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PHYSIOLOGICAL AND ANATOMICAL STUDIES ON THE EFFECT OF DIFFERENT FERTILIZERS ON THE LEAVES OF SOME MEDICINAL AND AROMATIC PLANTS
I- *MENTHA VIRIDIS* L.

Ibrahem, Zahira T. Z. and S . T. Boulos *
Dept. of Medicinal and Aromatic Plants, Desert Research Center, El-Matareya , Cairo, Egypt.
*Dept. of Range and Ecology, Desert Research Center, El- Matareya , Cairo, Egypt.

Field experiment was carried out during two successive seasons 2001/2002 and 2002/2003 on Spearmint (*Mentha viridis* L.) at Maryout research station, to study the effect of Ammonium sulphate 20.5% (60kg. N/Fed.) and a mixture of two a symbiotic nitrogen fixers micro-organisms (*Azotobacter chroococcum* and *Azosprillium lipoferum*) on the vegetative growth, herb yield, volatile oil content and its components, chlorophyll-a & b , carotenes and the effect on leaf structure. The anatomical study showed that fertilization had obvious effect on the size and area of the leaves , at the same time in the different tissue of the leaf. The chemical fertilizer caused to precipitate calcium oxalate as druses in the lower epidermis and in vascular tissues. It had been found that the essential oil were stored in the inner layers of the spongy close to the plisade tissue. Yield characters i.e. fresh and dry weight were affected with nitrogen application . The maximum yield was obtained as a result of 60 Kg N/ fed. which increased the total fresh yield / feddan with 43.1% and 44.5 % in the first and second season, respectively. While a symbiotic nitrogen fixers micro-organisms increased the total fresh yield / feddan with 26.4 % and 29.9% in both seasons compared with the control . The percentage of the volatile oil , yield and its components were affected by fertilizer treatments. Carvon was the main constituents of volatile oil in all treatments, and reached the maximum value of 84.87 % in the bio-fertilizer treatment. Both chemical and bio- fertilizer treatments increased pigment contents in the leaf than the untreated plants.

Keywords: *Mentha viridis* L., volatile oil, carvone, pigment contents, leaf structure, ammonium sulphate , *Azotobacter chroococcum, Azosprillium lipoferum* Sudan IV.

One of the most important culinary herb is Spearmint (*Mentha viridis* L.) , which belongs to Lamiaceae (Labiatae), the mint family. The Labiates form one of the largest and most highly evolved plant families, which a world-wide distribution (excluding the Arctic and Antarctic only), containing around 200 genera and between 2000 and 5000 species of aromatic herbs and low shrubs (Good, 1974 and Heywood, 1978).

Spearmint and its oil is used as aromatic, stomachic, stimulant, antiseptic, local anethetic and antispasmodic in treating indigestion , nausea ,sore throat, diarrhea, colds, headaches, toothaches and cramps.The oil is used primarily as a flavoring agent, carminative and as fragrance components in toothpastes, mouthwashes, gargles, soaps, detergents, creams, lotions, and perfumes. Maximum use levels reported for the oil is 0.4 % in perfumes and about 0.132 % in baked foods. Spearmint leaves and extracts are reported used in alcoholic and nonalcoholic beverages. The leaves are also used in baked foods, gelatin and pudding and meat and meat products. Highest average maximum use level is about 0.597 % in baked foods (Leung 1980).

While infusion of leaves are used as refreshing, carminative, stomach ache, aphrodisiac, odontalgic, appetizer, especially when mixed with tea, mixed with vinegar and indigo it forms an emetic (Boulos, 1983).

The main constituent of spearmint oil is Carvone up to 70 % (Balbaa, *et al*., 1981).

Many investigations were carried out dealing with N fertilization and its effect on growth and yield of medicinal and aromatic plants (Bhardwaj and Kaushal, 1990; El-Ghadban, 1994 Kothari and Singh, 1995; Saxena and Singh, 1995; Swaefy, 1996; Zheljazkov and Margina, 1996; Mitchell and Farris, 1996; Youssef *et al.,* 1998; Jeliazkova *et al.,* 1999 and Sakr Weam, 2001). They confirmed that one of the main factors that increasing vegetative growth and yield in plants is the use of nitrogen fertilization .

On the other hand, the intensive use of mineral fertilizers in recent years results environmental pollution problems. Chemical fertilizer at extremely high rates for a long period, decreased the potential activity of micro flora and the stability of organic matters (Pokorna, 1984). Hence, the attention had been focused on the researches of the possibility of substitution with bio-fertilizers.

Bio-fertilizers increasing the number of micro-organisms and accelerate certain microbial processes in the rhizosphere of inoculated plants or soils. Such microbiological processes can change unavailable forms of nutrients into available ones that can be easily assimilated by plants. Also, they increase the amounts of fixed nitrogen in the plants and amount of nitrogen left in the soil (Subb Rao, 1981 and Alaa El-Din, 1982) .

The effect of bio-fertilization on plant growth and chemical composition was studied by many workers (El-Sawy *et al.,* 1986; Maheshwari *et al.,* 1988 and 1991; Nawar, 1994; Govedarica *et al.,* 1996; Attia, 2000; Badawi, 2000 and Ibrahem, 2000).

The present work aimed to study the influence of ammonium sulphate as a source of nitrogen fertilization as well as bio-fertilization with two a symbiotic nitrogen fixers on growth, yield and active constituents of *Mentha viridis* L .

Anatomical study had been carried out on the leaves taken from different treatments to find out the effect of fertilizers on leaf structure.

MATERIALS AND METHODS

The experiment was carried out during two successive seasons 2001/2002 and 2002/2003 at Maryout research station, Desert Research Center. *Mentha viridis* L. runners were planted on 15th March. for the two seasons. A complete randomized block design with three replicates was used.

Treatments included two sources of nitrogen . Ammonium sulphate 20.5 % at the rate of 60 Kg. N / fed. divided into three portions , after 30 , 45 and 60 days from planting , respectively . The other source of nitrogen was a mixture of two a symbiotic nitrogen fixers micro-organisms namely *Azotobacter chroococcum* and *Azosprillium lipoferum* beside the control (i.e. no nitrogen applied).

Three cuts were taken every season after 45, 105 and 165 days from planting date, respectively. Plant growth parameters were recorded i.e. fresh and dry weigh per plant and per feddan, the volatile oil percentage of the herb and oil yield per plant and per feddan .

The volatile oil of the leaves was extracted by water distillation according to Guenther (1961). The chemical composition of the essential oils from the different treatments were studied by GLC analysis.

The identification of the different compounds of each essential oil was achieved by comparing their retention times with those of authentic samples injected into instrument under the same condition. The relative percentage of each individual compound was calculated on the basis of peak corresponding to each compound.

The chlorophyll -a & -b and carotene pigments were extracted with acetone as described by Ranganna (1978).The pigments were quantified by using spectrophotometer measurements of tissues extracts .

The data statistical analyzed by computer SAS program. LSD. test was used to compare the average means of treatments .

Histological study

Samples of leaves were taken from the mature leaves (Fourth node on the shoot from the terminal end) during August. Each sample comprised of three mature leaves. The specimens were taken from the leaf between the mid vein and the leaf margin. Samples were killed and fixed in FAA 70 %. Dehydration, infiltration and imbedding processes of samples were carried out. (Johansen 1940). Transverse sections were cut by a rotary microtome with a thickness of 8-10 microns. Sections were stained by safranin FCF methanol then mounted in Canada balsam .

Measurements were taken from the cells of the upper and lower epidermis , palisade and spongy cells.Ten cells were recorded(Radial and tangential of each cell), number of cells, also the area was recorded to find out the surface area of the air spaces in the spongy tissue according to the following formula (El – Toumi et al., 1973):-

(1) No. of palisade cells per unit area Lamina

(Unit area 10.000 sq . ų .) $=N^2 / S^2 \times 10.000$

where N = number of cells

S = Sample width.

(2) Area of a cell = $1 \times \pi \times AB$

where $\pi = 22 / 7$, A and B the two dimensions of the cell .

$$\text{Percentage of air spaces} = \frac{\text{Area of examined tissue} - \text{area of cells}}{\text{Area of examined tissue}} \times 100$$

Thickness of leaves were recoded from ten sections of each treatments. No. of cells were calculated per microscope field (290 μ^2).

To put the finger on the tissue where the essential oil occurred and stored, free hand sections were taken from the med of the leaf and bleached with chlorine water to remove the chlorophyll from the cells. Sections were stained with Sudan IV which gives the oil and fats red color then washed in running water . Mounted in gleasren gill and examined and photographed. (Johansen 1940)

RESULTS AND DISCCUSION

Vegetative growth parameters

The data in table (1) showed that the fresh and air dried weight of spearmint herb were high significantly affected by nitrogen application in both seasons. Fresh weight per plant was increased as a result of adding chemical and bio-fertilizers as the recorded values in the first season were 184.52, 233.20 and 264.00 gm per plant at the control, bio-fertilizer and chemical fertilizer, respectively. In the second season, similar trend was showed as the corresponding values were 215.5. 280.03 and 311.28 gm per plant for the 3 treatments, respectively .These differences in fresh weight per plant may be attributed to the effect of the amount of nitrogen fertilization in addition to inoculation with a symbiotic nitrogen fixers on the enzymatic systems which are responsible for the biosynthesis of stored foods and this may be reflected on a higher average fresh weight of the herb.

The same trend was observed in total fresh weight per feddan. The highest value was 7920.00 Kg and 9338.40 Kg using 60 Kg N / fed with increment 43.1 % and 44.5 % in the first and second season, respectively. Mean while the bio-fertilizer treatment gave increment of 26.4 % and 29.9 % in both seasons, respectively more than the control.

The same trend was observed for the dry weight per plant and per feddan in the two seasons.

TABLE (1). Effect of fertilization treatments on fresh and dry weights of *Mentha viridis* L. Herb.

Treatments	Fresh weight (gm)				Dry weight (gm)				Total fresh weight/fed. (Kg)	Total dry weight/ fed. (Kg)
	1st Cut	2nd Cut	3rd Cut	Total / Plant	1st Cut	2nd Cut	3rd Cut	Total / Plant		
First Season										
Control	47.33	75.93	61.26	184.52	6.55	10.98	8.62	26.15	5535.60	784.50
Bio-fert.	59.40	89.30	84.50	233.20	8.38	12.38	11.71	32.47	6996.00	974.10
Chem.fert.	69.20	96.82	97.98	264.00	9.68	15.57	13.33	38.58	7920.00	1157.40
L.S.D.0.05	6.14	7.18	6.00	14.51	0.90	1.40	0.70	2.04	--------	--------
Second Season										
Control	56.33	85.20	73.97	215.50	7.88	12.29	9.99	30.16	6465.00	904.80
Bio-fert.	81.42	102.60	96.01	280.03	11.32	14.27	13.35	38.94	8400.00	1168.20
Chem.fert.	85.11	112.24	113.93	311.28	12.01	15.84	16.08	43.93	9338.40	1317.90
L.S.D.0.05	3.41	5.24	3.85	8.25	0.46	1.13	0.65	1.16	--------	--------

Volatile oil productivity

The relation between nitrogen treatments and volatile oil percentage and yield is shown in Table (2). It can be noticed that the increment of volatile oil per plant and per feddan was parallel to increasing fresh weight per plant and per feddan in the different treatments. The maximum values of volatile oil percentage and yield were obtained as a result of 60 Kg N / feddan then the treatment of bio-fertilizer. In this connection, nitrogen fertilization might enhance volatile oil bio-synthesis, through biosynthesis processes.

TABLE(2). Effect of fertilization treatments on volatile oil productivity of *Mentha viridis* L. fresh herb.

Treatments	1st Season			2nd Season		
	Vol.oil %	Volatile oil yield		Vol.oil %	Volatile yield	
		ml.L/Plant	L./feddan		ml.L/Plant	L./feddan
Control	0.220	0.405	12.178	0.230	0.495	14.869
Bio-fert.	0.250	0.583	17.490	0.260	0.728	21.842
Chem.fert	0.260	0.686	20.592	0.260	0.809	24.279

Volatile oil constituents

Data in table (3) indicated that chemical and bio-fertilization treatments affected the main constituent Carvone of the volatile oil of *Mentha viridis* L. leaves. The highest value of carvone 84.87 % was obtained from bio-fertilization treatment compared with 75.42 and 60.51 % for the chemical and the control treatments, respectively. The phellandrene limonene and menthone percentage recorded the lowest values as 6.94 , 3.37 and 1.66 %, respectively using bio-fertilizer followed by 8.64, 7.29 and 2.42 % using the chemical fertilizer treatment and then the control treatment as 20.76, 6.07 and 3.79 %, respectively for the three constituents. This can be explained in the light of the fact that Azotobacter and Azospirillum have the capability to fix atmospheric nitrogen and convert it to inorganic form. This nitrogen can be utilized by the plant and leads to enhance volatile oil biosynthesis, through playing direct or indirect role in this process. It could be concluded that inoculation with Azotobacter and Azospirillum increased the main constituent of the volatile oil of *Mentha viridis* L. leaves i.e. Carvone and decreased the other constituents as this is judged by Albert (1980) and Balbaa (1981) on spearmint (*Mentha viridis* L.) that L. carvone (mono-cyclic terpene ketone) is the main component of the oil.

TABLE(3). Volatile oil constituents percentage of *Mentha viridis* L. leaves as affected by different treatments

Components	Control	Bio-fert.	Chem. fert.
a – Pinene	0.65	0.38	0.64
B – Pinene	0.66	0.27	0.63
Limonene	6.07	3.37	7.29
Phellandrene	20.76	6.94	8.64
Comphene	0.28	0.17	0.07
Myrcenen	1.20	0.65	1.08
Menthone	3.79	1.66	2.42
Citronellol	0.38	0.04	0.17
Carvone	60.51	84.87	75.42
Pulegone	1.79	1.48	1.64
Cineole	0.39	0.18	0.28
Unidentified	3.52	------	1.72

Pigment contents

As shown in table (4) the chemical fertilizer displayed the highest amount of total chlorophyll-a and b compared with the bio-fertilization and control treatments which were 1.619 mg / g and 1.679 mg / g fresh weight in the first and second season, respectively. Whereas bio-fertilizer attained 1.538 and 1.521 mg/g fresh weight in the first and second season, respectively. The control has the lowest values which were 0.670 mg /g and 0.854 mg /g fresh weight in the first and second season, respectively.

It noticed that content of chlorophyll – a was higher than chlorophyll – b in all treatments.

On the other hand, carotenes content was much lower than that of chlorophyll-a and chlorophyll-b where they recorded 0.614, 0.412 and 0.246 and 0.639, 0.427 and 0.267 for chemical, bio-fertilizers and control treatments in the first and second season, respectively.

These results were in agreement with those mentioned by Salah, (1980) on lemon grass and Attia, (2000) on *Lawsonia inermis* L. They found that nitrogen fertilization increased both chlorophyll – a, b – and total carotenes than the untreated plants.

TABLE (4). Effect of fertilization treatments on pigments contents (mg/g fresh weight) in *Mentha viridis* L. leaves.

Treatments	Leaves chlorophyll (mg /g fresh weight)						Carotenes	
	1st Season			2nd Season			1st Season	2nd Season
	Chlor. A	Chlor. B	Total A&B	Chlor. A	Chlor. B	Total A&B		
Control	0.452	0.218	0.670	0.563	0.291	0.854	0.246	0.267
Bio-fert.	0.998	0.540	1.538	1.014	0.507	1.521	0.412	0.427
Chem.fert.	1.030	0.589	1.619	1.191	0.488	1.679	0.614	0.639

Histological study

In this study the leaf structure had been significantly affected by the different applications of treatments. Table (5) showed that leaf area increased with both the chemical and bio-fertilizers. The maximum value was 8.09 cm^2 for the chemical fertilizer compared with 7.77 cm^2 and 5.32 cm^2 for bio-fertilizer and the control, respectively. These results were in agreement with those obtained by (Kothari and Singh 1995) on spearmint (*Mentha gracilis* Sok.) and Attia (2000) on *Lawsonia inermis* L. It was observed that leaf area in the chemical fertilizer treatment was larger than that of the bio-fertilizer. On the other hand, leaf thickens increased in the bio-fertilization treatment (Table 5).

TABLE (5). Effect of chemical and bio-fertilization treatments on leaf structure of *Mentha viridis* L.

Measurements/Treatments	Control	Bio-fert.	Chem.fert.	L.S.D.0.05
Leaf area (cm^2)	5.32	7.77	8.09	1.193
Leaf thickness (u)	136.6	214.6	201	4.523
Palisade depth (u)	75.6	105.6	89.2	2.183
No. of palisade / unit area 10000	39.06	43.06	35.25	1.698
Spongy depth (u)	33	79	96	3.584
No. of spacing	31	36	23	4.755
Percentage of air spacing	14.09	19.09	23.14	--------

The anatomy of *Mentha viridis* L. studied is similar of the anatomy of dicotyledons which is composed of the upper and lower epidermis and Mesophyll in between (Metcalfe and Chalk, 1950 and Esau, 1965).

I-The epidermis

A- The upper epidermis

The mature epidermis cells are tabular in shape and relatively small extent in depth. It was found a thin layer of cuticle covering the outer layer of the epidermis. It also found that the epidermis cells of the control were meanly similar in shape and size (Fig .1 A) while there in the treated leaves, the epidermal cells became larger in size (Fig. 1B&C) but in the bio-fertilizer treatment were nearly the same in size while those from the chemical treated, two types of cells (Fig. 1C) some were small and isodiametric, the second are few elongated and larger in size.

B - The lower epidermis

Is composed of a single layer of tabular cells which are small extent in depth. Few trachoma were observed in the lower epidermis which are multicelluler composed of a foot which is imbedded in the epidermis and two small cells only. No essential oil was formed in these hairs (Fig. 2). Stomata were nearly the same in number in the treated and control leaves.

It had been observed the occurrence of druses of calcium oxalate in the lower epidermis cells (Fig. 3A) in the leaves of the chemical treated, but these druses were absent in the control and the bio-fertilized treated. These druses were also found in the bundle sheath of the midrib of the leaf and also in xylem tissue (Fig. 3B). The present of these druses in the treated plants with chemical fertilizer which contains 20.5 % nitrogen may be due to increase of N2 in the plant which cause of the present of calcium oxalates in this tissue.

A thin layer of cuticle occurred on the outer layer wall of the epidermal cells.

II- Mesophyll

The ground tissue of the leaf which enclosed the epidermis is the Mesophyll which is specialized as the photosynthetic tissue and is differentiated into plisade and spongy parenchyma.

A – The plisade tissue

In this study the palisade were elongate prismatic in shape which is several times longer than its width. From table (5) the depth of the palisade tissue was 75.6 u in the control while it reached its maximum in the bio-fertilizer treated 105.6 u while in chemical treatment was 89.2 μ.

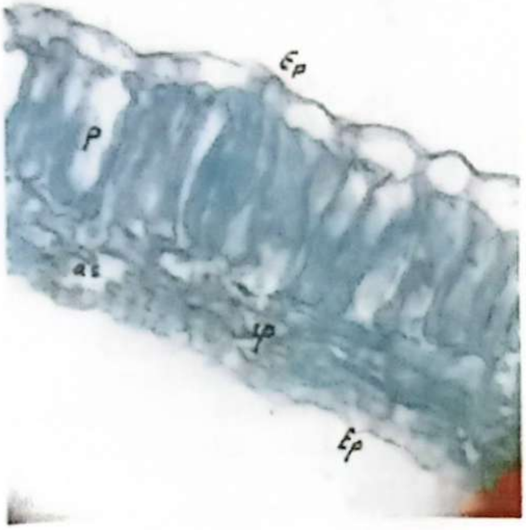
At the same time it was observed the No. of palisade cells per unit area Lomina (El- Toumi *et al.,* 1973) in bio-fertilizer treatment reached 43.06u while in the chemical treatment was less than the control one.

B – Spongy tissue

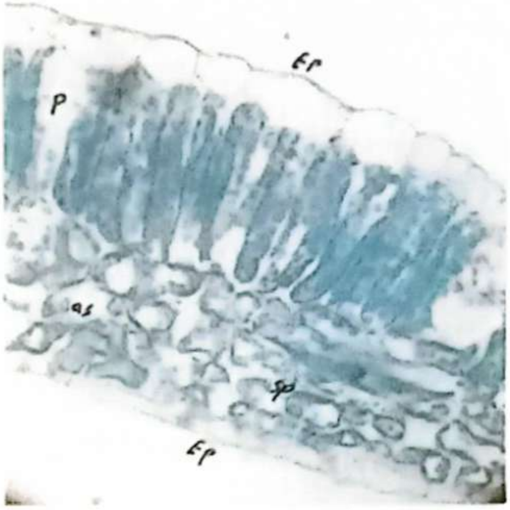
The spongy zone followed inword the palisade layer which composed of several layers of different forms of cells , some are isodiametric and few elongated . Large intercellure spaces were found .

It was found that both the palisade and spongy parenchyma had chloroplasts but most of them were in the palisade cells .

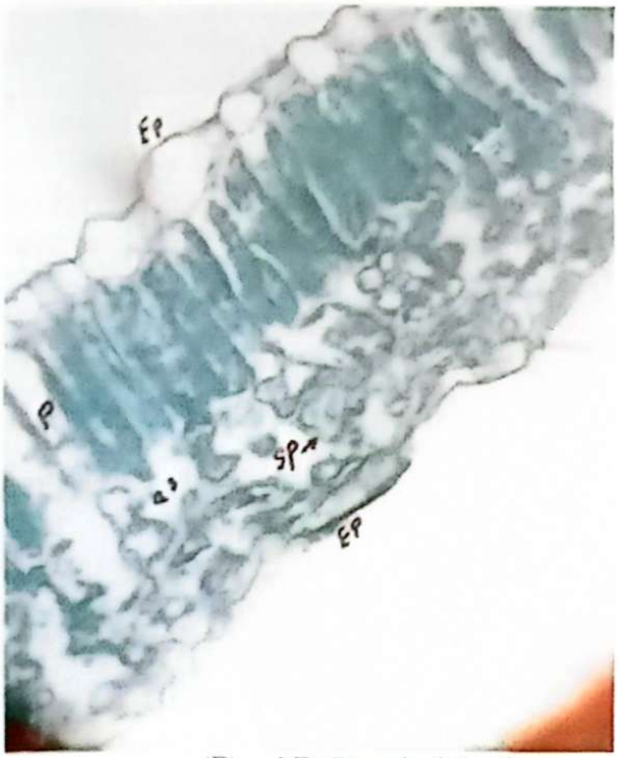
In this study it was observed that the thickness of the spongy tissue had been affected by the different applications of fertilizers .



[Fig . 1 (A)] Control



[Fig . 1 (B)] Bio-fertilizer



(Fig . 1C) Chemical fertilizer

Fig . (1). The effect of different treatments of fertilizers on the leaf structure of *Mentha viridis* L.X250.

EP : Epidermis
P : Palisade.
sp : Spongy
as : Air espaces.

Fig. (2). A.T. S. in the leaf showing the distribution of the oils that are concentrated in the spongy cells mainly in the inner layers. (A) palisade tissue (B) spongy tissue (C) essential oil. X 280.

[Fig . 3 (A)]

[Fig . 3 (B)]

Fig . (3). Druses of calcium oxalate in the lower epidermis (A) and in the midribe (B) in the leaves of the chemical treatment plants X 250.

As shown in Table (5) and [Fig.1 (A)] it was narrow in the control (33 u) and increased by the bio-fertilizer and reached the maximum in the chemical treatment. At the same time the percentage of air spacing increased the spongy area, but the number of the air spacing were less in the chemical treatment due to the large spaces of the air spaces .

In this study it was found that the essential oil of Mentha was found in the spongy parenchyma cells. It was concentrated in the inner layers of cells that close to the palisade parenchyma [Fig. 2 (C)].

REFERENCES

Alaa El-Din, M.M. (1982). Bio-fertilizers requirements and application. *FAO Soils Bulletin*, 45: 164-174 .

Attia, E.M. (2000). Using different forms of agricultural management to produce Henna *Lawsonia inerms* L. with minimized pollution under north Sinai conditions. *Ph.D.Thesis*, Agric. Sci. Inst. of Envir. Studies Research Ain Shams Univ., Cairo, Egypt .

Badawi, A.M. (2000). Effect of some agricultural treatments on growth and active ingredients in Roselle plants in north Sinai. *Ph.D.Thesis*, Fac. Agric., Zagazig Univ., Egypt.

Balbaa, S.I.; S.H. Hilal and A.Y. Zaki (1981). In"*Medicinal Plants constituents*". 3rd ed. General Organization for Univ. Books, Cario, Egypt, 644 pp.

Bhardwaj, S.D. and A.N. Kaushal (1990). N levels and harvesting mangement studies on fresh herbage and oil yield in peppermint cultivars (*Mentha piperita* L.). *Indian Perfumer*, 34 (1): 30-41 (c.f. *Hort. Abst.*, Vol. 61 No. 8).

Boulos, L. (1983). In "*Medicinal Plants of North Africa*" Public, Inc. Clair River Drive, Algonac, Michigan 4800 / 286pp.

El-Ghadban, E.A.E. (1994). The effect of some trace elements on growth and oil yield of spearmint (*Mentha viridis* L.). *M.Sc.Thesis*, Fac. Agric., Cairo Univ., Egypt.

El-Sawy, M.; E.A. Salah; M.K. Abdel Fattah; M.A. El-Borollosy and M. Sharaf (1986). Hyoscyamin content of *Hyoscyamus muticus* L. as affected by inoculation with Azotobacter and/or Azospirillum strains. *Proc. 2nd Conf. AABNF, Cairo, Egypt.* p498-513.

El-Toumi, A.L.; S.T. Boulos and G.M. Nour (1973). Studies on the Effect of nitrogen fertilization on the epidermis and misophyll of shemlali olive leaves. *Desret Inst. Bull.*, Egypt, 23(2): 457-462.

Esau, K. (1965). In "*Plant Anatomy*". John Wiley & Sons, Inc., New York. London, Sydney, Toppan company, LTD., Tokyo, Japan. 767pp.

Good, R. (1974). In "The geography of the flowering plants". 4th edition, London, Longman.

Govedarica, M.; N. Milosvic; M. Jarak; D. Milosev and S. Djuric (1996). Diazotrophs and their activity in pepper. *Acta Hort.,*: 462: 725-732.

Guenther, G. (1961). In "*The Essential Oils*". D. Van Nastrand Company Inc. Toronto, New York, London. Vol.3.

Heywood, V.H. (Ed.) (1978). In "*Flowering plants of the world*". Oxford. Oxford University Press.

Ibrahem, Zahira T.Z. (2000). Effect of some cultural practices on growth and chemical composition of some medicinal plants under conditions of north Sinai. *Ph.D.Thesis*, Fac. Agric., Kafer El-Sheikh, Tanta University, Egypt.

Johansen, D.A .(1940).In "*Plant microtechnqiue*". McGraw Hill Book Co. Inc., New York, London.

Kothari, S.K. and U.B. Singh (1995). The effect of row spacing and nitrogen fertilization on Scotch spearmint (*Mentha gracilis* Sole). *Journal of Essential Oil Reserch*, 7 (3):287-297 (*Hort. Abst.*, 65:9197).

Leung, Albert, Y. (1980). In "*Encyclopedia of Common Natural Ingredients Used in Food, Drugs, and Cosmetics*". Johhn Willey & Sons, New York, U.S.A.

Maheshwari, S. K.; S. K. Gangrade; S. N. Mishra and K. C. Trivedi (1988). Effect of Azotobacter and nitrogen on herb yield and alkaloid content of blak henbane . *J. Agron* . , 33 (3) : 308 – 311.

Maheshwari, S. K.; S. K. Gangrade and K. C. Trivedi (1991). Comparative responses of palmarosa to Azotobacter and nitrogen under rainfall and irrigated swards. Indian – Perfume., 35 (2):108–111.

Metcalfe, C. R. and L. Chalk (1950). In "*Anatomy of the dicotyledons*". 2 vols. Oxford, Clarendon Press. p1041-1053.

Mitchell, A. R. and N.A. Farris (1996). Peppermint response to nitrogen fertilizer in an arid climate. *J. Plant Nutrition*, 19 (6): 988.

Nawar, A. I. (1994). Response of sunflower varieties to mineral and bio-fertilization with nitrogen. *Com. Sci. and Dev. Res.*, 723 (47): 163-178 .

Pokorna, K. J. (1984). Effect of long term fertilization on the dynamics of changes of soil organic matter. *Zentralblatt – fur Mikrobiologie*, 139 (7): 497 – 504 (*Soil and Fertil. Abst*, 45: 3225).

Ranganna, S. (1978). In "*Manual of analysis of fruits and vegetative products*". 2nd reprint plant pigments 72–93. Tata McGraw–Hill publishing company, Limited, New Delhi, 634 pp .

Sakr Weam, R. F. (2001). Effect of some organic and inorganic fertilizers on *Mentha piperita* L. *M.Sc.Thesis*, Fac. Agric. Cairo Univ., Egypt.

Salah, M. (1980). Study on the effect of some agriculture treatment on the quantity of lemon grass oil. *M.Sc. Thesis*, Faculty of Agric., Al-Azhar Univ., Cairo, Egypt.

Saxena, A. and J. N. Singh (1995). Effect of irrigation , mulch and nitrogen on yield and composition of Japanese mint (*Mentha arvensis* L.) *Oil. J. Agron. and Crop Sci.*, 175(3):183–188. (Hort. Abst., 66:2530).

Subb Rao, N . S . (1981). In "*Bio-fertilizers in Agriculture*". Oxford and IBH Publishing Co. New Delhi, Bombay, Calcutta . p.6 – 9, 16 – 22, 77–92 and 142–160.

Swaefy, H. M. F. A. (1996). Effect of chemical fertilization and some trace elements on growth and productivity of *Mentha piperita* L. plant. *M.Sc.Thesis*, Fac. Agric., Cairo Univ., Egypt.

Youssef , A. A.; I. M. Talaat and E. A. Omer (1998). Physiological response of basil green ruffles (*Ocimum basilicum* L.) to nitrogen fertilization in different soil types . *Egypt . J. Hort.*, 25(2): 253–269.

Zheljazkov, V. and A. Margina (1996). Effect of increasing doses of fertilizer application on quantitative and qualitative characters of mint. *Act. Hort.*, 426:579-592. (*Hort. Abst.*, 67 (7): 6277).

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دراسات فسيولوجية وتشريحية علي تأثير بعض الأسمدة المختلفة علي أوراق بعض النباتات الطبية والعطرية
١ -النعناع البلدى

زهيرة توفيق زكى وصدقي توفيق بولس*
قسم النباتات الطبية والعطرية - مركز بحوث الصحراء- المطرية - القاهرة - مصر.
* قسم البيئة والمراعى - مركز بحوث الصحراء- المطرية - القاهرة - مصر.

يعتبر النعناع من النباتات العطرية والطبية الهامة حيث تستعمل الأوراق وكذلك الزيت الناتج من تقطير الأوراق في كثير من الأغراض الطبية.
وقد تم إجراء تجربة حقلية خلال موسمين متتالين ٢٠٠٢/٢٠٠١ و ٢٠٠٣/٢٠٠٢ بمحطة بحوث مريوط لدراسة تأثير التسميد النيتروجيني بسماد سلفات النشادر بمعدل ٦٠كجم نيتروجين للفدان وسماد حيوي عبارة عن خليط من بكتيريا الأزوتوباكتر والأزوسبيرلليم لدراسة تأثيرهما علي المحصول لنباتات النعناع وعلي كمية الزيت الطيار ومكونات الزيت وقد تم تقدير كل من كلوروفيل أ ، ب ، الكاروتين كما تم قياس مساحة الأوراق في المعاملات المختلفة وتشريح الأوراق لمعرفة التأثير الناتج عن التسميد علي التركيب التشريحي للأوراق.
وقد أوضحت التجارب أن كل من الوزن الطازج والوزن الجاف قد تأثر بإضافة النيتروجين وقد حصل علي أعلي محصول نتيجة إضافة (٦٠ كجم نيتروجين للفدان) بزيادة ٤٣,١% ، ٤٤,٥% في الموسم الأول والثاني علي التوالي بينما في معاملة التسميد الحيوي فقد زاد المحصول بنسبة ٢٦,٤% ، ٢٩,٩% في الموسمين الأول والثاني مقارنة بالكنترول .نسبة الزيت ومحصول الزيت ومكوناته تأثرت بمعاملات التسميد. وكان الكارفون هو المكون الرئيسي في الزيت في جميع المعاملات ووصل إلى أعلي قيمة ٨٤,٨٧% في معاملة التسميد الحيوي وقد زاد المحتوي الكلي للكلوروفيل والكار وتين في معاملة التسميد الكيماوي عن معاملة التسميد الحيوي والكنترول وقد اتضح أن هناك تغير ملحوظ في التركيب التشريحي للورقة حيث وجد كبر مساحة الورقة مع رقة سمكها في التسميد الكيميائي مقارنة بالتسميد الحيوي كما يوجد اختلاف واضح بين التركيب التشريحي لأوراق النباتات المسمدة والكنترول. كما وجد أن عدد خلايا النسيج العمادي كان عددها أكثر في التسميد الحيوي.وقد وجد أن الزيت العطري يتركز في خلايا النسيج الإسفنجي الملاصقة للنسيج العمادي و لوحظ في أوراق النباتات المسمدة بالتسميد الكيماوي وجود بلورات من اكسالات الكالسيوم في البشرة السفلي وكذلك في الخلايا المحيطة بالحزمة الوعائية، وهذه صفة غير مرغوبة.