Indian Journal of Agricultural Sciences **92** (2): 245–8, February 2022/Article
https://doi.org/ 10.56093/ijas.v92i2.122230

Variation in yield and essential oil composition of *Mentha spicata* cultivars

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Received: 18 December 2020; Accepted: 25 August 2021

ABSTRACT

Mentha spicata L. is cultivated for its essential oil which has various industrial uses. There are several species of *Mentha* genus but *M. spicata* is the only one which is rich in carvone. The present study was carried out at Central Institute of Medicinal and Aromatic Plants (CIMAP), Research Centre, Pantnagar in 2020 to evaluate the yield potential and essential oil quality of five spearmint cultivars grown in sub-tropical condition of Uttarakhand, so that farmers could select, choose and cultivate the right cultivar to get maximum benefits. CIM-Mohak and MSS-5 showed significant higher yield (herb yield, essential oil yield) i.e. 124.10 and 116.20 q/ha and 68.88 and 70.58 kg/ha, respectively. The analysis of essential oils from five spearmint cultivars revealed that limonene was more in Neer kalka (35.1%) and carvone was more in Arka (64.35%) but cultivars CIM-Mohak and MSS-5 had almost same concentration of carvone and limonene. The study concludes that CIM-Mohak and MSS-5 can be grown for spearmint cultivation in sub-tropical conditions for higher returns.

Keywords: Carvone, Cultivars, Essential oil yield, Limonene, *Mentha spicata*

Mint is a prominent industrial crop grown for essential oil in northern Indian plains. It belongs to *Lamiaceae* family and includes about 25-30 species cultivated in moderate temperature in Eurasia, Australia and South Africa. Out of all, *M. arvensis, M. piperita, M. citrate* and *M. spicata* are the principal kinds which are grown at moderate temperature, Mediterranean region and hot & humid areas (Lawrence *et al.* 2007) because of yield and chemo-diversity and used in different industries (Mathella *et al.* 2005). *M. spicata* is the only one, were which is rich in carvone and has a characteristic odour. Traditionally, *Mentha* spp. are used for treatment of migraine, fever, stomach related issues and different diseases. The plant parts and oil are widely utilized in food, confectionary, chewing gums, toothpastes etc. (Jirovetz *et al.* 2002). The production and synthesis of essential oil in *M. spicata* is mainly controlled by different agro-climatic conditions.

India is probably the biggest producer of spearmint oil and produced around 325 MT in 2015. In correlation, the USA produces around 1,500 tonnes every year. As in other aromatic crops, the yield and essential oil configuration in mint species is impacted by interaction among genotypes, climate and season (Kofidis *et al.* 2004). Different analysts revealed that oil yield and its configuration in aromatic crops is primarily identified with their heredity (Shafie *et al.* 2009), climate, edaphic, height and topography (Ghasemi and Mohammadi 2013). Several experiments and studies have been reported on spearmint in warm climates but few have so far been mentioned or published from sub-temperate regions. The study was done to assess the production potential, essential oil yield and composition of promising cultivars of *M. spicata* in sub-tropical conditions.

MATERIALS AND METHODS

The experiment was conducted with spearmint cultivars 'CIM-Mohak', MSS-5, Arka, Neera and Neer Kalka in complete Randomized Block Design (RBD) at Central Institute of Medicinal and Aromatic Plants (CIMAP), Research Centre, Pantnagar in 2020 located at 29°3'N latitude, 79°31'E longitude and at an altitude of 243.8 m above mean sea level having sub-tropical climatic condition. The soil of the experimental site was clay-loam in texture with *p*H 7.8 and organic carbon 1.2%. The status of available N, P and K were 220, 39 and 118 kg/ha, respectively. Nursery beds were prepared by adding 4 kg FYM/m^2 properly added in the soil and 200 g runners of each spearmint cultivars were sown in the last week of September 2019. To ensure proper germination and growth, light irrigations were applied as per the need. Afterwards, 95–105 days old runners of all spearmint cultivars were planted in the second week of

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January, 2020 at a spacing of 50 cm × 10 cm. After primary tillage operations in the main field, recommended uniform basal dose of 60 kg P_2O_5 and 40 kg K_2O/ha were applied through di-ammonium phosphate and murate of potash at the time of planting. Nitrogen in the form urea was applied in three equal splits (one-third at planting and remaining dose at 30 and 60 days after planting). Plots were irrigated just after planting for proper establishment of the crop. Later, total 6–7 irrigations were applied till harvesting. Two manual weedings were done at 30 and 60 days after planting and two hoeings were done at 45 and 75 days after planting. Data on plant height, canopy, internodal length, leaf length, leaf width, leaf/stem ratio, herb yield, oil content (%) and oil yield were collected at crop maturity (110 days old crop).

Freshly harvested samples from all spearmint cultivars were hydro-distilled in a Clevenger apparatus for 3 h to extract essential oil. The oil quantity was calculated directly within the extract measuring device. Calculation of oil percentage was done as volume (ml) of oil per 100 g of plant sample. Isolated oil was dried over anhydrous Na_2SO_4 in sealed bottle and stored in cold condition in dark place for further investigation.

The essential oils were analysed using GC-FID and GC-MS techniques. Analysis of essential oil by GC-FID was carried on Thermo Fisher Trace GC-1300 with TG-5 fused silica capillary column (30 m × 0.25 mm; 0.25 µm) and flame ionization detector (FID). Nitrogen at flow rate of 1.0 ml/min was used as carrier gas. The oven temperature had initially risen from 60–230°C at 3°C/min. The temperature of injector and detector was maintained at 250°C and 280°C, respectively. The injection volume was 0.02 µl neat with a split ratio of 1:40. GC-MS was performed on Clarus 680 GC coupled with Clarus SQ 8C mass spectrometer of PerkinElmer equipped with Elite-5 fused silica capillary column (30 m × 0.25 mm × 0.25 µm). The oven column temperature program was 60–240°C at 3°C/min with initial and final hold time of 2 min. Helium was employed as carrier gas at 1.0 ml/min and the sample was split using a ratio of 1:30. The injector and detector temperature was 250°C. Different compounds were ionised using an ionisation potential of 70 eV. Most of the compounds were distinguished by looking at their mass spectra (MS) data with literature data (Adams 2007). OPSTAT statistical software (Sheoran *et al.* 1998) was used for statistical analysis at 0.05% probability.

RESULTS AND DISCUSSION

Growth characters: The data on morphological characters, herbage yield and oil yield of all spearmint cultivars are given in Table 1. It represents the average values of plant height, canopy, internodal length, leaf length, leaf width and leaf/stem ratio. Results demonstrated that plant height differed significantly between spearmint cultivars except Neera (83.3 cm) and MSS-5 (77.0 cm). Height of plants varied from 52.0–100.5 cm. The maximum plant height was recorded in CIM-Mohak (100.50 cm) cultivar and minimum in the Neer Kalka (52.00 cm) cultivar (Table 1). Variations in plant height in spearmint cultivars might be due to change in geographical location or inherent genetic variations (Desai *et al.* 2018). Data on canopy diameter also varied in different cultivars. The canopy diameter varied from 37.70–63.70 cm^2. Among the diverse spearmint cultivars, the utmost canopy was recorded in Neera cultivar (63.7 cm^2) and minimum was recorded in Neer Kalka (37.7 cm^2). The cultivars CIM-Mohak (49.5 cm^2), MSS-5 (55.0 cm^2) and Arka (49.0 cm^2) were statistically at par with each other in canopy diameter (Table 1). Internodal length in spearmint cultivars varied from 1.5–3.7 cm. The interndoal length of MSS-5 (3.7 cm) and Arka (3.5 cm) were statistically at par with each other but significantly higher than CIM-Mohak (3.0 cm), Neera (3.0 cm) and Neer Kalka (1.5 cm). Leaf length in all spearmint cultivars varied from 3.3 cm to 4.0 cm, and there was no significant difference in leaf length of all cultivars (Table 1). Data of leaf width presented in Table 1, showed that leaf width varied between 0.5 cm and 1.8 cm. Maximum leaf width was found in CIM-Mohak (1.8 cm) which was significantly higher than leaf width of all cultivars and minimum leaf width was recorded in Neera cultivar (0.5 cm). Leaf/stem ratio varied from 1.19 to 1.50. The maximum leaf/stem ratio was recorded in CIM-Mohak (1.50) and least in Neer Kalka (1.19). Variations in agronomic parameters of plant might be due to the congenial weather conditions and soil which play major role in increasing all growth parameters as reported by Desai *et al.* (2018) and Nilofer *et al.* (2015). It is need of the hour to evaluate the full potential of species

Table 1 Variability in growth and yield of *M. spicata* cultivars

Variety	Growth parameters						Yield parameters		
	Plant height (cm)	Canopy (cm^2)	Internodal length (cm)	Leaf length (cm)	Leaf width (cm)	L:S ratio	Herb yield (q/ha)	Essential oil content (%)	Essential oil yield (Kg/ha)
CIM-Mohak	100.50	49.50	3.00	3.70	1.80	1.50	124.10	0.61	68.88
MSS-5	77.00	55.00	3.70	3.50	1.50	1.32	116.20	0.66	70.58
Arka	65.00	49.00	3.50	3.50	1.60	1.27	72.30	0.83	54.70
Neera	83.30	63.70	3.00	3.30	0.50	1.42	75.80	0.63	43.43
Neer Kalka	52.00	37.70	1.50	4.00	1.50	1.19	46.30	0.88	37.43
CD at 5%	7.76	6.38	0.48	NS	0.16	0.17	12.60	0.05	6.13

and cultivars with consideration of genetic diversity to judge the full performance of cultivars among the population.

Yield parameters: Data on yield attributes are presented in Table 1. Data showed that the herb yield varied from 46.30 to 124.10 q/ha among the cultivars of *M. spicata*. The maximum herb yield was recorded in CIM-Mohak (124.10 q/ha) and the minimum in Neer Kalka (46.30 q/ha). Cultivars CIM-Mohak (124.10 q/ha) and MSS-5 (116.20 q/ ha) recorded significantly higher herb yield compared to other cultivars of *M. spicata* i.e. Neera (75.80 q/ha), Arka (72.30 q/ha) and Neer Kalka (46.30 q/ha). Herb yield of CIM-Mohak, MSS-5 and Arka, Neera were statistically at par with each other. Essential oil content varied from 0.61 to 0.88% (Table 1). Highest essential oil content was noted in Neer Kalka (0.88%) and least in CIM-Mohak (0.61%). In context to essential oil content, cultivars Arka and Neer Kalka were statistically at par with each other, similarly cultivars CIM-Mohak, MSS-5 and Neera were statistically at par with each other. Essential oil yield varied among the *M. spicata* cultivars when grown in sub-tropical condition of Uttarakhand. It showed that essential oil yield varied between 37.43 to 70.58 kg/ha among the different cultivars of *M. spicata*. The maximum essential oil yield was found in MSS-5 (70.58 kg/ ha) which was statistically at par with CIM-Mohak (68.88 kg/ ha) and both cultivars showed significantly higher essential oil yield than other remaining three cultivars (Table 1). Essential oil yield of Arka (54.70 kg/ ha) cultivar was significantly higher than Neera (43.43 kg/ha) and Neer Kalka (37.43 kg/ha). These variations in yield parameters among *M. spicata* cultivars might be due to cyclic and prime variations, geographical origin, hereditary variation, development stages, portion of plant utilized as previously mentioned by several researchers such as Desai *et al.* (2018), Inan *et al.* (2011) and Barakat *et al.* (2013) in *T. spicata* var. *spicata*. Variation in essential oil content in *Thymus vulgaris* was due to better growing conditions in different temperatures and elevation as reported by Pirabalouti *et al.* (2013). Desai *et al.* (2018) reported that if the geographical location or genetic variation may cause variations in growth parameters and yield.

Chemical composition: The essential oil obtained from spearmint cultivars samples was analysed by GC-MS technique. Altogether, in five cultivars of *M. spicata*, 29 compounds were identified, out of these, eight compounds represented 74.5 to 89.7% composition of total essential oil (Table 2). Carvone and Limonene were noted as marker compounds in essential oil of five cultivars of M. *spicata*. The maximum accumulation of Limonene was recorded in Neer Kalka (35.1%) followed by CIM-Mohak (26.35%) and Neera (23.35%) and least accumulation of Limonene was detected in MSS-5 (16.65%). Similarly, Carvone varied from 51.4–64.35% in *M. spicata* cultivars, the maximum carvone accumulation was recorded in Arka (64.35%) cultivar followed by CIM-Mohak (57.9%) and least in Neer Kalka (51.4%). Inan *et al.* (2011) and Barakat *et al.* (2013) reported that seasonal, geographical, genetic variation and growth stages have significant effect on essential oil yield and distribution of volatile compounds in *Thymus spicata*. Padalia *et al.* (2014) reported that the chemical composition of essential oil in Mint spp. shows much chemical diversity because of the cultivar nature, as various chemocultivars exist together within climatic and geographical variations. Padalia *et al.* (2013) reported carvone and limonene as marker compounds present in all mentioned *M. spicata* cultivars (Table 2) in Tarai region of Uttarakhand.

Table 2 Essential oil composition of *M. spicata* cultivars

Compound	CIM-Mohak	MSS 5	Arka	Neera	Neer Kalka
α-Pinene	0.80	1.00	1.05	1.15	0.85
Sabinene	0.35	0.75	0.20	1.55	0.30
β-Pinene	0.85	1.15	0.85	4.30	0.95
Limonene	26.35	16.65	20.00	23.35	35.10
1,8-Cineole	0.10	1.50	0.10	3.15	0.10
Carvone	57.9	51.55	64.35	51.95	51.40
β-Caryophyllene	1.10	0.90	0.70	2.30	0.85
Germacrene-D	0.20	1.00	0.75	0.40	0.15
Total	87.65	74.50	88.00	88.15	89.70

Hence, the study concludes that crop development, yield and essential oil composition in spearmint cultivars vary with geographical location and genetic factors. Herb and essential oil yield in CIM-Mohak and MSS-5 were significantly higher compared to other cultivars under sub-tropical condition of Uttarakhand. So, CIM-Mohak and MSS-5 can be used as potential source of genetic material not only for horizontal expansion in area for spearmint cultivation, but also for bridging the gap between industrial demand and essential oil supply, and increasing farm income and livelihood security of farmers.

ACKNOWLEDGEMENT

The authors are grateful to CSIR for providing the financial support.

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