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# Effect of frequent harvest on yield and essential oil of lemon verbena grow under Egyptian conditions

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## Abstract
Lemon verbena is considered as a new source of essential oil with relatively high yield used in perfumery with its strong citral. Field experiments were performed at the Experimental Farm of Qalyupia Governate, Egypt to study the effect of frequent harvesting on yield and essential oil of lemon verbena shrubs under the environmental conditions of Egypt. The one harvest during October was favorable compared with 2 and 3 harvests since the highest values of the fresh and dry weight values were recorded. Different frequent harvest significantly affected essential oil content (%, ml plant$^{-1}$ and liter ha$^{-1}$). Thus the essential oil contents in general increased with 3 harvests per year compared with other harvests per year. The main components were D - limonene, 1, 8 cineol and citral. The highest values of major constituent were recorded under one harvest per year compared with 2 or 3 harvests per year.

*Keywords:* Lemon verbena, yield, essential oil, D - limonene, 1, 8 cineol and citral, frequent harvest.

## 1. Introduction
True verbena oil comes from *Lippia citriodora* Kunth, syn. *Verbena triphylla L* Herit., *Aloysia citriodora* Ort. (Family Verbenaceae), verbena a small shrub that grows to 1.5 m high. Although native to South America. *Lippia citriodora* plant is available in Egypt [1], it is considered as a new source of essential oil with relatively high yield used in perfumery with its strong citral content. Lemon verbena essential oils have been studied by several researchers. The essential oils are popular among the herbalists because the oil contains citral and limonene. Lemon verbena oil is obtained by steam distillation of the freshly cut leaves and twigs in approximately 0.1 % yields; it is a yellow-greenish liquid with a characteristic fresh, lemon like odor. Catherine [2] found that the main constituents of *Lippia citriodora* oil under conditions of grass were geranial, neral and limonene constituting 66.3% of the total essential oil yield in May and increasing to 69% in September. Santos-Gomes [3] found that under Portugal conditions 63 compounds were identified in hydro-distillates from leaves of lemon verbena plants. The three main essential oil compounds were geranial (38.3%), neral (29.6%), and limonene (20.6%).

Most perennial aromatic crops experience reduction in biomass yield due to repeat harvesting, thus affecting essential oil yield. Repeated harvests can be beneficial or detrimental to oil production, depending on other environmental factors. For example, herbage yield is usually high at first harvest, becomes constant and declines with repeated harvests [4-5]. Gunether [6] reported that the first harvest of *Lippia citriodora* shrubs takes place in July, as soon as the first inflorescence appear, the second harvest in October before the plants are billed up to protect them from the cold of the winter, some growers harvest three times viz the end of June the middle of August and the end of October Distillation gives only a very small yield of oil from fresh leaves and twigs was 0.012. Gunether [7] stated that the number of harvests per year varied in different mint growing districts. Stengele [8] found that the highest mint yield of green material was obtained with cutting at 75 - day intervals and this permitted 3 crops in the year, but the highest of oil yield was found with 2 cuts at 105 day apart. Clikvanaja [9] found that the highest average green matter yield of geranium plants resulted from cutting the plant once a year at the end of October while Agena [10] working with *Pelargonium graveolens*, pointed out that the highest values of total vegetative growth weight and oil yield of *Pelargonium graveolens resulted* from three harvests per year which more than one harvest a year. Hamza [11] found that the highest geranium fresh weight yield was obtained when the crop was harvested on 3 occasions, early May, late August and late December .Weiss [5] stated that the time of cutting affected fresh material and oil yield of geranium plants. Khalil [12] mentioned that cutting of mint every three months significantly increased the weight of fresh and dry weight of herb, leaves and the oil yield over cutting every four months. El-Sayed [13] found that the highest plant, herb, dry matter productions and oil yield of geranium resulted in April cutting; also he found that the oil percentage in leaves of geranium reached maximum values in summer samples and minimum ones in winter samples. Osman [14] found that that harvesting parsley or dill for one or two times to obtain fresh herb caused reduction in plant weight at flowering stage, consequently less umbels numbers, seed and oil yield compared with non harvested plants. On the other side the total income fresh herb and seed yield was

higher than harvested two times before obtaining seeds. Nozipho [15] found that, harvesting frequency had a highly significant effect herbage yield and oil yield of rose-scented geranium. Closer harvesting intervals (after two months) produced less herbage yield and oil yield while longer harvesting intervals produced more herbage yield mid oil yield. Harvesting at two month intervals improved the oil composition of rose-scented geranium but did not improve oil yield. Kothari [16] found that biomass yield was great in the first harvest and gradually declined in the subsequent harvests of *Ocimum tenuiflorum.* The method of harvesting had no effect on biomass yield. Essential oil content in general was lower in the first harvest and increased gradually in subsequent harvests to reach maximum in the fourth harvest. Rose geranium *(Pelargonium* sp.) has a life span of six to eight years under commercial production and the first harvest is carried out at 6 - 8 months after planting. Subsequent harvests are then conducted at 3 - 4 month intervals. Considerable time is taken before harvesting the first crop so that it could establish itself, thereafter, harvested at close intervals (3 - 4 months) to avoid losses in oil yield due to leaf senescence [5, 17]. Harvesting of secondary branches of *O. Tenuiflorum* led to maximum plant height. Plant spread and number of secondary branches during second and subsequent harvests [16]. Rose-scented geranium plants are normally cut at 15 to 20 cm above the ground to allow reestablishment of new leaves for the process of photosynthesis [5, 18]. Lemon verbena oil could be considered as source of hard currency in Egypt. This point itself needed a thorough and accurate study *of Lippia citriodora* plant from both the agricultural and chemical points of view. Such a study will undoubtedly elucidate the best conditions of producing the best quality and quantity of *Lippia citriodora* oil. The present investigation is the first attempt to survey the influence of some different agricultural factors on the growth, oil yield and its constituents under the Egyptian conditions. Therefore this study included revealing the effect of number of harvests per year on the plant growth and essential oil were carried out.

## 2. Materials and methods

*2. 1. Plant materials*

Two field experiments were performed at the Experimental Farm of Qalyupia Governate, Egypt. to study the effect of frequent harvest on yield and essential oil of lemon verbena shrubs under the environmental conditions of Egypt during the years of 2010 and 2011. The plants of lemon verbena were grown on rows 60 cm. apart and the plants were spaced at 100 cm in between. Plants were divided into 3 main groups. The first group was harvested 3 times during July, August, and October of both seasons. The second group was harvested 2 times during July and October. On the other hand the third group was harvested one time during October. Fresh and dry weights of herb per plant and per hectare were recorded. The age of lemon verbena shrubs was one year in the first season while it was two years in the second season. All agricultural practices were conducted according to the recommendations by the Egyptian Ministry of Agriculture.

*2. 2. Essential oil isolation*

Fresh mass was deviled into small pieces (0.5 - 1 cm) was collected from each treatment, and then 500 g from each replicate of all treatments was subjected to hydro-distillation for 3 h using a Clevenger-type apparatus [19]. The essential oil content was calculated as a relative percentage (v/w). In addition, total essential oil (ml plant$^{-1}$ and liter ha$^{-1}$) was calculated by using the fresh mass. The essential oils which were extracted from the plant materials were collected from each harvest and dried over anhydrous sodium sulphate to identify the chemical constituents of the essential oil.

*2. 3. Gas chromatography*

GC analyses were performed using a Shimadzu GC-9A gas chromatograph equipped with a DB5 fused silica column (30 m x 0.25 mm i.d., film thickness 0.25 μm). Oven temperature was held at 40°C for 5 min and then programmed until 250°C at a rate of 4°C/min. Injector and detector (FID) temperature were 260°C; helium was used as carrier gas with a linear velocity of 32 cm/s.

*2. 4. Gas chromatography-Mass spectrometry*

GC-MS analyses were carried out on a Varian 3400 system equipped with a DB-5 fused silica column (30 m x 0.25 mm i.d.); Oven temperature was 40 to 240°C at a rate of 4°C/min, transfer line temperature 260°C, injector temperature 250°C, carrier gas helium with a linear velocity of 31.5 cm/s, split ratio 1/60, flow rate 1.1 ml/ min, Ionization energy 70 eV; scan time 1 s ; mass range 40-350 amu.

*2. 5. Identification compounds*

The components of the oils were identified by comparison of their mass-spectra with those of a computer library or with authentic compounds and confirmed by comparison of their retention indices either with those of authentic compounds. Kovat's indices [20] were determined by co-injection of the sample with a solution containing a homologous series of n-hydrocarbons, in a temperature run identical to that described above.

*2. 6. Statistical analysis*

The experimental design was in completely block randomized with three replicates. The averages of data were statistically analyzed using analysis of variance (ANOVA) and values of least significant difference (LSD) at 5% according to Snedecor [21].

## 3. Results and discussion

*3. 1. Effect of frequent harvest on fresh and dry weight of herb*

The data obtained for the fresh and dry weight measurements of herb [g plant $^{-1}$ and ton ha$^{-1}$] of lemon verbena were recorded and are presented in Table 1. It is clear from the data that the fresh and dry weight measurements of herb were significantly affected by different harvests number. The one harvest during October was favorable compared with 2 and 3 harvests since the highest values of the fresh and dry weight values of herb were recorded during both seasons. The highest values of the fresh weight were 463.0 and 281 g plant $^{-1}$; 14.0 and 8.9 ton ha$^{-1}$ for the first and second season respectively. On the other hand the highest values of the dry weight were 168.2 and 118 g plant $^{-1}$; 5.9 and 3.8 ton ha$^{-1}$ for the first and second seasons respectively. These results are in accordance with those obtained by Gunether [7], he stated that the number of harvests per year varied in different mint growing districts. On the other hand Agena [10] working on *Pelargonium graveolens*, he pointed out that the total vegetative growth weight resulted from three harvests per year were more than one harvest a year. Hamza [11] found that the highest geranium fresh weight yield was obtained when the crop was harvested on 3 occasions.

**Table 1:** Effect of frequent harvest on the fresh and dry weight of herb

| First season | | | | | | |
|---|---|---|---|---|---|---|
| Number of harvests | | Harvest date | Fresh weight | | Dry weight | |
| | | | g plant $^{-1}$ | Ton ha$^{-1}$ | g plant $^{-1}$ | ton ha$^{-1}$ |
| 3 harvests | 1st | July | 79.1 | 2.5 | 33.0 | 1.1 |
| | 2nd | August | 128.2 | 4.1 | 45.5 | 1.5 |
| | 3rd | October | 140.8 | 4.5 | 59.6 | 1.1 |
| Total of 3 harvests | | | 348.1 | 11.1 | 138.1 | 3.6 |
| 2 harvests | 1st | July | 102.1 | 3.2 | 38.1 | 1.1 |
| | 2nd | October | 174.2 | 5.5 | 56.1 | 2.1 |
| Total of 2 harvests | | | 276.3 | 8.8 | 94.2 | 3.2 |
| 1 harvest | 1st | October | 463.0 | 14.0 | 168.2 | 5.9 |
| LSD at | | | | | | |
| 0.05 | | | 67.2 | 2.1 | 17.4 | 0.5 |
| 0.01 | | | 111.2 | 3.5 | 28.2 | 0.9 |
| Second season | | | | | | |
| Number of harvests | | Harvest date | Fresh weight | | Dry weight | |
| | | | g plant $^{-1}$ | Ton ha$^{-1}$ | g plant $^{-1}$ | ton ha$^{-1}$ |
| 3 harvests | 1st | July | 136.7 | 4.3 | 59.9 | 1.9 |
| | 2nd | August | 52.2 | 1.7 | 18.8 | 0.7 |
| | 3rd | October | 67.0 | 2.1 | 24.7 | 0.8 |
| Total of 3 harvests | | | 255.9 | 8.1 | 103.4 | 3.3 |
| 2 harvests | 1st | July | 130.5 | 4.1 | 49.5 | 1.6 |
| | 2nd | October | 79.5 | 2.4 | 30.7 | 1.0 |
| Total of 2 harvests | | | 210.0 | 6.5 | 80.2 | 2.6 |
| 1 harvest | 1st | October | 281.0 | 8.9 | 118.0 | 3.8 |
| LSD at | | | | | | |
| 0.05 | | | 14.3 | 0.8 | 12.9 | 0.3 |
| 0.01 | | | 23.7 | 1.3 | 21.3 | 0.5 |

*3. 2. Effect of frequent harvest on essential oil content (extracted from the herb) and its main constituents*

Different frequent harvest significantly affected essential oil content (%, ml plant $^{-1}$ and liter ha$^{-1}$) (Table 2). Thus the essential oil contents in general increased 3 harvests per year compared with 2 and one harvest per year. Greatest essential oil was obtained from the 3 harvests per year with the values of 0.5 and 0.5%; 1.6 and 1.3 ml plant $^{-1}$; 45.4 and 46.2 liter ha$^{-1}$ at the first and second season respectively. Three main constituents amounting 38.7 - 49.6 % of the oil were found in the lemon verbena leaves essential oil extracted by the hydro - distillation method during both seasons (Table3). The main components were D - limonene, 1, 8 cineol and citral. The highest values of major constituent were recorded under one harvest per year compared with 2 or 3 harvests per year. The highest values were 15.3 and 14.9 % for D - limonene; 6.9 and 6.4% for 1, 8 cineol; 25.5 and 28.3 % for citral during first and second seasons respectively. The variations in essential oil content and composition could be due to its effect of frequent harvest on enzymes activity and metabolism improvements [22]. On the other hand these results agree with those obtained by Mtutag [4, 5] they mentioned that most perennial aromatic crops experience reduction in biomass yield due to repeat harvesting, thus affecting essential oil yield.

**Table 2:** Effect of frequent harvest on the essential oil content

| Number of harvests | | Harvest date | Essential oil | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | First season | | | Second season | | |
| | | | % | ml plant $^{-1}$ | liter ha$^{-1}$ | % | ml plant $^{-1}$ | liter ha$^{-1}$ |
| 3 harvests | 1st | July | 0.5 | 0.4 | 11.5 | 0.5 | 0.7 | 20.1 |
| | 2nd | August | 0.4 | 0.6 | 17.9 | 0.7 | 0.4 | 10.1 |
| | 3rd | October | 0.4 | 0.6 | 16.0 | 0.4 | 0.6 | 15..2 |
| Total of 3 harvests | | | 0.5 | 1.6 | 45.4 | 0.5 | 1.3 | 45.4 |
| 2 harvests | 1st | July | 0.4 | 0.5 | 15.0 | 0.5 | 0.7 | 20.0 |
| | 2nd | October | 0.4 | 0.7 | 20.1 | 0.4 | 0.3 | 8.1 |
| Total of 2 harvests | | | 0.4 | 1.2 | 35.1 | 0.5 | 1.0 | 28.1 |
| 1 harvest | 1st | October | 0.3 | 1.2 | 33.3 | 0.3 | 0.9 | 25.0 |
| LSD at | | | | | | | | |
| 0.05 | | | 0.1 | 0.3 | 7.5 | 0.1 | 0.1 | 4.1 |
| 0.01 | | | 0.2 | 0.4 | 12.5 | 0.2 | 0.2 | 5.0 |

**Table 3:** Effect of frequent harvest on the main constituents of essential oil

| First season | | | | | | |
|---|---|---|---|---|---|---|
| Number of harvests | | Harvest date | D - limonene % | 1,8 cineol % | citral % | Total |
| 3 harvests | 1st | July | 13.2 | 5.1 | 21.4 | 39.7 |
| | 2nd | August | 11.5 | 5.6 | 14.7 | 31.8 |
| | 3rd | October | 14.3 | 5.7 | 22.6 | 42.6 |
| Overall 3 harvests | | | 13.0 | 6.1 | 19.6 | 38.7 |
| 2 harvests | 1st | July | 13.4 | 5.2 | 20.9 | 39.5 |
| | 2nd | October | 14.7 | 6.1 | 22.0 | 42.8 |
| Overall 2 harvests | | | 14.1 | 5.7 | 21.5 | 41.3 |
| 1 harvest | 1st | October | 15.3 | 6.9 | 25.5 | 47.7 |
| Second season | | | | | | |
| Number of harvests | | Harvest date | D - limonene % | 1,8 cineol % | citral % | Total |
| 3 harvests | 1st | July | 12.4 | 6.1 | 24.2 | 42.7 |
| | 2nd | August | 10.0 | 5.5 | 22.2 | 37.7 |
| | 3rd | October | 11.3 | 6.3 | 25.8 | 43.4 |
| Overall 3 harvests | | | 11.2 | 6.0 | 24.1 | 41.3 |
| 2 harvests | 1st | July | 11.6 | 5.7 | 27.8 | 45.1 |
| | 2nd | October | 11.4 | 6.1 | 24.8 | 42.3 |
| Overall 2 harvests | | | 11.5 | 5.9 | 26.3 | 43.7 |
| 1 harvest | 1st | October | 14.9 | 6.4 | 28.3 | 49.6 |

## Conclusion

It may be concluded that The one harvest during July was favorable compared with 2 and 3 harvests since the highest values of the fresh and dry weight values of herb were recorded. Different frequent harvest significantly affected essential oil content (%, ml plant $^{-1}$ and liter ha$^{-1}$). The highest values of major constituent were recorded under one harvest per year compared with 2 or 3 harvests per year.

## References

1. El Hamidi A., Ahmed S.S. and Shaarawy F. *Acta Hort.,* 132 (1983) 31.
2. Catherine A., Dimitra D., Petros A., Tarantilis C.F. and Moschos P., *Bioch, Sys. Eco.,* 35 (2007) 831.
3. Santos G., Paula C., Fernandes F., Manuel V. and Ana M., *J Ess. Oil Res.*, 17 (2005) 73.
4. Murtagh G.J., *Aus. J. Agric. Res.,* 47 (1996) 801.
5. Weiss E.A., Essential oil crops. Centre for Agriculture and Biosciences (CAB) International, New York and UK (1997).
6. Gunether E., The Essential Oils vol VI, Ed Van Nostrand New York (1952).
7. Gunether E., The essential oils vol (IV) von Nostrand, New.York (1961).
8. Stengele, M., Stahl-Biskup, E., *Acta Hort.,* 344 (1993) 41.
9. Clikvanaja E.E., Kotlgarova M.V., *Trudy Suhum Opyt Stan Efironmasl Kalto.,* 16 (1965) 13.

10. Agena E.A., Effect of some cultural treatments on the growth and oil yield of geranium plants (*Pelarqonium graveolens*). M.S. Thesis, Faculty of Agric. Ain Shams Univ. Cairo, Egypt (1966).
11. Hamza A., El Mahmoudi E., *Res. Rev Cairo.,* 44 (1966) 1.
12. Khalil M., Effect of some cultural treatments on the growth and oil yield of some Mint varieties M.Sc. Thesis Ain-Shams Unv. Cairo-Egypt (1973).
13. El-Sayed A.G., Phraseological study of Geranium plants *Pelargonium graveolnes* as affected by GA and CCC. Ph.D Thesis. Fac. of Agric. Cairo Univ., (1979).
14. Osman, Y., Abd El-Wahab, M., Res., *J. Agric. Bio. Sci.*, 5 (2009) 218.
15. Nozipho M. M., Essential oil yield and composition of Rose-scented Geranium *(Pelargonium SP.)* as influenced by harvesting frequency and plant shoot age M. Sc., (Agric) Agronomy,Faculty of Natural and Agricultural Sciences University of Pretoria (2006 ).
16. Kothari S.K., Bhattachary A. K., Rame S., *J. Hrom.,* 1054 (2004) 67.
17. Rajeswara A., Rao B.R., Cultivation and distillation of Geranium oil from *Pelargonium* species in India. The genera Geranium and *Pelargonium* In. Maria Lis- Balchin (ed.). Medicinal and Aromatic Plants-Industrial Profiles. Published by Taylor and Francis, London. Pp. 212–217 (2002).
18. Demarne F.E., 'Rose-scented geraniuni' *a Pelargonurm* growin for the perfume industry. Geraniums and Pelargoniums: The genera Geranium and *Pelargoinurn. In.* Maria Lis-Balclain (ed.)l Medicinal and Aromatic Plant-Industrial Profiles. Published by Taylor and Francis. London. Pp. 193-211 (2002).
19. Clevenger J.F., *J. Amer. Pharma..,* 17 (1928) 346.
20. Kováts E., *Helv. Chim. Acta.,* 41 (1958) 1915.
21. Snedecor G.W. and Cochran W.G., Statistical Methods 11th ed. The Iowa State. Univ. Press, Iowa, USA (1990).
22. Burbott A.J., Loomis, D., *Plant Phys.,* 44 (1969) 173.