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Response of the newly introduced plant species *Monarda citriodora* in Egypt to nitrogen fertilization and plant density

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Abstract : The present study was aimed to develop basic production information of *Monarda citriodora* in Egypt and to determine the appropriate levels of nitrogen and plant densities for growth and essential oil content and composition. Therefore, an experiment was conducted to study the effect of three nitrogen doses (33.5, 50.25 or 67 kg N/fedden = 4200 m^2) and four plant spacing (20, 25, 30, or 35 cm between plants) in 2009/2010 and 2010/2011 seasons at the Experimental Station of the Faculty of Agriculture, Cairo University, Giza, Egypt. The medium level of nitrogen produced the highest growth parameters, yields of fresh and dry herb as well as oil yield while plant spacing of 30 cm seemed to be the optimum for plant fresh and dry weights. Plants spaced 20 cm apart gave the highest fresh, dry herb and oil yields.

Nitrogen fertilization and plant spacing slightly affected or did not dramatically change the chemical composition of the essential oil of *Monarda citriodora* **.** The chemical composition of the essential oil of the aerial parts of *Monarda citriodora* identified 17 constituents when analyzed by GC-MS. Thymol was the dominating compound in the all analyzed samples (32.73 – 63.88 %), followed by carvacrol (6.54 - 29.56%), which constituted almost 80 % of the essential oil, followed by p-cymene (1.24 - 17.72 %) and γ-terpinene (0.37-19.6%).

Keywords: Nitrogen fertilization, Plant density, *Monarda citriodora,* Yield, Essential oil.

Introduction

Genus monarda belonging to the family lamiaceae comprises about 30 species of annual and perennial medicinal, aromatic and ornamental plants. It is typical of the North American continent in its natural distribution. Species of this genus have been used in landscaping, gardening and as food flavoring or additive. As with most species in the lamiaceae, the leaves produce an essential oil that is spicy and fragrant composed mostly of monoterpenes in glandular trichomes located on the surface of leaves, sepals, flower petals and to some extent on stems. The best known species of this genus are: *Monarda didyma* L., *M. fistulosa* L. and *M.citriodora*.[1]

Lemon bergamot (*Monarda citriodora*), also called horsemint, has citrus-scented leaves, indigenous to the United States, well-developed root system, stems are branched, squared, approximately 25-90 cm tall, with leaves arranged oppositely on the stem, petiolate, narrowly lanceolate or oblong-lanceolate in shape. The leaves are pubescent, highly aromatic with a strong lemon scent. Flowers are pink-purple, ¾ inches diameter, grow in clusters on a stem and occur from late May through July depending on weather conditions. Monarda is grown as an ornamental, aromatic and medicinal plant throughout most regions of the world[2].

The plant is a natural source of thymol, an active ingredient in some mouthwashes. Medicinally, Native American and early settlers used the plant to treat minor wounds, infections, headache, fever, stomach and bronchial ailments, mouth and throat infections caused by cavities and gum disease[3].

Eighteen compounds were identified in the oil of *M. citriodora* and accounted for 95.9% of the oil. Thymol (70.6%), p-cymene (10.6%) and carvacrol (6.1%) were identified as the dominant components. The oil demonstrated a range of bioactive properties. It is clear that *M. citriodora* var. citriodora oil is a potential preservative against free radical-mediated deterioration of lipid-rich cosmetics, foods and pharmaceuticals[4].

Also, the composition of the essential oil from the leaves and flowers of *Monarda citriodora* var. *citriodora* was analyzed by GC and GC/MS in another experiment. Thymol was the dominant component in the oil from both the flowers (61.77%) and leaves (50.69%). The second most important compound of the leaf oil was p-cymene, although yield from the flower oil was lower (4.19%). A more abundant component of the flower oil was γ-terpinene (13.30%), but only a trace of this was detected in the oil from leaves. Since α -terpine is an undesirable compound found in extracts from lemon bergamot it is important to produce greater amounts of thymol methylester and thymol, and production of less α –terpine[5].

Recently, in vitro studies have confirmed the antimicrobial and antifungal activities of *M. citriodora* oil. The oil exhibited a high level of antifungal activity by direct contact and in the vapor phase. It was generally active particularly against more rapidly growing fungal species [6]. Moreover, an antioxidant activity was described for the oil of *M. citriodora* in three avian assay systems[7].

Fertilization has been reported as one of the agricultural factors that have a critical effect on plant growth and quantitative and qualitative characteristics of essential oil (EO) biosynthesis. The largest growth and yield responses usually result from nitrogen application. Nitrogen has a vital role in the growth and active substances in medicinal and aromatic plants[8].

The relationship between N levels and plant responses had been observed in different species. Some researches deal with thyme species, For example it was found that fertilizers increase thyme crop, but differences in the yield of essential oil were not remarkable. However, it resulted in higher yields of essential oil obtainable from the cultivation area unit. In addition, very slight changes in the percentage of compounds in thyme herb cultivated under different fertilization doses were detected[9].

In another work, Results indicated that the level of nitrogen fertilizer significantly altered growth characteristics, oil percentage and oil yield of garden thyme. The highest values of shoot dry weight and oil percentage were obtained at 100 mg N/kg of ammonium nitrate[10].

Also, nitrogen fertilization had a significant effect on herb yield and essential oil content of 3-year-old garden thyme but did not change the thymol content[11]. Nitrogen rates ranging from 50 to 150 kg ha-1 were optimum for thyme production depending on soil fertility[12].

In fact, optimum planting density is a key to achieve maximum crop production. While thyme plants biomass and oil production were lower at the highest planting density, hyssop plants showed no response to planting density [13]. In another experiment on thyme, planting space had a significant effect on plant diameter and very significant effect on other measured parameters except oil content, which was not significant. The maximum yields of dry and fresh herbage, yield and content of oil and thymol yield were obtained in 15 cm space. Maximum thymol content was observed in 45 cm space. However, 15 cm spacing was the best treatment in respect of yield of dry matter, oil and thymol per unit area[14].

Similarly, at 30x45 cm spacing, maximum plant height, herb yield, oil content and oil yield of *M. piperita* were recorded. These coupled with the use of NPK (120:50:40 kg/ha) fertilizer[15].

No agronomic information about *Monarda citriodora* is available. So, this study was aimed to develop basic production information of *Monarda citriodora* in Egypt and to determine the appropriate levels of nitrogen and plant spacing for growth and essential oil content and composition.

Materials and Methods

The present study was conducted in 2009/2010 and 2010/2011 seasons at the Experimental Station of the Faculty of Agriculture, Cairo University, Giza, Egypt (30 ° 05′ N, 31 ° 22′ E).

A fertilizer application of 200 kg/fed super phosphate was applied during ploughing and 150kg/fed potassium sulphate was applied after the second cut on May 18. Seeds were obtained from Jelitto seed company, Germany and were sown for both seasons in a nursery on October under shade in the open field. Individual plots contain four rows; each row was 2 m long and 50 cm wide. A plant spacing within the row of 30 cm was used for the fertilizer experiment, and the N dose of 50.25 kg N/fed was used in the spacing experiment. The seedlings cultivated in the field on December 23. Three nitrogen doses were applied (33.5, 50.25 or 67 kg N/fedden = 4200 m^2) and four plant spacing (20, 25, 30, or 35 cm between plants) were studied. In the experiment reported herein, plants spaced 20 cm apart in the row had a stand approximating 40000 plants per fed, those spaced 25 cm apart had a stand approximating 32000 pant per fed, those spaced 30 cm apart had a stand approximating 26000 pant per fed and those spaced 35 cm apart had a stand approximating 22000 pant per fed.

Treatments were arranged in a randomized complete block design with three replicates. The plants were side-dressed three times with N fertilizer; the first dosage was applied eight weeks after transplanting, the second was one week after the first cut and the third was one week after the second cut. Furrow irrigation was applied as required.

Physical and chemical analyses of the field experiment soil were carried out before planting and shown in Table 1.

Tab. 1: Properties of the soil used for growing monarda

Clay (%)	Silt (%)	Sand (%)	Organ. C (%)	OM (%)	CaCO3 (%)	pH	EC (Sm^{-1})	N (ppm)	P (ppm)	K (ppm)
37.2	24.6	38.2	0.23	1.7	3.2	7.9	2.35	47	14	67

Three cuts were carried out 10 cm above the soil surface on March 31, May 5 and June 19 during both seasons. For dry matter determination, four replicates of 10 g material of each treatment were dried in an electrical oven at 105 °C for several hours until constant weight was reached. Then the dry matter was determined.

Samples of fresh herb of each replicate at each cut in both seasons were subjected to hydro-distillation for 3 h using Clevenger apparatus to extract and to determine the essential oil percentage according to the Egyptian Pharmacopoeia[16]. The resulted essential oil was separately dehydrated over anhydrous sodium sulphate and kept in silica vials with Teflon-sealed caps and stored at 2°C in the absence of light till GC analysis.

The essential oil constituents were analyzed and determined in the oil samples of the three cut of the first season. The dehydrated oil of each treatment was subsequently analyzed using a gas liquid chromatography-mass spectrometer (GC-MS) to evaluate oil quality. The gas chromatography–mass spectrometry (GC-MS) analysis of the essential oil samples was carried out using gas chromatography-mass spectrometry instrument stands at the Department of Medicinal and Aromatic Plants Research, National Research Center, Egypt with the following specifications. Instrument: a TRACE GC Ultra Gas Chromatographs (THERMO Scientific Corp., USA), coupled with a THERMO mass spectrometer detector (ISQ Single Quadruple Mass Spectrometer). The GC-MS system was equipped with a TG-WAX MS column (30 m x 0.25 mm i.d., 0.25 μm film thickness). Analyses were carried out using helium as carrier gas at a flow rate of 1.0 ml/min and a split ratio of 1:10 using the following temperature program: 40°C for 1 min; rising at 4.0°C/min to 160°C and held for 6 min; rising at 6°C /min to 210°C and held for 1 min. The injector and detector were held at 210°C. Diluted samples (1:10 hexane, v/v) of 0.2 μL of the mixtures were always injected. Mass spectra were obtained by electron ionization (EI) at 70 eV, using a spectral range of m/z 40 to 450. Most of the compounds were identified using mass spectra (authentic chemicals, Wiley spectral library collection and NIST library).

The collected data were subjected to the analysis of variance in Randomized Complete Block Design (RCBD) arrangement[17] using MSTAT-C V.2.1 software package[18]. Differences among means were compared for each trait by Duncan multiple range test (DNMRT) [19].

Results and Discussion

Effect of Nitrogen fertilization:

Individual plant growth characteristics of *Monarda citriodora* as affected by nitrogen fertilization for both seasons are shown in Table 2. Plant height was unresponsive to nitrogen fertilization. Although plant fresh and dry weights reach their maximum values at the medium level of nitrogen, the differences compared to the low level failed to be significant in the first two cuts.

In the third cut plants that received the medium level of nitrogen fertilizer were higher in plant fresh weight by 16 and 84 % than plants received the low level in the first and second seasons, respectively. Also, the increments in plant dry weight were 32 and 112% for the same respective treatments.

Table (2): Monarda growth characteristics as affected by nitrogen fertilization during two seasons

Nitrogen fertilizer	**Plant height (cm)**			**Fresh weight (g/plant)**			**Dry weight (g/plant)**		
	1st cut	**2nd cut**	**3rd cut**	**1st cut**	**2nd cut**	**3rd cut**	**1st cut**	**2nd cut**	**3rd cut**
1st season									
N1	26.60 a	38.43 a	35.90 a	48.19 a	251.30 a	353.33 b	8.96 a	51.54 a	75.03 b
N2	26.52 a	40.19 a	37.08 a	49.46 a	263.56 a	410.00 a	9.27 a	54.49 a	100.05 a
N3	29.09 a	41.62 a	37.22 a	46.73 a	239.11 a	266.67 c	8.64 a	50.69 a	61.47 b
2nd season									
N1	25.29 a	39.40 a	38.45 a	59.09 a	223.10 a	253.33 c	11.21 a	47.19 a	55.54 b
N2	29.97 a	41.53 a	39.18 a	62.33 a	264.53 a	466.67 a	11.40 a	57.44 a	117.83 a
N3	30.48 a	39.92 a	39.92 a	58.81 a	250.94 a	310.00 b	10.76 a	53.71 a	76.04 b

Values within columns followed by the same letter are not significantly different at the $P \leq 0.05$ level.
N1 the low level = 33.5 kg N/fedden N2 the medium level = 50.25 kg N/fedden N3 the high level = 67 kg N/fedden

Negative trend of decreasing yield with increasing nitrogen fertilizer above the medium level suggests no more than 50.25 kg N/fed is necessary for monarda production and possibly lower N levels may be more appropriate for these species. This result is in harmony with the findings of cox[8] who mentioned that further increase in N application generally do not result in large increase or may actually reduce plant growth.

Most authors report that higher growth and yield response in herbs were obtained with higher nitrogen application because nitrogen has the important functions of building amino acids, proteins, nucleotides, nucleic acids, chlorophylls, and coenzymes[20]. For example, sweet basil, sweet marjoram, pot marjoram and oregano responded favorably to 168 and 252 N kg /ha when using various levels of ammonium nitrate in combination with P and K and micronutrients[21].

In contrast, rosemary does not respond well to high levels of fertilizer where low levels of fertilizer promoted the growth of rosemary more than high levels[22] and our outcomes support these findings. The rise of EC and pH in the higher fertilizer treatments may have caused the depressed root and shoot dry weights. On the other hand, excess fertilizer contaminating the environment makes it important for growers to use as little fertilizer as possible to produce marketable plants[23].

It was determined that nitrogen fertilizer almost has no effect on the oil percentage which varied from 0.29 to 1.14 %.

The productivity of monarda (fresh herb yield, dry herb yield and oil yield) followed the same pattern as in the growth characters and was affected by the level of nitrogen only in the third cut of both seasons (Table 3). It was found that the medium level of nitrogen produced the highest yields of fresh and dry herb as well as oil yield.

Table (3): Monarda yield as affected by nitrogen fertilization during two seasons

Nitrogen fertilizer	Fresh herb yield (ton/fed)			Dry herb yield (ton/fed)			Oil yield (L/fed)		
	1st cut	2nd cut	3rd cut	1st cut	2nd cut	3rd cut	1st cut	2nd cut	3rd cut
1st season									
N1	1.285 a	6.701 a	9.421 a	0.238 a	1.374 a	2.001 a	3.765 a	55.610 a	81.423 b
N2	1.319 a	7.028 a	10.933 a	0.247 a	1.453 a	2.668 a	3.450 a	56.623 a	125.047 a
N3	1.246 a	6.376 a	7.110 b	0.230 a	1.351 a	1.639 a	3.707 a	56.927 a	74.343 b
2nd season									
N1	1.575 a	5.949 a	6.755 b	0.299 a	1.259 a	1.481 b	3.947 a	55.040 a	57.953 bc
N2	1.662 a	7.054 a	12.444 a	0.304 a	1.532 a	3.142 a	4.670 a	57.483 a	129.953 a
N3	1.568 a	6.692 a	8.266 b	0.287 a	1.432 a	2.027 ab	3.220 a	71.707 a	86.646 b

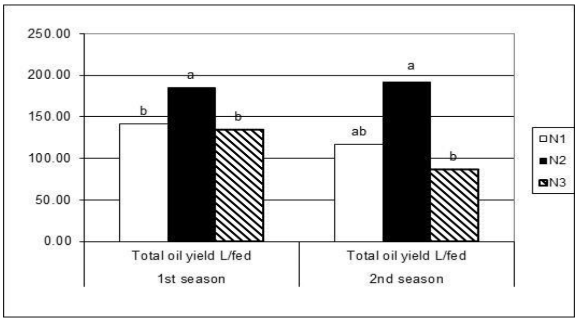
Values within columns followed by the same letter are not significantly different at the $P \leq 0.05$ level.
N1 the low level = 33.5 kg N/fedden N2 the medium level = 50.25 kg N/fedden N3 the high level = 67 kg N/fedden

In the third cut plants that received the medium level of nitrogen fertilizer were higher in the fresh and dry herb yields by 16, 84 % and by 33, 112 % than plants received the low level in the first and second seasons, respectively. In the same manner, the increments in essential oil yield were 53 and 124 % for the same respective treatments.

Although, the trend of response of yield parameters to nitrogen fertilization was similar in all cuts, monarda had four to five times as much yield in the second and third cuts as in the first cut.

Cumulative fresh and herb yield varied significantly among N levels in the second season only but not in the first one (Fig1). Results revealed that increasing nitrogen application increased the cumulative yields of fresh and dry herb over cuts. The highest yields (fresh and dry herb) of 21.16 and 4.98 ton/fed were obtained by application of 50.25kN/fed. Those were increases of about 48 and 64% respectively, compared to the first level.

The maximum oil yields (19.28 and 21.16 L/fed in the first and second seasons, respectively) were obtained from the medium level of nitrogen. Increasing nitrogen level from 33.5 to 50.25 kg/fed increased essential oil yield by 32 and 64 % in the first and second seasons respectively.



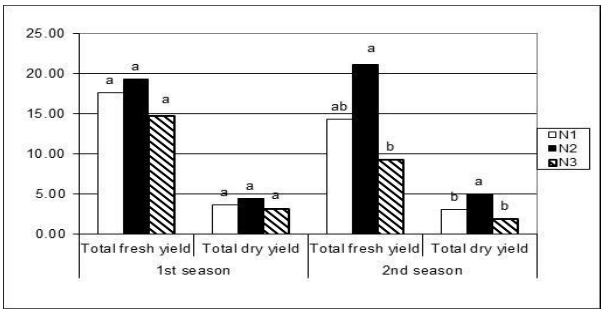


Fig 1: Cumulative Monarda yield as affected by nitrogen fertilization during two seasons

From the previous results, It was said the low or medium levels of nitrogen added to the soil are almost effective and enough to give considerable growth and yield parameters of *Monarda citriodora*. This may be because the levels applied were sufficient to warrant a good response. Maximum fresh and dry herb yields as well as oil yield were occurred at 30-50 kg of applied N which equal to application rate of approximately 80-120 kg N ha−1. These results are consistent with the reports on lemon balm[24] and the reports on origanum[25].

In another work it was concluded that nitrogen rates ranging from 50 to 150 kg ha-1 were optimum for thyme production; within this range, the specific rate will vary depending on soil fertility [9,12]. Highest biological yield and plant height of lemon balm were produced by application of 90 kg N ha-1 and highest tiller number, essential oil percentage and essential oil content were obtained under application of 60 kg N ha^{-1}.[26].

Effect of plant spacing:

Individual plant growth characteristics as affected by spacing between plants in both seasons are shown in Table (4). Plant height significantly affected by plant spacing especially in the first season where the tallest plants were at 30 cm apart while the shortest plants were at 20 cm apart.

Plants spaced 30 cm apart in the row had significantly higher plant fresh weight than those at other spacing. The trend was reflected also in the plant dry weight data, which shows that plants spaced 30 cm apart have greater dry weight/plant.

Widening spacing between plants from 20 to 25 cm or from 30 to 35 cm slightly affected the growth parameters in all cuts in both seasons and the differences in most cases did not reach the level of significance.

Table (4) Monarda growth characteristics as affected by plant spacing during two seasons

Plant spacing	Plant height (cm)			Fresh weight (g/plant)			Dry weight (g/plant)		
	1st cut	2nd cut	3rd cut	1st cut	2nd cut	3rd cut	1st cut	2nd cut	3rd cut
1st season									
S1	22.69 c	37.71 b	42.67 b	105.23 b	213.06 b	148.00 a	18.44 b	46.09 b	34.38 a
S2	28.53 b	43.19 a	44.67 ab	113.39 ab	252.78 b	153.33 a	20.06 ab	54.61 b	34.91 a
S3	33.04 a	41.32 ab	48.07 a	152.54 a	351.81 a	158.33 a	27.02 a	75.14 a	38.00 a
S4	27.16 b	40.63 ab	46.26 ab	123.47 ab	226.79 b	160.00 a	21.91 ab	49.10 b	35.15 a
2nd season									
S1	25.01 c	38.73 a	46.96 a	148.12 a	236.69 b	176.67 b	25.69 a	53.55 b	41.93 a
S2	26.46 bc	42.10 a	46.11 a	148.22 a	267.94 ab	157.67 b	25.76 a	59.13 ab	35.55 b
S3	32.89 a	44.76 a	47.10 a	151.39 a	311.51 a	206.67 a	27.02 a	72.29 a	45.91 a
S4	28.30 b	40.90 a	48.26 a	166.75 a	310.57 a	173.33 b	29.65 a	66.38 ab	35.59 b

Values within columns followed by the same letter are not significantly different at the $P \leq 0.05$ level.
S1= 20 cm between plants S2= 25 cm S3= 30 cm S4= 35 cm

This response was in agreement with the findings on spearmint in which fresh leaf weight, leaf to stem ratio and moisture content increased significantly with the decreasing inter row spacing. The value of fresh biomass and essential oil yield were significantly reduced with increase of inter row spacing from 30 cm to 60 cm. (17).

In contrast to our results, the highest plant height was achieved by the lowest spacing (15 cm) but our results may be explained by the fact that in close plant spacing, plants sense their neighbors to compete for light, so each plant is able to capture less light, which can reduce plant growth even plant height. For example, closely spaced plants can have thinner shoots and fewer branches and flowers than the same plants given more space [14].

The effect of spacing on growth and development is largely due to change in the interception of radiant energy. Narrow row spacing leads to higher interception and consequently to the greater growth of peppermint. As row spacing is reduced below a certain level, mutual shading and poor aeration may reduce the leaf-stem ratio, and consequently oil concentration, in the green herb[28].

Dry matter percentage (%) was constant across plant densities, but it was influenced by different cuts. It comprised the larger values at the third cut followed by the second and the first cuts (data not shown) and the differences in plant dry weight were mainly due to the differences in the plant fresh weight not to dry matter %.

Differences in plant spacing had no significant effect on oil percentage % or there is no clear trend was observed.

Plants at low density had greater fresh weight and of course more extensive branching, while plants at the high density had lower growth. On the hand, Low plant density resulted in greater fresh and dry herbage yield as well as oil yield while and significantly suppressed those at high plant density (Table 5).

At the higher density of 40000 plant/fed the plants compensated the low fresh weight of individual plants in lower density by producing a greater fresh herbage yield/fed and consequently greater dry herbage and oil yield /fed. The highest oil yield was recorded in plants at the high density which indicated that oil yield was mainly dependent on herb yield than oil content. Greater herbage or biomass yield produces higher oil yield irrespective of the oil content or percentage.

Table (5) Monarda yield as affected by plant spacing during two seasons

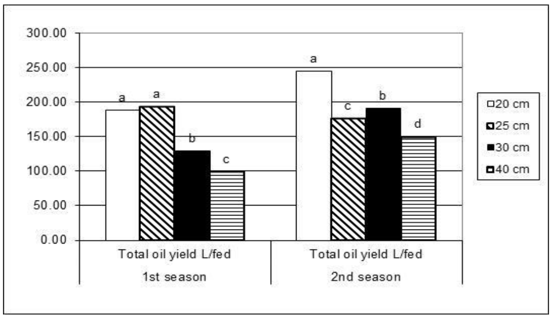
Plant spacing	Fresh herb yield (ton/fed)			Dry herb yield (ton/fed)			Oil yield (L/fed)		
	1st cut	2nd cut	3rd cut	1st cut	2nd cut	3rd cut	1st cut	2nd cut	3rd cut
1st season									
S1	4.21 a	8.52 b	5.92 a	0.74 a	1.84 b	1.38 a	8.72 a	80.99 a	98.66 a
S2	3.63 ab	8.09 b	4.91 b	0.64 ab	1.46 bc	1.12 b	11.33 a	72.20 a	82.47 a
S3	4.07 a	9.38 a	4.22 c	0.72 ab	2.40 a	1.01 b	13.45 a	86.29 a	53.56 b
S4	2.82 b	5.18 c	3.66 d	0.48 b	1.08 c	0.77 c	8.47 a	36.57 b	54.54 b
2nd season									
S1	5.93 a	9.47 a	7.07 a	1.03 a	2.14 a	1.68 a	20.53 a	106.33 a	118.03 a
S2	4.74 b	8.57 ab	5.05 b	0.82 ab	1.89 b	1.14 b	13.88 b	85.44 bc	76.89 b
S3	4.04 b	8.31 ab	5.51 b	0.72 b	1.93 b	1.22 b	12.83 b	93.23 b	85.33 b
S4	3.81 b	7.10 b	3.96 c	0.65 b	1.46 c	0.78 c	13.05 b	75.19 c	61.24 c

Values within columns followed by the same letter are not significantly different at the $P \leq 0.05$ level.
S1= 20 cm between plants S2= 25 cm S3= 30 cm S4= 35 cm

Previous studies of the relation of row and plant spacing to growth and yield of the labiateae family plants have yielded various results. Generally, closely spaced plants have yielded more fresh and dry herb yield per unit area where highest fresh and dry herb yield resulted from the highest population (15 cm spacing) and the lowest ones resulted from the lowest population (45 cm spacing). This is due to the increase of vegetative coverage of plants in high population that resulted in higher use of light [14].

Although the trend in the fresh herb yield with plant spacing was similar in the three cuts, the second cut recorded a higher fresh herb yields than the first cut of both seasons and declined in the third cut but still superior to the first cut. The higher yield in the second cut was a result of greater plant fresh weights. The second cut was generally superior to the first or the third cuts in all aspects of plant production.

The high oil yield in the third cut did not contribute to herb yield since there was a little herb in this cut. Data in Fig. 2 compared cumulative herb yield that could be obtained irrespective of cuts. Nevertheless, the plants spaced 30 cm apart in the row were significantly larger than those spaced 20 cm apart, it did not compensate the herb yield enough to counteract the deficiency in density. Plants spaced 20 cm apart gave an estimated fresh herb yield of 18.65 and 22.46 ton/fed in the first and second season respectively, which superseded by 5 and 26 % compared to plants spaced at 30 cm. In the same manner, plants spaced 20 cm apart gave an estimated dry herb yield of 4.85 ton/fed which superseded by 25 % compared to plants spaced at 30 cm in the second season.



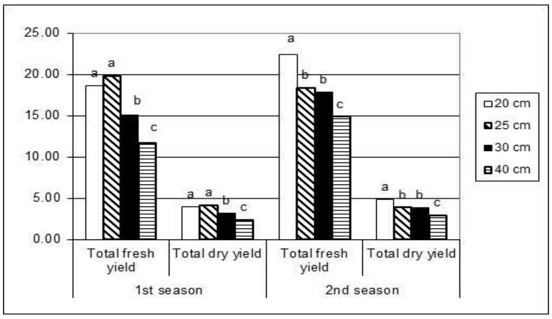


Fig 2: Cumulative Monarda yield as affected by plant spacing during two seasons

These results were in harmony with those obtained by a research conducted on *monarda citrodora* where the above-ground biomass per plant declined with each increase in density of 1 m^2 suggesting that increasing density increased the intensity of competition. The absolute decline in biomass per plant was particularly great as density was increased from low to medium densities (15–45 plants/ m^2). The proportion of incident light reaching the soil surface was lower at high and medium densities than at low density[29]. Based on another study was reported on thyme, the higher yield of thyme was achieved by dense cultivation and fertilization; however, the essential oil content was not influenced by either plant spacing or fertilization treatment [30].

Effect of nitrogen and plant spacing on chemical composition of the essential oil:

The hydrodistillation gave oil in 0.3-1.14 % (v/w; ml 100 g^{-1} fresh weight) yield. The chemical composition of the essential oils of the aerial parts of *Monarda citriodora* which analyzed by GC-MS is shown in Table 6 and 7. Totally, 17 constituents were identified in *Monarda citriodora* essential oil. Thymol was the dominating compound in the all analyzed oils (32.73 – 63.88 %), followed by carvacrol (6.54 - 29.56%), which constituted almost 80 % of the essential oil, followed by p-cymene (1.24 - 17.72 %), γ-terpinene (0.37-19.6%).

Table (6): Effect of nitrogen on chemical composition of essential oil of monarda

Compound	1st cut			2nd cut			3rd cut		
	N1	N2	N3	N1	N2	N3	N1	N2	N3
Thujene	0.94	0.07	0.05	1.56	3.12	3.02	3.81	1.42	3.51
α-Pinene	0.35	t	t	0.49	0.83	0.89	1.02	0.44	1.02
β-Myrcene	1.43	0.23	0.26	2.48	4.29	4.12	4.58	2.48	4.19
α-Phellandrene	0.14	0.02	0.05	0.54	0.98	0.98	1.03	0.45	0.75
α-Terpinene	0.18	0.17	0.4	7.79	7.51	7.22	8.07	7.13	5.03
p- cymene	1.30	1.24	2.93	10.19	14.15	12.38	14.14	13.49	17.72
D-Limonene	t	t	t	0.72	1.13	1.04	1.18	t	1.17
γ-Terpinene	1.37	0.37	1.34	19.9	19.6	18.13	17.33	11.96	11.22
Trans-Sabinene hydrate	0.68	0.85	1.03	0.48	0.98	1.04	1.37	1.18	1.22
4-Terpineol	0.32	0.43	0.95	0.79	0.97	0.85	0.84	0.69	0.72
Carvacrol methyl ether	0.92	1.47	1.87	0.16	0.29	0.15	0.07	t	0.22
Thymol	55.34	59.75	55.16	36.07	32.73	34.45	34.39	46.77	43.18
Carvacrol	20.65	29.56	26.8	10.1	8.36	10.22	7.66	8.67	6.85
Thymyl acetate	1.67	2.69	3.67	0.37	0.79	1.13	0.29	0.2	0.64
Carvacryl acetate	0.88	1.1	1.8	0.06	0.11	0.13	0.05	0.05	0.08
Caryophyllene	0.57	0.22	0.47	0.55	0.87	1	0.95	0.44	0.38
Germaene-D	t	t	t	0.09	0.75	0.81	0.83	t	t

N1 the low level = 33.5 kg N/fedden N2 the medium level = 50.25 kg N/fedden N3 the high level = 67 kg N/fedden

Nitrogen fertilization had no significant effect on chemical constituents of the essential oil of *Monarda citriodora* (Table 6). Similar findings were recorded by[31] who reported that oil content and quality of lemon grass were not influenced by spacing or nitrogen fertilization levels. Also, fertilization slightly affected or did not dramatically change the chemical composition of the essential oil of origanum [32].

In contrast of our results, cultivation without using N, P, and K fertilizer caused a slight decrease in carvacrol content against an increase of its main precursor p cymene. However, application of N, P, and K fertilizer increased the carvacrol content of 3.9 % for *T. maroccanus* and 9.4 % for *T. leptobotrys* oils compared with unfertilized plants[33].

Data in Table (7) showed that the differences in the chemical composition of the essential oils of monarda as affected by different plant spacing were not remarkable where essential oil composition was much influenced by different cuts. This may explained by the simple fact that essential oils are mainly genetically controlled or the enzymatic systems responsible for the biosyntheses of these compounds did not affect by the applied treatment [34].

Table (7): Effect of plant spacing on chemical composition of essential oil of monarda

Compound	1st cut				2nd cut				3rd cut			
	S1	S2	S3	S4	S1	S2	S3	S4	S1	S2	S3	S4
Thujene	0.51	0.53	0.56	0.78	1.92	2.2	2.22	2.16	2.4	3.25	3.5	3.15
α-Pinene	0.17	0.17	t	t	0.55	0.63	0.61	0.64	0.69	0.92	1.01	0.92
β-Myrcene	0.89	0.99	1.19	1.32	3	2.95	2.94	2.91	3.29	3.87	4.4	3.82
α-Phellandrene	0.22	0.25	t	t	0.64	0.73	0.73	0.66	0.7	0.83	0.94	0.79
α-Terpinene	2.01	2.11	2.26	1.23	4.71	5.31	5.71	4.69	5.24	5.73	6.67	5.34
p- cymene	5.94	5.76	6.9	6.0	11.09	10.09	10.09	10.91	12.54	13.72	16.08	14.65
D-Limonene	t	t	t	t	0.8	0.83	0.84	0.84	0.95	1.04	1.19	1.03
γ-Terpinene	5.15	6.57	6.29	4.91	11.15	11.23	13.79	9.37	10.41	12.56	14.3	10.1
Trans-Sabinene hydrate	0.55	0.37	0.6	0.22	0.79	0.54	0.73	0.57	1.01	0.82	0.8	0.77
4-Terpineol	0.48	0.35	t	0.52	0.85	0.65	0.63	0.63	0.74	0.78	1.1	0.84
Carvacrol methyl ether	1.54	0.93	0.93	1.54	0.2	0.1	0.1	0.1	0.22	t	t	t
Thymol	63.88	60.42	52.13	60.02	52.32	53.98	50.34	53.16	50.56	46.22	48.78	47.93
Carvacrol	18.81	17.98	22.3	13.92	7.74	6.54	6.85	9.02	8.17	7.44	7.19	8.13
Thymyl acetate	2.83	1.33	2.55	1.2	1.09	1.09	1.04	0.01	0.24	0.1	0.24	0.16
Carvacryl acetate	0.44	0.35	0.77	0.29	0.12	0.1	0.11	0.12	0.06	0.06	0.06	0.05
Caryophyllene	0.89	0.67	0.91	0.3	0.76	0.97	0.92	0.71	0.76	0.62	0.93	0.46
Germaene-D	0.04	0.3	0.28	t	0.29	0.52	0.8	0.31	0.13	0.13	0.11	0.07

S1= 20 cm between plants S2= 25 cm S3= 30 cm S4= 35 cm

Some variations in the amount of individual constituents in the different cuts were observed. Thymol concentration seems to decrease with the growing season. When the first cut is compared with the second and third cuts, thymol content reached their maximum values in the first cut (63.88 %) then decreased after wards in the second and the third cuts. The same pattern occurred with carvacrol from 22.30 % in the first cut up to 6-8% in the second and third cuts. The reduction in thymol and carvacrol contents in the second and third cuts had a corresponding increase in the contents of p–cymene and γ-terpinene which corresponding with previous studies on lemon verbena[35].

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