

Original article

Studies on the phytochemical traits and their correlation with quantitative characters of *Monarda citriodora* Cerv. ex Lag

S. R. Meena, F. A. Aga, S. Chandra*, R. Gochar*, B. Koli**, M. H. Khan*** and Shahid Rasool*
 Division of Agronomy, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir,
 Shalimar, Srinagar-190025, Jammu and Kashmir, India

*Genetic Resources and Agrotechnology Division, CSIR-Indian Institute of Integrative Medicine, Canal Road,
 Jammu-180001, Jammu and Kashmir, India

**Regional Fodder Station, Kalyani, N.S.S., Nadia-741251, West Bengal, India

***Department of Agriculture Kashmir, Lal Mandi, Srinagar-190018, Jammu and Kashmir, India

Received May 2, 2017; Revised May 30, 2017; Accepted June 5, 2017; Published online June 30, 2017

Abstract

Evaluation of phytochemical traits and their correlation studies were conducted with special reference to the quantitative characters of *Monarda citriodora* Cerv. ex Lag. A field experiment was carried out during two successive cropping seasons of 2014 and 2015 at Field Research Station, Chatha, CSIR - Indian Institute of Integrative Medicine, Jammu to study the effect of four irrigation frequencies (irrigation at 30 (F₁), 20 (F₂), 15 (F₃) and 12 (F₄) days interval with four nitrogen levels, viz., N₀ (0), N₁ (40), N₂ (80) and N₃ (120) kg N ha⁻¹ on quantitative characters and phytochemical traits in *M. citriodora*. The experiment was laid out in a split plot design with 3 replications. Results showed that plant height, number of branches per plant⁻¹, leaf area index, dry matter partitioning (leaf g/plant) and (stem g/plant) were significantly higher with treatment F₃ over F₂ and F₁, though remained at par with F₄ (12 days interval) during 2014 as well as 2015. F₁ treatment took significantly least number of days for initiation of primary and secondary branches, however, days taken to flowering at initiation, 50% and 75% was significantly least in F₄ treatment over other treatments. Thymol content was significantly higher with treatment F₂ over other treatments, while significantly highest carvacrol content was recorded with treatment F₃, though it remained at par with F₂ treatment. Nitrogen level N₂ significantly increased plant height, number of branches per plant⁻¹ and leaf area index, while in case of dry matter partitioning, N₂ and N₁ remaining at par recorded significantly higher dry matter partitioning over N₁ and N₀. Thymol content was significantly higher with N₁ level, while as carvacrol content was recorded significantly higher with N₂ level when compared to other treatments during the two years of study. In case of irrigation frequencies, it was observed that with increase in the quantitative characteristics of *M. citriodora*, the phytochemical traits, viz., thymol and carvacrol contents also showed the increased trend and visa-versa, while in case of nitrogen treatments, the increase in the quantitative characteristics showed the decrease in phytochemical traits.

Key words: *Monarda citriodora* Cerv. ex Lag, irrigation, nitrogen, growth, essential oil content

1. Introduction

Approximately 80% of the world population has their faith on natural products of plant kingdom for their secured health and prophylactic healing. Khalil *et al.* (2007) reported that though developing countries have their fashion to allelopathy for immediate cure, in this millennium, irrespective of developing / developed status, the global nations focus their vision on naturopathy for sustained relief without repercussion from health disorders. Jammu and Kashmir state has great potential of establishing and exploiting pharmaceutical and essential oil industry based on high value plants species like Lavender (*Lavandula officinalis*), Rose (*Rosa*

damascena), Clarysage (*Salvia sclarea*), Peppermint (*Mentha piperita*), Rosemary (*Rosmarinus officinalis*), Artemisia (*Artemisia annua*) and Monarda (*M. citriodora*). Among medicinal plants, horse mint (*M. citriodora*), commonly known as lemon bee-balm and lemon-mint is widely used as flavouring and garnishing agent in salads, soft drinks and as medicinal herb. Tea made from leaves can treat colds, coughs, fevers and respiratory problems (Garret and Odena, 2001). Its essential oil is used as insect repellent and in perfumery. Pathania *et al.* (2013) have reported anticancer activity of essential oil's of *M. citriodora* in human promycocytic leukemia.

Essential oil composition and yield of *M. citriodora* depends upon many factors and it is difficult to segregate these factors from each other, since many are interdependent and influence one another (Terblenche, 2000). These variables may include seasonal and maturity variations, geographical origin, genetic variation, growth stages, part of plant utilized, post harvest drying and storage. However, genetic make up of the plant is one of the most important contributors to their essential oil composition (Graven *et al.*, 1990).

Author for correspondence: Dr. Shahid Rasool
 Agronomist, Genetic Resources and Agrotechnology Division, CSIR-
 Indian Institute of Integrative Medicine, Canal Road, Jammu-180001,
 Jammu and Kashmir, India

E-mail: msrasool@iiim.ac.in

Tel.: +91-9469801947

Copyright © 2017 Ukaaz Publications. All rights reserved.

Email: ukaaz@yahoo.com; **Website:** www.ukaazpublications.com

Because of the presence of most volatile oil's and pharmaceutical metabolites like thymol and carvacrol, *M. citriodora* has attracted the interest of several research groups during past years and has left a room to improve the yield of *M. citriodora* quantitatively and qualitatively by varietal development and agronomical manipulations like proper irrigation and nutrient management which has given a fillip in its production scenario. Irrigation is a basic necessity for sustaining high production in areas prone to water deficit especially when the rainfall is seasonal, unassured and low. It influences soil microbial activity, mineral nutrition, germination, morphology and maturation of plants. During the winter season, less water is required at early stage of crop, while at later crop growth stages, water requirement increases due to rapid increase in evapo-transpiration demand. Optimum irrigation levels would help in enhancing both the seed and oil yield of *M. citriodora* apart from higher water use efficiency. Further, plant nutrition is a key input to increase the productivity of crops. Out of several nutrients provided to plants, nitrogen is a major and limiting nutrient for better plant growth and yield. It is considered as most important nutrient for the crop to activate the metabolic activity and transformation of energy, chlorophyll and protein synthesis. In view of this, the present study was envisaged during 2014 and 2015 to investigate effect of irrigation frequencies and nitrogen levels on the phytochemical traits and their correlation with quantitative characters of *M. citriodora*.

2. Materials and Methods

2.1 Experimental site

A field experiment was conducted at Research Station, Indian Institute of Integrative Medicines, Chatha, Jammu which is situated at 32° 40' N latitude and 74° 58' E longitude with an altitude of 332 m above mean sea level (msl). Climatically, the experimental area has sub-tropical climate endowed with hot and dry summers, followed by hot and humid monsoon season. The annual rainfall of the location varied from 1050 to 1115 mm of which 75 per cent is received from June to September and 25 per cent during rest of the months. Data recorded for temperature, relative humidity and rainfall during the experimental period are presented in Figure 1.

2.2 Plant material and nursery raising

Seed material of *M. citriodora* cultivar (IIM (J) MC-2) were taken from the germplasm Bank of IIM-CSIR, Jammu. Raised nursery beds were prepared at a height of 15 cm with 1m width and 10 m length. Recommended package of practices were followed for raising the nursery. *M. citriodora* seeds were mixed with fine sand in the ratio of 1:10 and thoroughly spread out on the prepared beds and seeds were covered with a fine layer of sand and farm yard manure. The beds were covered with paddy straw to avoid displacement of seeds. 45 days old seedlings were uprooted after watering the bed on the day of transplanting in the experimental field.

2.3 Experimental design

The experiment was laid in a split plot design with 4 irrigation frequencies, viz., F₁ (30 days interval), F₂ (20 days interval), F₃ (15 days interval) and F₄ (12 days interval) in main plot and 4 nitrogen levels N₀ (0), N₁ (40), N₂ (80) and N₃ (120) kg N ha⁻¹ as sub-plot treatments. A recommended uniform basal dose of phosphorus and potassium was also applied at the time of final preparation of the

field for planting of seedlings. Each treatment was replicated three times. The plants were kept free from weeds and other intercultural operations/plant protection measures were done as per the recommended package of practices.

2.4 Growth analysis

Five plants were selected randomly from each treatment for recording of data on plant height, number of branched per plant⁻¹ and leaf area index. For dry matter partitioning, three plants were selected (small, medium and large) and harvested which represented each plot. Leaves and stems were separated from each other and fresh weight taken separately. The samples were first air dried for two days and finally dried in an electric oven at 70°C till a constant weight was achieved. The weight was recorded and expressed as average dry matter partitioning per plant. Observations pertaining to crop phenology like days-to-initiation of primary and secondary branches and days -to-flowering were also recorded by observing in each plot from transplanting.

2.5 Phytochemical constituents

2.5.1 Oil extraction

Extract essential oil content from the fresh aerial parts of *M. citriodora* (500 gm) were subjected to hydro-distillation for 4 h in Clevenger type apparatus (Clevenger, 1928). The resulted oil was dehydrated over anhydrous sodium sulfate and stored in glass vials at freezer in the absence of light till used for gas liquid chromatography (GLC) analysis. Three replicates were prepared.

2.5.2 GC/MS analysis

GC-MS analysis was carried out on a Varian GC-MS 4000 fitted with a Varian Factor Four Vf-5 ms fused silica capillary column (30m X 0.25 mm id, film thickness 0.25µm). Temperature programming of oven was from 50°C to 240°C at 5°C/min rising rate. Helium was used as carrier gas at flow rate of 1ml/min. Mass spectra were recorded over 50-300 amu range at one scan per second with E.I. at 70 eV. The volatile constituents were identified by calculation of their retention indices under temperature programmed conditions for n-alkanes (C8-C20). Identification of individual compounds was made by comparison of their mass spectra with those of the internal reference mass spectra library (Wiley/NIST) or with authentic compounds and confirmed by comparison of their retention indices with authentic compounds or with those of reported in literature database

2.6 Statistical analysis

The data obtained in respect of various observations were statistically analyzed by the method described by Cochran and Cox (1957). The significance of "F" and "t" was tested at 5 per cent level of significance. The critical difference was determined when "F" test was significant.

3. Results and Discussion

3.1 Quantitative characters

Plant height which is an important index of general growth performance of the plant was found to be significantly affected by the irrigation intervals. Irrigation frequencies at 15 days interval (F₃) at par with 12 days interval (F₄) showed significant increase over 20 and 30 days interval during both the years of experimentation. Similar to plant height, number of branches per

plant¹ and leaf area index were also significantly higher under F₃ (irrigation frequencies at 15 days interval) treatment over all other treatments during 2014 as well as 2015 (Table 1). The increase in plant height and number of branches per plant¹ under frequent irrigations might be due to the increase in the cell enlargement and less leaf senescence, resulting from higher turgor pressure (Shao *et al.*, 2008). Similar results were reported by Omidbaigi *et al.* (2003) and Moeini Alishah *et al.* (2006). The increase in the leaf area index under irrigation frequencies of 15 days interval might be due to the fact that availability of sufficient moisture around the root zone, more intake of water takes place which causes an increase in the cell enlargement due to more turgor pressure. Dry matter partitioning (leaf and stem) was also significantly affected by irrigation frequencies. Dry matter partitioning (leaf and stem) recorded at F₃ (15 days interval) treatment was significantly higher over other treatments during both the years of study. Increase in dry weight of the plant might be due to an increase in plant growth, photosynthesis and canopy structure during the frequent irrigations. The trend of results in the present investigation agreed with those obtained by Leithy *et al.* (2006) on rosemary plant, Bettaieb *et al.* (2012) on cumin plant and El-Tahir *et al.* (2011), El-Mekawy (2013) on *Achillea santolina* L. Moreover, in present study, it was noticed that delay in irrigation intervals, *i.e.*, F₁ treatment (30 days irrigation intervals) recorded lowest mean values of all the above parameters. This decrease in the mean values might be due to the fact that increase in the irrigation intervals causes the water stress to the plant which reduces growth due to reduction in photosynthesis by low CO₂ availability, due to reduced stomata and mesophyll conductance. Similar results were reported by Omidbaigi *et al.* (2003) and Moeini Alishah *et al.* (2006). It is evident from the data (Table 2) that the treatment F₄ (12 days interval) took significantly least number of days for initiation of primary branches and secondary branches and with regard to the days-to-flowering. It was noticed that F₁ treatment took significantly least number of days-to-flower, while F₃ treatment recorded maximum number of days-to-flower. The results are in line with that of Khan *et al.* (2005) who reported that the plants under any kind of stress conditions, *i.e.*, long irrigation intervals tends to shortened their life span and tried to hasten completion of life cycle which causes the lowest days-to-flowering. These results are in accordance with the results obtained by Hassan and Ali (2013) in *Coriandrum sativum* L.

The results of the present study indicated that the treatment N₃ (120 kg N ha⁻¹) and N₂ (80 kg N ha⁻¹) level recorded significantly higher plant height, number of branches per plant¹ and leaf area index during both the years of study (Table 1). Significant effect on the increase of plant height in *M. citrodora* with the application of nitrogen may be attributed to the fact that nitrogen being an essential constituent of plant tissue, favours rapid cell division and its enlargement, which together with the adequate quantity of phosphorus and potassium helps in the rapid cell division and better development of the cell size. Further, increased number of leaves increased the photosynthetic activities and the resultant growth might be the reason for getting significantly more number of branches per plant¹ with increasing level of fertilizers under study. Similar results were also obtained by Abedi *et al.* (2014) and Patel and Kushwaha (2013) in basil, Moosavi (2012) in chicory and Sotiropoulou and Karamanos (2010) in greek oregano. Nitrogen is

an essential constituent of proteins, enzymes and chlorophyll and has been observed to influence the leaf growth and its expansion, resulting in increased leaf area index. Dry matter partitioning (leaf and stem) obtained in the present study revealed that N₃ (120 kg N ha⁻¹) and N₂ (80 kg N ha⁻¹) at par with one another significantly increased dry matter partitioning (leaf and stem) over other treatments during both the years of experimentation (Table 1). The increase in dry matter partitioning (leaf and stem) with application of nitrogen fertilizer may be attributed to increase in plant height and leaf area index, resulting thereby in better light interception by crop which accumulated and translocated more photosynthates and, thus produced more dry matter. These findings corroborate with those of Ehsanipour *et al.* (2012) in fennel and Rosete *et al.* (2014) in thyme. With regard to the days-to-flowering (Table 2), the treatment N₀ (no nitrogen) recorded significantly least number of days-to-flower, while N₃ treatment (120 kg N ha⁻¹) recorded highest number of days-to-flower. This may be attributed to the fact that higher doses of nitrogen enhance the vegetative growth of the plant and, thus the flowering.

3.2 Phytochemical characters

The results of the study showed that the phytochemical constituents of *M. citrodora*, *viz.*, thymol and carvacrol were significantly affected by irrigation intervals and nitrogen levels. Data (Table 3) indicated that the treatment F₂ (20 days interval) exhibited significant increase in thymol content (74.72 %) over rest of the treatment during 2014, however, during 2015, F₂ (20 days interval) (73.57 %) and F₃ (15 days interval) (72.49 %) while remaining at par recorded significantly higher thymol content over F₁ (30 days interval) (69.20 %) and F₄ (12 days interval) (70.48 %). With regard to the carvacrol content, it was observed that the treatment F₃ (15 days interval) (5.85 %) at par with F₄ (12 days interval) (5.79 %) and F₂ (20 days interval) (5.72 %) recorded significantly higher carvacrol content over F₁ (30 days interval) (5.35 %) during 2014, however, during 2015, F₃ (15 days interval) (6.15 %) and F₂ (20 days interval) (5.93 %) at par with one another produced significantly higher carvacrol content than F₄ (12 days interval) (5.58 %) and F₁ (30 days interval) (5.34 %). Irrigation regimes are known on the plant metabolism and subsequently on the production of the secondary metabolites which are dependent on the environmental conditions (Dudai, 2005). Therefore, it is not surprising that irrigation regimes induce a range of effects on essential oil composition under various experimentation conditions. The increase in oil content in long irrigation interval (stressed conditions) suggests that the apparent increase in essential oil concentration resulted from reduced leaf sizes and low leaf and stem water content. Such phenomena could lead to a reduction in fresh mass, the denominator in calculating percentage oil content. Further, the increase in the essential oil content under stressed conditions, can also be attributed to the effect of various environmental factors like temperate, humidity, sunshine, rainfall, soil mineral, soil pH, *etc.*, since *M. citrodora* grew in summer months under high temperatures and received more solar energy than those grown in the spring summers. These conditions accelerate the

transformation of starch to the essential oil compounds (Omer *et al.*, 1994). Moreover, this could also be attributed to high water loss from the large herbage growth of the plants grown under more frequent irrigation, accompanied by the high minimum and maximum

temperatures during the water withholding period. Similar results were also reported by Khalid (2006), Panrong *et al.* (2006), Khalil and Abdel-Kader (2011), Akhani *et al.* (2012), Aloghareh *et al.* (2013) and Mohamed *et al.* (2014).

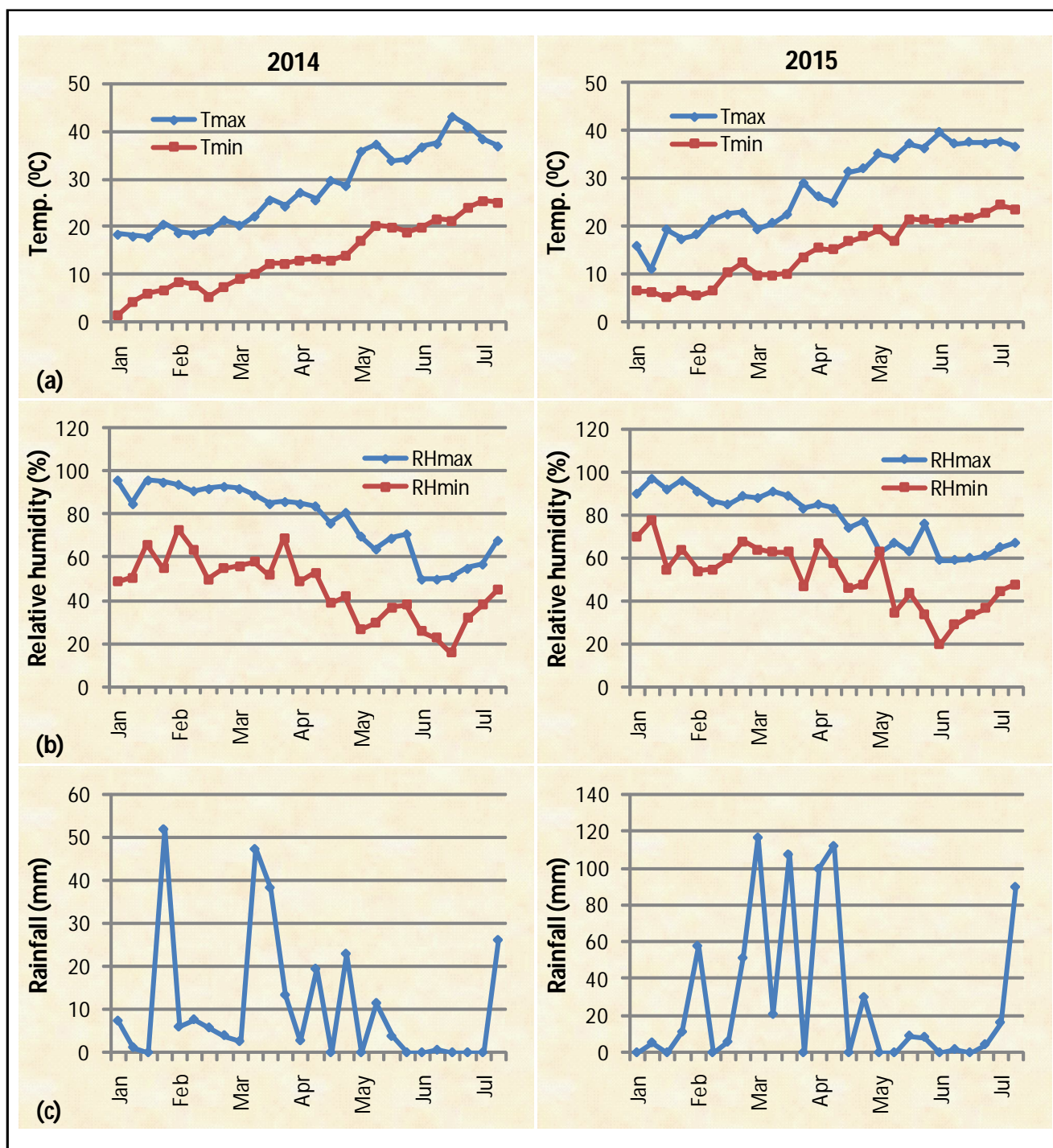


Figure 1: (a) The weekly minimum (T_{min}) and maximum (T_{max}) air temperatures (b) average relative humidity and (c) average weekly rainfall of the experimental field during 2014 and 2015.

Table 1: Effect of irrigation intervals and nitrogen levels on quantitative characteristics of *M. citriodora*

Treatments	Initiation of branches				Days taken to flowering						
	Primary branches		Secondary branches		Floral initiation		50% flowering		75% flowering		
	2014	2015	2014	2015	2014	2015	2014	2015	2014	2015	
Irrigation intervals											
30 days interval (F ₁)	74.88	73.62	62.53	70.70	2.40	2.21	28.96	30.83	35.63	39.92	
20 days interval (F ₂)	79.72	76.83	80.41	83.16	2.70	2.37	32.25	40.17	38.25	46.17	
15 days interval (F ₃)	85.82	79.76	90.64	89.79	2.93	3.02	35.38	43.00	40.13	49.00	
12 days interval (F ₄)	77.31	75.43	73.26	76.84	2.72	2.62	30.63	36.58	36.63	42.58	
$\pm SE_{\bar{X}}$	2.09	1.23	1.91	1.97	0.08	0.12	0.90	1.42	0.99	1.37	
CD ($p \leq 0.05$)	6.39	3.75	5.83	6.03	0.24	0.38	2.74	4.33	3.02	4.18	
Nitrogen levels											
0 kgNha ⁻¹ (N ₀)	76.31	74.19	60.01	68.99	2.46	2.09	26.50	32.67	33.17	38.67	
40 kgNha ⁻¹ (N ₁)	78.60	75.42	69.51	74.77	2.54	2.58	30.92	36.25	36.92	44.42	
80 kgNha ⁻¹ (N ₂)	84.64	79.40	95.13	92.30	2.96	2.94	35.75	42.67	40.75	49.42	
120 kgNha ⁻¹ (N ₃)	81.00	77.93	82.19	84.43	2.80	2.62	34.04	39.00	39.79	45.17	
$\pm SE_{\bar{X}}$	1.65	1.18	2.44	1.57	0.13	0.10	0.99	1.27	0.91	1.41	
CD ($p \leq 0.05$)	4.80	3.45	7.12	4.56	0.37	0.28	2.87	3.69	2.66	4.11	

Table 2: Effect of irrigation intervals on initiation of branches and days taken to flowering of *M. citriodora*

Treatments	Initiation of branches				Days taken to flowering						
	Primary branches		Secondary branches		Floral initiation		50% flowering		75% flowering		
	2014	2015	2014	2015	2014	2015	2014	2015	2014	2015	
Irrigation intervals											
30 days interval (F ₁)	14.00	13.75	66.67	64.67	76.25	82.42	86.50	91.48	96.33	99.12	
20 days interval (F ₂)	13.83	13.58	64.08	62.33	77.92	84.42	88.92	93.00	98.50	102.00	
15 days interval (F ₃)	12.67	12.50	62.75	60.75	78.25	85.00	89.58	94.58	99.17	102.48	
12 days interval (F ₄)	11.50	11.33	61.17	60.08	81.33	90.25	91.08	96.24	100.75	102.64	
$\pm SE_{\bar{X}}$	0.25	0.26	0.73	0.71	0.42	1.07	0.72	0.88	0.46	0.70	
CD ($p \leq 0.05$)	0.76	0.79	2.26	2.19	1.29	3.28	2.21	2.68	1.40	2.13	
Nitrogen levels											
0 kgNha ⁻¹ (N ₀)	14.07	13.67	67.84	64.92	76.17	81.50	87.75	90.25	97.67	98.95	
40 kgNha ⁻¹ (N ₁)	13.71	13.48	64.25	63.17	78.42	85.67	88.50	93.58	97.92	101.64	
80 kgNha ⁻¹ (N ₂)	12.58	12.38	61.83	60.33	78.92	86.75	89.83	94.78	98.92	102.22	
120 kgNha ⁻¹ (N ₃)	11.33	11.25	60.58	59.42	80.25	88.17	90.00	96.70	100.25	103.45	
$\pm SE_{\bar{X}}$	0.33	0.38	0.91	0.50	0.70	1.25	0.51	0.83	0.58	0.82	
CD ($p \leq 0.05$)	0.99	1.13	2.71	1.50	2.03	3.64	1.49	2.42	1.69	2.38	

Table 3: Effect of irrigation intervals on phytochemical traits of *M. citriodora*

Treatments	Thymol content (%)		Carvacrol content (%)	
	2014	2015	2014	2015
Irrigation intervals				
30 days interval (F ₁)	69.08	69.20	5.35	5.34
20 days interval (F ₂)	74.72	73.57	5.72	5.93
15 days interval (F ₃)	71.35	72.49	5.85	6.15
12 days interval (F ₄)	68.33	70.48	5.79	5.58
±SE \bar{x}	0.81	0.95	0.06	0.18
CD ($p \leq 0.05$)	2.48	2.91	0.17	0.56
Nitrogen levels				
0 kgNha ⁻¹ (N ₀)	70.42	70.61	5.02	5.30
40 kgNha ⁻¹ (N ₁)	73.70	74.59	6.05	5.99
80 kgNha ⁻¹ (N ₂)	71.12	71.08	6.80	6.63
120 kgNha ⁻¹ (N ₃)	68.23	69.48	4.83	5.09
±SE \bar{x}	0.67	0.77	0.10	0.26
CD ($p \leq 0.05$)	1.95	2.24	0.29	0.75

Nitrogen applied at an increased rate can also modify the chemical composition of essential oil (Sharma and Kumar, 2012). It is evident from the Table 3 that nitrogen level N₁ (40 kg N ha⁻¹) significantly increased thymol content, though it remained at par with N₀ (0 kg N ha⁻¹) during the two years of investigation. Similarly, with regard to the carvacrol content, application of nitrogen at the rate of 80 kg ha⁻¹ (N₂) significantly increased the carvacrol content, though it remained at par with nitrogen level of 40 kg ha⁻¹ (N₁) over other treatments. These results may be attributed to the fact that nitrogen being the essential mineral is used by plants to build many organic compounds, viz., amino acids, proteins, enzymes and nucleic acids. Amino acids and enzymes play a key role in the biosynthesis of numerous compounds which are essential oil constituents (Jabbari *et al.*, 2011; Zheljzakov *et al.*, 2011). These findings agree with those of Zheljzakov *et al.* (2008) who have reported that maximum basil oil yield was obtained when higher rates of nitrogen per hectare was applied. In addition, nitrogen significantly modifies the percentage of linalool, eugenol, bornyl acetate, and eucalyptol in basil essential oil. Higher nitrogen application increases the concentration of methyl chavicol and decreases the proportion of linalool in basil volatile oil. The concentration of linalool and eugenol was highest when the lowest rate of nitrogen was applied whereas the percentage of eucalyptol and bornyl acetate was highest at the higher rate (Nurzynska-Wierdak and Borowski, 2011). The oil of plants fed with the medium rate of nitrogen was marked by the highest concentration of linalool, *trans*-geraniol and linalyl acetate (Sharma and Kumar, 2012). These results are in close agreement with the present findings. Furthermore, the composition and yield of fennel essential oil was not modified by nitrogen rate (Chatzopoulou *et al.*, 2006). Environmental factors especially non-edaphic factors also play an important role in the synthesis of

essential oils, since *M. citriodora* grew in summer months under high temperatures and received more solar energy than those grown in the spring summers. These conditions accelerate the transformation of terpinene and p-cymene to phenolic compounds. These findings agree with those of Omer *et al.* (1994) on marjoram. Piccaglia *et al.* (1993) also reported that during two year study, differences in relative amounts of thymol, carvacrol, χ -terpinene, and p-cymene essential oils of *Satureja montana* were observed which could be attributed to the effects of environmental conditions. The above differences may arise from the genetic conditions of the biosynthesis of secondary metabolites as well as from environmental factors. With regard to the irrigation frequencies (20 and 15 days interval), it was observed that with increase in the quantitative characteristics of *M. citriodora*, viz., plant height, number of branches per plant⁻¹, leaf area index and dry matter partitioning, the phytochemical traits, viz., thymol and carvacrol contents also showed the increased trend and *visa-versa*. However, the increase in the quantitative characteristics of *M. citriodora* at nitrogen levels of 80 and 120 kg N ha⁻¹, the phytochemical traits, viz., thymol and carvacrol contents showed the decreasing trend.

4. Conclusion

According to the results obtained in the present study, improvement in the growth characters can be obtained by irrigating the crop at 15 days interval (F₃) with application of 80 kg N ha⁻¹ (N₂) and for improvement in essential quality characters, viz., thymol and carvacrol contents. Irrigation at 20 days interval (F₂) along with application of 40 kg N ha⁻¹ (N₁) were found to be appreciable. However, before giving final recommendations, the investigation needs to be carried out at different agroclimatic regions to arrive at final conclusions.

Conflict of interest

We declare that we have no conflict of interest.

References

- Abedi, M.H.; Seghatoleslami, M.J. and Mousavi, S.G.R. (2014). Effects of irrigation intervals and nitrogen fertilizer levels on vegetative and reproductive yields of basil (*Ocimum basilicum* L.) under Birjand conditions. *Agroecology*, 5(4):502.
- Akhani, A.; Darzi, M.T. and Hadi, M.H.S. (2012). Effects of biofertilizer and plant density on yield components and seed yield of coriander (*Coriandrum sativum*). *Int. J. Agri. Crop Sci.*, 4(16):1205-1211.
- Aloghareh, R.R.; Tahmasebi, B.K.; Safari, A.; Armand, R. and Odavi, A.G. (2013). Changes in essential oil content and yield components of anise (*Pimpinella anisum* L.) under different irrigation regimes. *Int. J. Agri. Crop. Sci.*, 6(7):364-369.
- Bettaieb, I.R.; Jabri-roul, I.; Hamrouni-Sellami, I.; Bourgou, S.; Limam, F. and Murzouk, B. (2012). Effect of drought on the biochemical composition and antioxidant activities of cumin (*Cuminum cyminum* L.) seeds. *Ind. Crop Prod.*, 36(1): 238-245.
- Chatzopoulou, P.S.; Koutsos, T.V. and Katsiotis, S.T. (2006). Study of nitrogen fertilization rate on fennel cultivars for essential oil yield and composition. *J. Veg. Sci.*, 12(2):85-93.
- Clevenger, J.H. (1928). Apparatus for the determination of volatile oil. *J. Am. Pharm. Assoc.*, 17:346.
- Cocharan, W.G. and Cox, G.M. (1957). *Experimental Designs*. 2nd Ed. Johan Wiley and Sons Inc., New York.
- Dudai, N. (2005). Factors affecting content and composition of essential oils in aromatic plants. In: Dris R (Ed.), *Crops Growth, Quality and Biotechnology*. Part III: Quality Management of Food Crops for Processing Technology. WFL Publisher, Helsinki, Finland, pp:77-90.
- Ehsanipour, A.; Razzmjoo, J. and Zeinali, H. (2012). Effect of nitrogen rates on yield and quality of fennel (*Foeniculum vulgare* Mill.) accessions. *Industrial Crops and Products*, 35(1):121-125.
- El-Mekawy, M.A.M. (2013). Response of *Achillea santolina* L. to fertilizers under different irrigation intervals. *Asi. J. Crop Sci.*, 5:338-359.
- El-Tahir, B.; El-Hawary, A. and Yagoub, S.O. (2011). Effect of different irrigation intervals on wheat (*Triticum aestivum*) in semi arid regions of Sudan. *J. Sci. Tech.*, 12(3):75.
- Garret, H. and Odena, B. (2001). *Herbs of Texas*. Press ISBM 978-0-292-72830-1, pp:112-113.
- Graven, E.H.; Webber, L.; Venter, M. and Gardner, J.B. (1990). The development of *Artemisia afra* (Jacq.) as a new essential oil crop. *J. Essen. Oil Res.*, 2:215-220.
- Hassan, F.A.S. and Ali, E.F. (2013). Impact of different water regimes based on class-A pan on growth, yield and oil content of *Coriandrum sativum* L. plant. *J. The Saudi Soc. Agri. Sci.*, 13(2):155-161.
- Jabbari, R.; Dehaghi M.A.; Sanavi A.M.M. and Agahi K. (2011). Nitrogen and iron fertilization methods affecting essential oil and chemical composition of thyme (*Thymus vulgaris* L.) medical plant. *Adv. Environ. Biol.*, 5(2):433-438.
- Khalid, K.A. (2006). Influence of water stress on growth, essential oil and chemical composition of herbs (*Ocimum* sp.). *Int. Agrophys*, 20(4):289-296.
- Khalil, M.Y.; Moustafa, A.A. and Naguib, N.Y. (2007). Growth, phenolic compounds and antioxidant activity of some medicinal plants under organic farming condition. *World J. Agric. Sci.*, 3:451-457.
- Khalil, S.E. and Abdel-Kader, A.A.S. (2011). The influence of soil moisture stress on growth, water relation and fruit quality of *Hibiscus sabdariffa* L., grown within different soil types. *Nature and Science*, 9(4):62-74.
- Khan, M.H.; Chattha, T.H. and Saleem, N. (2005). Influence of different irrigation intervals on growth and yield of bell pepper (*Capsicum annum* Grossum group). *Res. J. Agri. Biol. Sci.*, 1:125-28.
- Moeyni Alishah, H.; Heidari, R.; Hassani, A. and Asadi Dizaji, A. (2006). Effect of water stress on some morphological and biochemical characteristics of purple basil (*Ocimum basilicum*), *J. Biol. Sci.*, 6(4):763-767.
- Mohamed, M.A.; Wahba, H.E.; Ibrahim, M.E. and Yousef, A.A. (2014). Effect of irrigation intervals on growth and chemical composition of some *Curcuma* spp. plants. *Nusantara Bioscience*, 6(2):140-145.
- Moosavi, S.G.R. (2012). Effects of irrigation and nitrogen (N) fertilization levels on yield, morphological traits and water use efficiency of chicory (*Cichorium intybus* L.). *J. Med. Plants Res.*, 6(31): 4647-4652.
- Nurzynska-Wierdak, R. and Borowski, B. (2011). Changes in the content and chemical composition of sweet basil essential oil under the influence of fertilization of plants with nitrogen and potassium. *Annales UMCS, Sec. DDD, Pharmacia*, 3(15):133-145.
- Omer, E.A.; Ouda, H.E. and Ahmed, S.S. (1994). Cultivation of sweet marjoram, *Majorana hortensis* in newly reclaimed lands of Egypt. *J. Herbs, Spices and Med. Plants*, 2(2):9.
- Omidbaigi, R.; Hassani, A. and Sefidkon, F. (2003). Essential oil content and composition of sweet basil (*Ocimum basilicum* L.) at different irrigation regimes. *J. Ess. Oil Bear. Plants*, 6(2):104-108.
- Panrong, C.; Chunyan, L. and Kebin, L. (2006). Aromatic constituents in fresh leaves of *Lingtou dancong* tea induced by drought stress. *J. South China Agri. Uni.*, 27:17-20.
- Patel, K. and Kushwaha, N.K. (2013). Studies on influence of species, nitrogen and spacing on parameters of plant growth at various stages of basil. *Int. J. of Pharm. and Life Sci.*, 4(10): 3028-3034.
- Pathania, A.S.; Guru, S.K.; Verma, M.K.; Sharma, C.; Abdullah, S.T.; Malik, F.; Chandra, S.; Kataoch, M. and Bhushan, S. (2013). Disruption of PI3K/AKT/mTOR signaling cascade and induction of apoptosis in HL-60 cells by an essential oil from *Monarda citriodora*. *Food Chem. Toxicol.*, 62:246-254.
- Piccaglia, R.; Marotti, M.; Giovanelli, E.; Deans, S.G. and Eaglesham, E. (1993). Antibacterial and antioxidant properties of Mediterranean aromatic plants. *Industrial Crops Prod.*, 2:7-50.
- Rosete, J.C.R.; Castillo, J.A. and Mendoza, R.M.N. (2014). Fertilizer source in biomass production and quality of essential oils of thyme (*Thymus vulgaris* L.). *Eur. J. Med. Plants*, 4(7):865-871.
- Shao, H.B.; Chu, L.Y.; Jaleel, C.A. and Zhao, C.X. (2008). Water-deficit stress-induced anatomical changes in higher plants. *C.R. Biologies*, 331: 215-225.
- Sharma, S. and Kumar, A. (2012). Effect of nitrogen on growth, biomass and oil composition of clarysage (*Salvia sclarea* Linn.) under mid hills of North Western Himalayas. *Ind. J. Nat. Products Res.*, 3(1): 79-83.
- Sotiropoulou, D.E. and Karamanos A.J. (2010). Field studies of nitrogen application on growth and yield of greek oregano (*Origanum vulgare* ssp. *hirtum* (Link) Ietswaart). *Ind. Crops and Products*, 32:450-457.
- Terblanche, F.C. (2000). The characterization, utilization and manufacture of products recovered from *Lippia scaberrima* Sond. PhD. thesis, Pretoria, University of Pretoria.
- Zheljzkov, V.D.; Cantrell, C.L.; Astatkie, T. and Cannon, J.B. (2011). Lemongrass productivity, oil content, and composition as a function of nitrogen, sulphur, and harvest time. *Agron. J.*, 103(3):805-812.
- Zheljzkov, V.D.; Cantrell, C.L.; Ebelhar, M.W.; Rowe, D.E. and Coker, C.H. (2008). Productivity, oil content, and oil composition of sweet basil as a function of nitrogen and sulphur fertilization. *Hort. Sci.*, 43(5): 1415-1422.