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Introduction of *Ocimum tenuiflorum* plant to the Egyptian cultivation

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Abstract : Through our program for the introduction of new varieties of medicinal and aromatic plants, we decided to study the *Ocimum tenuiflorum* plant. Beginning from the germination of seeds, tracking its growth and productivity of essential oil and its components, under Egyptian conditions. Therefore, this study was designed to determine the influence of different soil types at different dates of the year on seed emergence. Also, plant growth characters, oil content and its constituents of *Ocimum tenuiflorum* plant, under Egyptian conditions were estimated. Preparing of the seeds was carried out using three different soil types for germination, pure sand soil (M1) and two level of mixture of sand and clay loamy soil (M2=1 vol. sand + 1 vol. clay loamy & M3= 2 vol. sand + 1 vol. clay loamy) were used as germination medium. The seeds of *Ocimum tenuiflorum* plant were sown every month in nursery, started on the first of October, and lasted for seven months. Using M3 treatment and sown at February month gave the best result during two seasons. These seedlings were planted in the field. The growth of *O. tenuiflorum* plants during the growing seasons under the Egyptian conditions, were found that the average height of the plants up to 74 cm and 78 cm and the fresh yield of herb recorded 465 and 443 g/ plant in the first and second cut respectively. The highest percentage of volatile oil found in the first cut (15 %), compared with the second cut (10%). GC-MS analysis of *O. tenuiflorum* oil indicated that, eugenol, bisabolene, estrigole and 1,8- cineol were found as the main constituents of *Ocimum tenuiflorum* oil under Egyptian atmosphere conditions.
Keywords : *Ocimum tenuiflorum*, *Egyptian atmosphere*, soil type, germination, growth parameters, essential oil.

Introduction

Ocimum tenuiflorum,(holy basil- Lamiaceae Fam.).It is cultivated on a large scale in India and in the southeast Asian tropics (Staples and Kristiansen[1], Warrier[2]). It is cultivated for medicinal purposes, and for religious belief. It has long been documented as a diverse and rich source of essential oils. At the same time,it is used in cooking for its flavor and fragrance, so the fresh or dried leaves add to many foods, such as rice, pasta, and salads (Mabey et al [3]), in addition the, herb of *O. tenuiflorum* has medicinal and cosmetic uses (Norr and Wagner[4]). Traditionally the various parts like leaves, flowers, and stemsare being used in the treatments various disorders such as skin disorders, cold, fever, vomiting, cough swelling ect. *O.tenuiflorum* was reported to have anti-cancer, antimicrobial, antiseptic, antispasmodic, antifungal, antiviral, anti-inflammatory, analgesic and immune-stimulatory properties. The powder is used for treating jaundice and for alleviating blood pressure. The volatile oil of *O.tenuiflorum* is characterized by remarkably high concentrations of eugenol, methylchavicol as well as bisabolene, camphor, linalool, β-caryophyllene and thymol compound (Palla et al[5], Cedric et al [6]).The available information about the best production processes for this plant under Egyptian conditions is little. Due to the increasing interest in medicinal and aromatic plants in Egypt as a source of Egyptian national income of

hard currency, this research aims to work on the introduction of new varieties of medicinal and aromatic plants to the Egyptian market by working on the regionalization of those plants under Egyptian weather conditions. However, all species of ocimum are propagated from a seeds. The factors which influence the germination of *Ocimum tenuiflorum* in a known micro-environment are soil type and date of sowing (Mabey et al[3], Norr and Wagner[4]). The effect of different soil types and date of sowing on seed germination of some medicinal and aromatic plants has been reported. Seeds germination are influenced by several factors such as the substrate type used, ecological factors such as water, oxygen, temperature and light. Generally, soil type is identified factors to get the seedlings of high quality (Hartmann et al [7], Baiyeri and Mbah[8], Dickens[9], Omokhua et al[10], Süleyman et al [11]). Also, several environmental factors have been observed to affect the growth, chemical content and composition of essential oil. (Julian[12]). So, the main purpose of the current study was to determine the best possible soil type and sowing time that maximizes the seed germination as well as study the effect of the Egyptian conditions on plant growth and oil content of *Ocimum tenuiflorum*.

Materials and Methods

In previous experiment seeds of *Ocimum tenuiflorum* (*Syn.Ocimum sanctum*) were imported from the (J.L. Hudson, Seeds man Redwood City ,California 94064, USA.) and had been cultivated at the farm of ornamental plants unit in National Research Centre-Giza Egypt during two successive seasons 2013 and 2014. The experiment included studying the propagation of *Ocimum tenuiflorum* plants under the conditions of Egypt. The seeds of *Ocimum tenuiflorum* were sown every month in greenhouse to study the effect the date of sowing on germination percentage. Using three different types of soil, pure sand soil [M1] and two level of mixture of sand and clay loamy soil [M2= (1 vol. sand + 1 vol. clay loamy)] & [M3= (2 vol. sand + 1 vol. clay loamy)] were used as germination medium. The medium were put in pots (20 cm in diameter) and the pots were kept in the nursery. Sowing was started on the first of October, and lasted for seven months. Healthy seedlings of *O. tenuiflorum* which resulted from experiment of germination (media M3 with sown in February) were planted in the field on 15th April in hills 25 cm apart on rows 60 cm in-between. Calcium superphosphate 15.5% 100kg/fed. was added during preparing of the soil. Ammonium sulphate 21% 200kg/fed. and potassium sulphate 48% 100kg/fed., divided into two equal portions, the first one added after one month from transplanting and the second one after first cut.The treatments in these experiments were arranged in completely randomized design. Each treatments includes three replicates. Data during of two seasons were statistically analyzed, the least significant difference (LSD at level of 5%) was used to compare between different means according to Snedecor and Cochran [13].

1-Soil analysis:

Physical and chemical analysis of the different soil types for germination, determined by hydrometer method Piper [14] (Table 1).

Table (1). Mechanical and chemical analysis for the different germination soil.

Mechanical analysis clay and sand soil			
Soil sample	**Coarse sand %**	**Fine sand %**	**Silt + Clay %**
Clay	40	18	42
Sand	73	17	12
Chemical analysis			
Items	M1 (pure sand soil)	M2 (1vol. sand+1 vol. clay loamy)	M3 (2vol. sand+2 vol. clay loamy)
PH	8.6	7.7	7.9
Total N ppm	120	210	190
Water soluble P ppm	0.6	0.5	1.1
Water soluble k ppm	2.7	1.1	4.2
EC (dSm^1)	1.2	1.1	1.4

Samples from the soil were taken before cultivation of the seedlings, and were subject to physical and chemical analysis in the Soil Science Department, National Research Centre, according to the method of Jackson [15] and presented in (Table, 2).

Table 2. The physical and chemical properties of the experimental soil during the two seasons.

Characters		**First season**	**Second season**
Physical properties	Clay %	22	22
	Silt %	53.7	51.7
	Sand %	24.3	26.3
	Soil texture	Sandy loam	Sandy loam
Chemical properties	pH	8.08	8.0
	E.C (dsm^{-1})	1.35	1.15
	Organic matter %	0.49	0.60
	Available N %	1.12	1.4
	Available P %	0.71	0.83
	Available K %	0.23	0.27
Cations:(Milliequivalent/L)	Ca^{++}	11.5	12.2
	Mg^{+2}	3.5	3.7
	Na^{+1}	0.68	0.72
	K^{+1}	0.42	0.53
Anions: (Milliequivalent/L)	CO_3^{--}	0.0	0.0
	HCO_3^{-}	1.0	1.1
	Cl^{-1}	1.3	1.4
	SO_4^{--}	13.8	13.5

2-**Vegetative growth characters:**

During the two cuts (the first cut was at June month while, the second cut was at October month) the following parameters were recorded: plant height, fresh and dry weight of herb g/plant and ton/fed.

3-Isolated and analysis of essential oils:

The volatile oil from fresh herb (50g) was isolated by hydrodistillation for 3hr in order to extract according to Gunether[16]. The isolated volatile oil was dehydrated over anhydrous sodium sulfate and was stored in refrigerator until analyzed. Samples of essential oil of *Ocimum tenuiflorum* were isolated from fresh herb of plants during in the two cuts. Generally, oil constituents of each sample were investigated by the means of GC and GC-MS. And also estimated the percentage of volatile oil from the organs of plants which were harvest at different growth stages and separated into leaves, stems, flowers and seeds.

Gas chromatography: FID Hewlett-Packard 5890 using a DB-5 (methyl-silicone containing 5 % phenyl groups) column 25 m x 0.31 mm i.d. Temperature program was 2 min at 60 °C, 60-100 °C (2°C/min) and 100-250°C (5°C/min), carrier gas was helium at flow rate of 1.0 ml/min.

Gas chromatography-Mass spectrometry: A Hewlett packard 5989A GC-MS system equipped with library software Wiley 138 and NBS75 was used. Capillary GC conditions as mentioned above were employed for DB-5 column. Injection volume was 1.0ul at 1:50 split. Significant MS operating parameter: ionization voltage 70 ev, scan mass range 40-350u.

Identification of components: Compounds were identified by matching their mass spectra with those recorded in the MS library (Adams [17]) and further confirmed by injecting the authentic samples of different compounds with the volatile oil and by comparing the mass spectra with those of reference compounds or with the published data (Kovats [18]).

Results and Discussion

1-Seed germination

Data in Table (3) recorded that in general, in both years in our study, the percentage of seed germination of *Ocimum tenuiflorum* between 30 to 90 % were achieved from the seeds sown between 1th October and 1th April under the all conditions of soil type. On the other hand, the healthy seedlings rates derived from the seeds of same sowing condition were higher than 50 % in the period from 1thNovember until1th April of each year. Concerning the germination media, it is obvious from our results that, the third treatment (M3) gave the high germination percentage in the first and second season. The average values recorded throughout the year as a result of the use of such treatment, recorded 73.57 and 65.3% for the first and second season, respectively. The best results of germination percentage were obtained when the seeds of *O. tenuiflorum* were sown under the nursery condition in (early February to early March) in the soil media M3 composed of a mixture(2 vol. sand + 1 vol. clay loamy). The percentage of germination in this respect amounted to 90 and 85 % .These trend was observed during two seasons. In conclusion, it is possible to obtain successful result in springs until March month using soil media (M3) Consisting of a mixture (2 vol. sand + 1 vol. clay loamy).The same results, which illustrated the effect of some environmental factors on germination of seeds, environmental factors such as temperature, light, pH, and soil moisture are known to affect seed germination (Martins et al [19], Canossa et al [20], Ikeda et al [21], Rizzardi et al [22]).

Table (3) Seeds germination percentage of *Ocimum tenuiflorum* plant as affected by soil type and sowing date. (During two seasons 2013 and 2014).

Media / Months	First season (Germination %)				Second season (Germination %)			
	M1	M2	M3	Mean	M1	M2	M3	Mean
Oct.	35	35	45	38.33	30	35	35	33.33
Nov.	55	60	65	60.00	50	60	56	55.33
Dec.	55	65	70	63.33	60	70	66	65.33
Jan.	70	80	85	78.33	70	75	75	73.33
Feb.	80	80	90	83.33	80	85	85	83.33
Mar.	75	80	85	80.00	75	75	78	76.00
Apr.	50	65	75	63.33	55	60	62	59.00
Mean	60.0	66.43	73.57	----	60	65.7	65.3	-----
L.S.D. at 0.05								
Months	3.643				3.459			
Media	2.385				2.265			
Months × Media	5.266				4.999			

2-Vegetative growth character.

For evaluation of *O.tenuiflorum* plant under the weather conditions of Egypt, healthy seedlings (seeds of *O.tenuiflorum* were sown under the nursery condition in early February to early March, in the soil media M3 (composed of a mixture 2 vol. sand + 1 vol. clay loamy) were transferred to the field on 15 th April of each year. The monitoring of the growth of *O. tenuiflorum* plants during the growing season under the Egyptian conditions, were found that the average height of the plants up to 74 cm. and 78 cm. and the fresh yield of *O. tenuiflorum* herb recorded 465 and 443 g/plant, 25.57 and 24.37 ton/hec. in the first and second cut respectively. While, the total of the two harvests in this respect recorded 49.94 ton/hec. fresh weight. At the same time the yield of dry weight recorded 62.5 g/plant and 3.44 ton/hec in the first cut against 75.5g/plant and 4.15 ton / hec. in the second cut with a total amounting to 7.59 ton\ hec. Study of the climate and ecological factors in determining the agriculture crop species of each region has become an essential issue. Today, agricultural organization and increased production per unit area require optimal use of natural resources and further knowledge about these resources. The conditions of climatic such as temperature, light intensity, air humidity, rainfall, wind speed, and their changes are the major factors that determine the kind of plants that are able of growing and developing in a certain region. In order to grow farming (Hassan Rahmanpour [23]). Soonthern et al [24] on mint, found that, maximum leaves, stems and root dry matter was produced under 30°C day temperature

and the maximum oil yield occurred under v30/ 18°Cc regime. Ibrahim [25] found that, the highest yield of leaves and twigs of Lusa plant was recorded during September and October.

3-Essential oil production:

Essential oil production.

The percentage of essential oil isolated from the herb of *O. tenuiflorum* recorded 0.15 % in the first harvest, while it was 0.10% in the second harvest. The yield of herb oil per plant amounted to 0.698 ml/ plant in the first harvest and 0.443 ml/plant in the second harvest. The total oil yield from the two harvests was 62.77 l/ hec. Cedric et al, Juliani, Franz, and Palevitch [6, 12,26,27] have been observed the effect of some environmental factors and harvest times as well as aagriculture conditions on optimization of oil yields. In the context of the follow-up to oil content in different parts of the plant (leaves, flower and seeds) during different growth stages. In all cases it was found that (fig. 1), the fresh leaves of *O. tenuiflorum* gave significant quantities of volatile oil especially in the flowering stage (0.20%). Also it was observed that, the percentage of volatile oil in the appropriate quantity in flowers (0.14%), while the seeds gave a high proportion of volatile oil in the final stage of growth (0.21%). Very small traces of essential oil was observed in the stems in different growth stages.

Essential oil constituents.

Table (4) Studies on the main oil constituents of the *O. tenuiflorum* L have been undertaken. Sixteen components were found, they represent about (67.76%) of the volatile fraction *O. tenuiflorum*in the first cut, while it recorded (82.76%) in the second cut. In the first cut the main oil constituents of *Ocimum tenuiflorum* L. were, eugenol (26.90) %, bisabolene (17.20 %), estrigole (8.3 %) and 1,8- cineol (4.3 %). In the second cut these values recorded, 32.2, 16.15, 11.90 and 10.1 % for the same main constituents respectively. Oil composition of *Ocimum tenuiflorum* could be divided into three groups. These groups includes, G1 (monoterpenes hydrocarbon), G2 (Oxygenated monoterpenes) and G3 (Sesquterpenes hydrocarbon). It is clear from the data that, *Ocimum tenuiflorum* oil is characterized by its content-rich Oxygenated monoterpenes compounds, which recorded 40.42% during the first harvest, while increased to 54.25% in the second harvest. This trend was observed on the *O. tenuiflorum* oils by Hassan et al [23], found that yield and composition of essential oil were affected by planting dates and harvest times. Several environmental factors and harvest times have been observed to affect the chemical content and composition of essential oil Juliani et al [12]. The development of oil production by holy basil accessions subjected to cultivated conditions could lead to optimization of oil yields, consistent oil quality, Franz [26], Palevitch[27].

Table (4) Vegetative growth characters and essential oil content in fresh herb of *Ocimum tenuiflorum* plant under the conditions of Egypt.

Characteristics **Treatments**	**Plant height cm/plant.**	**Fresh weight g/plant**	**Fresh weight Ton/hec.**	**Dry weight g/plant.**	**Dry weight Ton/hec.**	**Oil % (fresh weight herb)**	**Oil yield ml/plant.**	**Oil yield L/hec.**
First cut	74.5	465.0	25.57	62.5	3.44	0.15	0.698	38.37
Second cut	78.0	443.0	24.37	75.5	4.15	0.10	0.443	24.40
Total	152.5	908.0	49.94	138.0	7.59	0.25	1.140	62.77
Means	76.25	454.0	24.97	69.0	3.79	0.125	0.570	31.38
L.S.D. at 0.05	12.50	25.28	1.39	5.17	0.288	0.190	0.119	

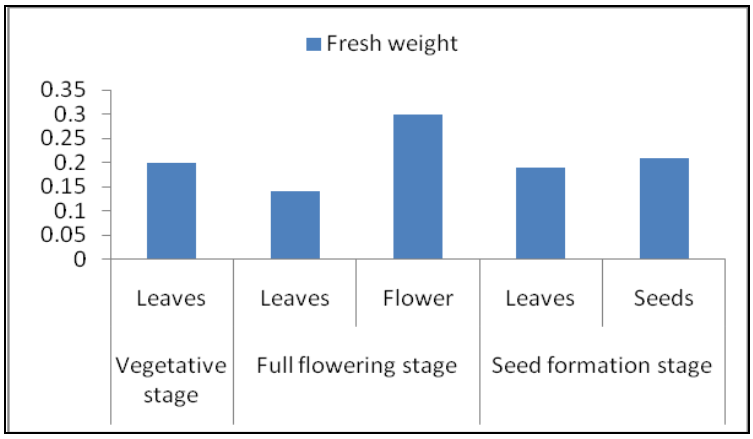

Fig.1 Volatile oil percentage of different organs of *Ocimum tenuiflorum* plant under the conditions of Egypt.

Table(5). Oil constituents of thefresh herb of *Ocimum tenuiflorum* plant under Egyptian conditions.

No.	Compound/Geoup	Classification	KI	First cut %	Second cut %
1.	α-Thujene	G1	931	0.07	0.04
2.	β–Pinene	G1	939	0.61	0.88
3.	Sabinene	G1	976	0.24	0.51
4.	β–Pinene	G1	980	1.9	1.30
5.	Trans-beta-ocimene	G1	1050	4.0	4.20
6.	Terpinene-gamma	G1	1062	0.27	1.20
Total monoterpenes hydrocarbon				**7.09**	**8.13**
7.	1,8 Cineol	G2	1033	4.3	10.10
8.	Borenol	G2	1165	0.92	0.05
9.	Estragol	G2	1195	8.3	11.90
12	Eugenol.	G2	1356	26.90	32.2
Total Oxygenated monoterpene.				**40.42**	**54.25**
13	β–elemene	G3	1375	1.01	1.2
14	β–Caryophyllene	G3	1418	0.44	0.33
15	α–*humulene*	G3	1454	1.9	2.70
16	β–Bisabolene	G3	1509	17.20	16.15
Total Sesquterpenes hydrocarbon.				**20.55**	**20.38**
Total identification of the compounds.				**68.06**	**82.76**

G1.Monoterpenes hydrocarbon. G2.Oxygenated monoterpene. G3.Sesquterpenes hydrocarbon

Conclusion

It can be concluded that the cultivation of the plant *Ocimum tenuiflorum* under Egyptian atmosphere conditions gave encouraging results and can be considered as one of the new sources of essential oils in Egypt, which requires further study of the post-harvest to produce high quality essential oils which are compatible with the global market.

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