Effect of Moringa Leaf Extract Spray on Sage (*Salvia officinalis* L.) Plant under Sandy Soil Conditions

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Abstract: The present study was carried out at the Experimental Farm, Horticulture Department, Faculty of Agriculture, Suez Canal University during two successive seasons (2012 & 2013). The objective was to study the effect of moringa leaf extract (MLE) spray at 0.0, 2.5, 5 and 10 gm /L on vegetative growth, yield, leaf area, oil percentage and the anatomical leaf structure of sage plant under sandy soil conditions. Spraying with MLE especially at highest concentration (10gm/L) significantly increased the plant height, number of leaves, number of branches, yield and essential oil percentage in herb. In addition such treatment markedly increased the percentage of most essential oil component and enhancement the characteristic of leaf anatomy of sage plants.

Keywords: Moringa, sage, leaf extract, (MLE), *Salvia officinalis* L. (Sage) essential oil component and anatomical leaf structure.

INTRODUCTION

Sage (*Salvia officinalis*, L) plant is a perennial herb, rich in volatile oils which contain rosmarinic acid and α camphoraceous oil consisting of about 50% thujone. It has been used for different diseases including respiratory infection, menstrual difficulties and digestive complaints.

In Egypt, the total cultivated area of sage plants in 2016 reached about 220 fed. and produced about 1.59 ton of herbs/fed. (Ministry of Agric., 2016). However, we must expansion in medicinal and aromatic plants production because of our suitable climate conditions, abundance of employment and abundance of new reclaimed sandy soil.

Chemical fertilizers are very important source of plant nutrition, but they are expensive and represent a source of environmental pollution. Also, chemical fertilizers at high rates for long time may increase the dangerous effect of chemicals reside in plant tissues on the human health and animal consumers.

Thus, it has led growers of medicinal and aromatic plants in countries to use many plant extracts to improve and stimulate plant growth as well as increase the active ingredients instead of chemicals fertilization. Among these extracts as (MLE) which produced from the leaves of (*Moringa olifera, Lam.*) plants.

(MLE) has been identified contain macro & micro nutrients, amino acids, plant growth hormones, vitamins, allele chemical a and anti- oxidants (Palada and Chang, 2003; Anwar *et al*., 2007; Basra *et al*., 2009; Pachauri and Flora 2011; Dhakar *et al*., 2013 and Hussain *et al*., 2013)

Various plant extract such as moringa and yeast extracts have found wide use in increasing growth and yield of different kinds of plants, *i.e.* onions, soya, sorghum, coffee, melon, peanut, tea, chili, maize, rice, wheat, cowpea, tomato and bean (Price. 1985; Foidl *et. al.,* 1996; Mathur, 2006; Fuglie, 2008; Phiri, 2010; Culver *et al*, 2012; Yasmeen *et al*., 2012; Culver *et al*., 2013; Muhamman *et al*., 2013).

Very little work have been done on the effect of moringa leaf extract on medicinal and aromatic plant such as Pradhu *et al*. (2010) on basil. However, the available data of the effect of (MLE) on vegetative growth, oil production and leaf anatomical structure of sage plant or other related species are not available in the literature.

Thus, the present work aimed to study the effect of foliar spraying with (MLE) on vegetative growth, yield and essential oil components as well as anatomical leaf structure of sage plants under sandy soil conditions.

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MATERIALS AND METHODS

This work was carried out at the Experimental Nursery of the Department of Horticulture, Faculty of Agriculture Suez Canal University, Ismailia, Egypt during the two successive seasons of (2012&2013) to study the effect of foliar spraying with (MLE) on vegetative growth, yield, essential oil components and leaf anatomical structure of sage (*Salvia officinalis,* L.) plants under sandy soil conditions. The soil of the experimental was sandy with pH values of 7.22 and 7.52 and contained 28.00 & 22.00mg kg-¹ available N, 14.51 &20.50 mg kg-¹ available P, 105 & 139 mg kg-¹ available K and 0.357 & 0.288 mg kg-¹ organic matter in the two seasons, respectively.

In early January of both seasons, the soil was divided into plots (2.5×2.5m) and amounted of cattle manure at the rate 25m³/fed. Cattle manure was mixed with the soil during the preparation process, five weeks before planting. The physical and chemical characteristics of cattle manure are presented in Table (1).

The plot containing 3 rows of 60cm apart and 2m length. Sage plants of (which previously prepared) by cutting were planted in 20th and 7th February in the first and second seasons, respectively. The spacing between plants was 30cm, since the number of plants was 3 in each row and 9 plants per plot.

Fresh leaves of moringa (*Moringa olifera, Lam*) were dried at room temperature then grounded into their powder and mixed with 80% ethanol to extract three times as suggested by (Makker and Becker, 1996). The

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suspension was stirred using rotatory evaporator under vacuum and kept in refrigerator until using. The drying extract was used at 2.5, 5 and 10 gm/L water and used for spraying of sage plants at three equal doses for every plant during the two growing seasons. The first addition was done one month after cultivations or after first cut of herbs, while the other addition was applied 15 days intervals after the first application, respectively. The control plants were sprayed with water.

Table (1): Physical and chemical characteristics of the cattle manure used for fertilization of sage plants during 2012 and 2013 seasons.

Properties	First season	Second season
EC dSm-1	0.85	1.09
pH	7.11	7.66
Cations meq/L		
Ca^2+	4.00	5.00
Mg^2+	8.00	6.00
Na+	17.00	12.0
K+	22	15
Anions meq/L		
Cl-	20	17
HCO-3	6.00	7.00
SO_4	25.0	14.0
CO_3^{2-}	0.0	0.0
Total N mgkg-¹	1.63	1.07
Available N mgkg-¹	254	294
Available P mgkg-¹	160	127
Available K mgkg-¹	1040	1200
C/N ratio	12.58	16.14
Organic Carbon gkg-¹	205	279
Organic Matter gkg-¹	353	48.2
Moisture%	4.00	7.90
Fe mgkg-¹	2388	2966
Cu mgkg-¹	16.61	11.67
Mn mgkg-¹	190.3	189.8
Zn mgkg-¹	30.34	39.45

So the experimental design was a randomized complete blocks, where the experiment involved 4 treatments with three replicates (plots).

The plants were irrigated whenever they needed and all of the cultural practices were followed as normal.

The harvesting process was taken place on 29th May 2012, 15th May 2013 for the first cut, and in 3rd September 2012, 15th August 2013 for the second cut, respectively. At the harvesting time, data were recorded concerting the vegetative growth presented in plant height (cm), leaf number/plant, average of leaf area, and number of main branches/plant, fresh weight of herb/plant (gm) and dry weight of herb/plant (gm).

The essential oil percentage of each treatment of sage plants was determined in the fresh herb using water distillation method according to the British Pharmacopoeia (1963).

Essential oil constituents were analyzed [for the most effective treatments to essential oil production of sage plants (10gm MLE/L) and control treatment in the two cuts of the two studied seasons] using gas liquid chromatography (GLC) to determine the main constituents according to Hoftman (1967).

For anatomical study, leaf samples for the most effective treatments to essential oil production of sage plants (10gm MLE/L) and control treatment were taken in the second season at the time of the second cutting, killing and fixation in FAA solution (10ml formalin+ 5ml glacial acetic acid + 50ml ethyl alcohol 95% + 35 ml distilled water), dehydration, clearing with n- butyl alcohol series and embedded in pararaffin wax of 56-58ºc, using a rotary microtome, sections (20µ) were cut and stained with safranin and light green before mounting in Canada balsam. Slides were examined microscopically and photomicrography (Nassar and El-Sahhar, 1998).

Data were statistically analyzed with the analysis of variance using a computer statistical software SPSS program Version 12.0, (copy right© 2003 for SPSS Inc., USA), was used for handling data. Results were displayed as the differences between the means of treatments and tested using modified L.S.D. The means were significantly different if the P value was =0.5 according to Snedecor and Cochran (1980).

RESULTS AND DISCUSSION

Effect of Moringa Leaf Extract (MLE) on vegetative growth

Plant height (cm): It is clear from Tables (2&3) that all MLE treatments significantly increased plant height comparing to untreated one in both seasons. In addition, a gradual increase in plant height was induced as MLE rate increased. The higher rate of MLE (10 gm/L water) produced the tallest plants as 59.30- 64.18 and 58.10 - 59.49 cm compared to control plants as 44.55- 47.34 and 43.70 - 44.48 cm in the first and second seasons, respectively.

The increase in plant height that occurs may be from (MLE) can be used to produce an effective plant growth, hormone and increasing yield by 25-30%. In addition to the active substances is zeatin a plant hormone from the cytokinines group this foliar spray should be used other fertilizer, watering and sound agricultural practices (Fuglie, 2000).

Similar stimulative effect on plant height as result of MLE application have been reported by Pal *et al.*

(1995), Chawla *et al.* (1988); Pezzuto and Park (2002) and Fuglie (2008).

Number of leaves/plant:

Data presented in Tables (2 & 3) indicated that the number of leaves per plant was increased by increasing the rates of MLE. The least mean number of leaves per plant (46.25, 48.58 and 48.75, 46.58) was produced from the untreated plants, while the higher numbers of leaves per plant as (69.08 & 75.33) in the first season and (71.33 & 73.83) in the second season were obtained with those treated by MLE at rate (10 gm/L). At the same time, the statistical differences between treatments were significant. The plants treated with the lower (2.5 gm/L water) and medium (5 gm/L) rates of MLE produced (60.33, 66.42 and 64.08, 65.50) and (64.75, 69.67 and 67.83, 70.00) leaves /plant in the two season, respectively.

The possible reason for this acceleration of growth might be due to the enriched content of moringa leaf extract of crude proteins (43.5%) and growth promoting hormones, as auxis and cytokinins, Protein is essential for the formation of the protoplasm, while growth hormones favored rapid cell division, cell multiplication and enlargement (Makkar and Becker, 1996).

In this connection, Mathur (2006) Fuglie (2008) and Moyo *et al*. (2011) concluded that increasing MLE rate resulted in an increase in number of leaves per plant.

Average of leaf area (cm²):

Data from Tables (2 & 3) showed that MLE at all levels caused a significant decrease in leaf area of leaves than the check treatment in both seasons. Moreover, the two levels of MLE at (5 &10 gm/L) had significant decrease effect than that lower level (2.5 gm/L).

This result disagree with Yasmeen *et al*. (2012) who's reported that applying MLE on wheat exhibits longer seasonal leaf area duration compared with the control.

It worth to mentioned that the decrement in such leaf area of sage plants mainly due to negative correlation between leaf area and both numbers of leaves and branches per plant.

Number of main branches /plant:

As shown in Tables (2 & 3) in both seasons, MLE generally had significant effect in number of branches per plant than control. In addition level of 10 gm/L exhibited a significant increase in number of branches per plant compared to 2.5 gm/L or control. In this regard, the number of main branches/were (11.00& 10.17 - 9.66 &9.66), with the low level, (11.83, 11.08 and 11.77, 10.44) and with medium level, (12.27, 11.89 and 13.55, 11.41) with high level when compared to (7.66& 6.58 - 5.30 & 6.72) with the control in the first and second seasons, respectively.

The increment of such parameter could be attributing to the role of nutrient element of MLE in cell division, which led to increasing the number of branches/plant. These results are in agreement with those finding reported by Foidl *et al*. (2001) and Phiri (2010) on rice plant.

Table (2): Effect of (MLE) on vegetative growth and oil percentage of two cuts of sage (*Salvia officinalis* L.) during first season ٢٠١٢ and its two cuts.

Moringa leaf extract treatment (gm/L)	Plant height (cm)	Number of leaves /plant	Leaf area (cm²)	Number of main branches /plant	Fresh weight of herb/ plant (gm)	Dry weight of herb/ plant (gm)	Oil percentage (%)/100 (gm) F.W.
First cut of season (2012)							
0.0	44.55	46.25	7.38	7.66	26.34	17.57	1.27
2.5	53.64	60.33	5.57	11.00	44.03	34.14	1.44
5	56.17	64.75	4.71	11.83	48.17	37.33	1.51
10	59.30	69.08	4.44	12.27	52.52	41.05	1.54
L.S.D 0.05	2.32	2.93	0.89	0.55	2.40	1.61	0.49
Second cut of season (2012)							
0.0	47.34	48.58	9.01	6.58	25.96	19.64	1.05
2.5	58.84	66.42	6.38	10.17	41.47	34.14	1.42
5	62.04	69.67	5.49	11.08	45.16	37.32	1.48
10	64.18	75.33	5.01	11.89	51.33	43.21	1.51
L.S.D 0.05	1.79	3.07	0.39	0.62	2.31	2.28	0.19

Table (3): Effect of moringa leaf extract (MLE) on vegetative growth and oil percentage of two cuts of sage (*Salvia officinalis* L.) during second season 2013 and its two cuts.

Moringa leaf extract treatment (gm/L)	Plant height (cm)	Number of leaves /plant	Leaf area (cm^2)	Number of main branches /plant	Fresh weight of herb/ plant (gm)	Dry weight of herb/ plant(gm)	Oil percentage (%)/100 (*gm*) F.W.
First cut of season (2013)							
0.0	43.70	48.75	7.40	5.30	25.78	19.47	1.12
2.5	52.88	64.08	5.58	9.66	40.90	35.00	1.36
5	55.77	67.83	5.10	11.77	44.79	41.93	1.39
10	58.10	71.33	4.41	13.55	49.86	43.19	1.41
L.S.D 0.05	2.06	3.67	0.31	0.47	2.01	4.25	0.26
Second cut of season (2013)							
0.0	44.48	46.58	9.07	6.72	25.73	19.18	1.08
2.5	55.47	65.50	5.78	9.66	45.80	27.34	1.34
5	57.49	70.00	5.15	10.44	49.53	31.32	1.43
10	59.49	73.83	4.64	11.41	52.62	45.22	1.52
L.S.D 0.05	2.16	2.51	0.23	0.55	2.36	2.45	0.14

Fresh and dry weight of herb/plant (gm):

Data recorded in Tables (2 & 3) clearly indicate that by increasing MLE rate, and dry weights of leaves /plant were increased as compared to control.

Moreover, the high rate (10 gm/L) recorded the heaviest fresh weight as 52.52, 51.33 and 49.86, 2.62 gm while the lowest values as 26.34, 25.96 and 25.78, 25.73 gm were obtained in the control for the first and second seasons, and their cuts, respectively.

This effect may be due to beneficial effect of MLE properties. In addition to supply the growing plants with the required micro and macronutrient elements. Naturally, these elements play important roles in the metabolic processes like photosynthesis, respiration and carbohydrate synthesis. In this respect (Culver *et al*., 2012) on different crops, found that MLE especially at high rates of moringa significantly increased fresh and dry weights of leaves/plant in comparison with control. However, the dry weight of herbs/plants fallowed the same pattern as the herb fresh weight.

Essential oil percentage in the herb:

Results in Tables (2 & 3) revealed that the all levels of MLE gradually increased essential oil percentage in both seasons. These increments were statistically significant compared to untreated plants, except the lower concentration of MLE at 2.5 gm /L for the first cut in the two studied seasons. The most effective treatments were the medium and higher concentrations (5 & 10 gm/L). Likewise, the highest values of the essential oil percentage were ranged between 1.54 and 1.41% compared to the control as 1.27, 1.05 and 1.12, 1.08% in the both seasons.

The increase in the essential oil percentage when sage plants received the high level of MLE may be attributed to the higher energy in synthesis biochemical metabolites as result to applied MLE.

Effect of MLE on essential oil components:

It is clear from Tables (4 & 5) that the main components separated from sage essential oil were α-pinene, camphene, β-pinene, 1,8 cineloe, thyjone, terpinene 4-01, borneol and eugenol. In addition the major components of sage oil were 1,8 cineloe and thujone which presented about 60% from oil components.

It is clear also, the treatment of MLE at 10 mg/L markedly increased the percentage of α pinene, camphene, β- pinene, 1,8 cineole and borneol in essential oil of sage plants for the two cuts in the two studied seasons than that of the control, except camphene for the second cut in the first season, β-pinene for the first cut in the second season and borneol for the second cut in the two season. However, weak decreasing trend in percentage of thujone, terpinene 4-01 and eugenol was noted due to MLE treatment in both cuts and in the two seasons than the control.

Table (4): Effect of (MLE) on essential oil components (%) on two cuts of *Salvia officialis* L. in seasons 2012, 2013.

Treatments	Essential oil components (%)							
	α - pinene	Camphene	β - Pinene	1,8 cineole	Thujone	Terpinene-4-01	Borneol	Eugenol
				First season				
Control	2.92	4.31	4.80	27.32	24.13	7.72	2.91	6.69
10 gm MLE	5.15	9.33	7.63	35.45	21.98	1.02	4.75	5.34
				Second season				
Control	1.05	5.06	7.37	32.83	21.46	4.77	3.67	4.87
10 gm MLE	4.90	6.30	8.32	36.25	17.31	5.15	3.71	6.35

Table (5): Effect of moringa leaf extract(MLE) on essential oil components (%) of *Salvia officinalis* L. in the seasons of 2012 and 2013.

Treatments	Essential oil components (%)							
	α - pinene	Camphene	β - Pinene	1,8 cineole	Thujone	Terpinene-4-01	Borneol	Eugenol
				First season				
Control	3.76	5.92	8.46	30.72	26.99	6.49	2.01	6.62
10 gm MLE	4.60	7.03	8.81	36.06	25.44	5.53	1.27	4.77
				Second season				
Control	4.56	7.92	9.22	34.58	26.22	4.96	1.54	4.81
10 gm MLE	5.17	6.86	7.81	37.69	19.38	4.18	1.35	4.70

Effect of moringa leaf extract (MLE) on anatomical leaf structure of sage plant:

It is obvious from Table (6) and Fig. (1) That foliar application of sage plants with 10gm (MLE)/L increased the thickness of leaf blade, palisade tissue, spongy tissue and mesophyll tissue by 36.9, 49.4, 116.8 and 32.3 %, respectively, more than the control. In addition, vascular bundle of midvein was slightly increased in size as a result of spraying with MLE. The increament was mainly due to slight increament in width (+9.2%) and slight decreament in Length (-11.2%) as compared to the control.

The obtained results indicated that the increase in vascular bundle due to such application was accompanied with the increment in length and width of xylem and width of phloem tissues which were 6.3, 8.0 and 5.9% over the control, respectively. However, phloem length was decreased by 6.2% in this respect. Likewise, MLE at 10 gm/L increased both number of xylem arm/ midvein bundle and xylem vessels arm by 2.2 and 9.4% more than the control, respectively.

The increment in anatomical structure in leaf of sage plants treated with MLE could be attributed to their composition which involved to amino acids, vitamins, protein and mineral nutrition which act to stimulate many biological processes in cluding cell division and enlargement. However, no available data was found in the literatures in this respect.

Table (6): Effect of (MLE) at 10gm/L on anatomical structure of sage leaf plant (means of three sections from three specimens).

Anatomical structure	Treatments		
	Control	40ml (ALE) /L	±% to control
Thickness of blade (µm)	201.4	275.8	+36.9
Thickness of palisade tissue (µm)	103.7	154.9	+49.4
Thickness of spongy tissue (µm)	49.3	106.9	+116.8
Thickness mesophyll tissue (µm)	162.8	215.3	+32.3
Thickness of midvein (µm)	632.5	610.7	- 3.5
Length of vascular bundle (µm)	271.2	240.9	- 11.2
Width of vascular bundle (µm)	359.1	392.1	+9.2
Length of xylem tissue (µm)	169.8	180.5	+6.3
Width of xylem tissue (µm)	337.7	364.6	+8.0
Length of phloem tissue (µm)	99.1	93.0	- 6.2
Width of phloem tissue (µm)	368.8	390.7	+5.9
No. of xylem arm/midvein bundle	27.7	28.3	+2.2
No. of xylem vessels/ arm	10.7	11.7	+9.4

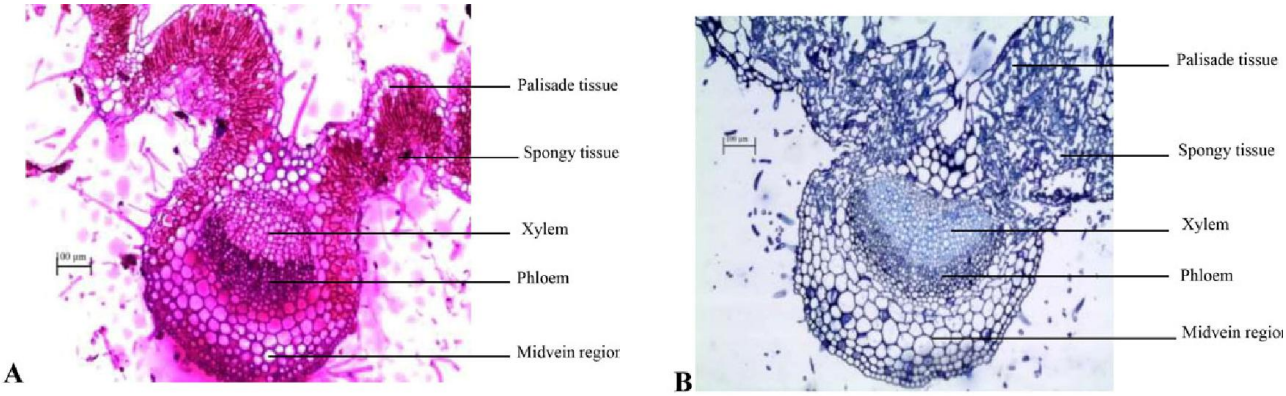

Fig. (1): Transverse sections through blade leaf of sage plant as affected by foliar application with moringa leave extract (MLE) at 10gm/L. **A-** From untreated plant (control). **B-** From plant sprayed with 10 gm (MLE)/L.

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تأثير الرش بمستخلص أوراق المورنجا على نبات المريمية تحت ظروف الأراضي الرملية

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أجرى هدا البحث بمشتل نباتات الزينة بمزرعة كليه الزراعة جامعة قناة السويس بمحافظة الاسماعيليه جمهورية مصر العربية خلال موسمين متتاليين ٢٠١٢ - ٢٠١٣ وذلك بهدف دراسة تأثير الرش بمستخلص أوراق المورنجا بتركيز صفر، ٢,٥، ٥، ١٠ جم/لتر على النمو الخضري والمحصول ونسبه الزيت ومكوناته وكذلك التركيب التشريحي لأوراق نبات المريميه تحت ظروف الاراضى الرملية.
وأوضحت النتائج أن الرش بمستخلص أوراق المورنجا خاصة عند التركيز العالي (١٠جم/لتر) أدى إلى زيادة معنوية في النبات، عدد الأوراق، عدد الأفرع، المحصول، نسبة الزيت الطيار كذلك أدى هدا المستخلص إلى زيادة جودة الزيت الطيار وكذلك تحسين الصفات التشريحية للأوراق لنبات المريمية عن مثيلتها في النباتات المقارنة.