composition) are not available.

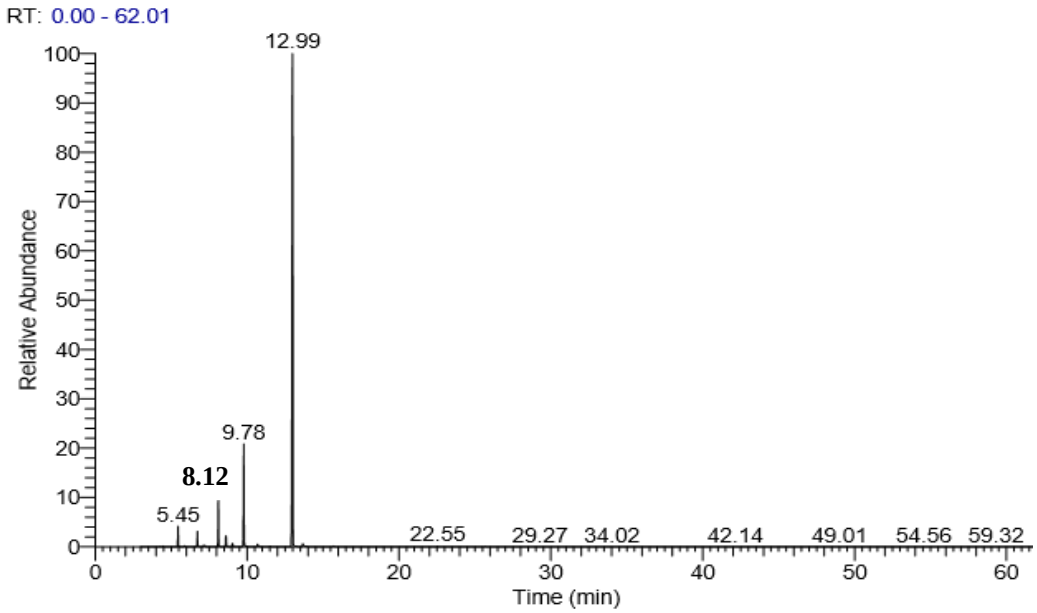
Table 4: Components and percentage of essential oil of sweet marjoram herb as affected by exogenous application with 1ml seaweed extract/L.

No. of peaks	Retention time (min.)	Components (%)	treatments	
			Control	1 ml/L seaweed extract/L.
1	4.28	β-Thujene	0.11	0.04
2	4.47	α-Pinene	0.20	0.08
3	**5.45**	**Sabinene**	**4.58**	**1.83**
4	5.61	β-Pinene	0.45	0.17
5	**6.73**	**α-Terpinene**	**5.08**	**1.56**
6	7.13	Limonen	0.37	0.14
7	7.22	β-Phellandrene	0.60	0.24
8	**8.12**	**γ-Terpinene**	**15.48**	**4.75**
9	8.62	Trans-Sabinene hydrate	3.47	1.32
10	9.04	α-Terpinolen	0.87	0.46
11	**9.78**	**β-Terpineol**	**8.73**	**10.30**
12	10.70	trans-p-2-Menthen-1-ol	0.39	0.41
13	11.50	1-Terpineol	0.10	0.08
14	**12.99**	**4-Terpineol**	**58.40**	**77.80**
15	13.67	α-Terpineol	0.51	0.50
16	15.71	Linalyl acetate	0.28	0.13
17	17.68	4-Terpinenyl acetate	0.07	0.04
18	22.55	Caryophyllene	0.18	0.09
19	25.70	Bicyclogermacrene	0.11	0.06
% of Essential oil in sweet marjoram herb			**0.75**	**0.88**



Constituents and their retention time, min.

Fig. 4: GC/ MS of essential oil in sweet marjoram plant of untreated plant at blooming stage.



Constituents and their retention time, min

Fig. 5: GC/MS of essential oil in sweet marjoram herb of treated plant with 1m/L. seaweed extract at blooming stage.

Conclusion

It could be concluded that sprayed seaweed extract at concentration of 1ml/L induced significant increase in fresh herb yield due to enhancing growth and anatomical structure as well as percentage of essential oil.

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