

Original Research Article

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Influence of Row Spacing and Nitrogen Levels on Biochemical and Quality Parameters of Japanese Mint (*Mentha arvensis* L.)

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ABSTRACT

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Field study was conducted during 2015 to investigate the influence of row spacing (30 cm, 45 cm and 60 cm) and nitrogen levels (50,100,150 and 200 kg/ha) on biochemical and quality parameters of Japanese mint (*Mentha arvensis* L.). The result revealed that at 90 days after planting, the row spacing of 60 cm recorded the highest total chlorophyll (3.09 mg/g of tissue) and carotenoid content (2.73 mg/g of tissue). Among the nitrogen level of 150 kg per ha recorded highest total chlorophyll (2.89 mg/g of tissue) and carotenoid content (2.71 mg/g of tissue). There was no significant effect of row spacing and nitrogen levels on menthol content. However, row spacing of 60 cm and application of 150 kg N per ha recorded maximum menthol content (64.57% and 64.58 % respectively).

Introduction

Japanese mint is one of the important essential oil bearing crop in northern semi-arid and subtropical region of India. Menthol mint is a potential source of natural menthol and other constituents like mint terpenes, menthone, isomenthone, methyl acetate, etc.

Japanese mint oil has a characteristic strong minty odour. It is used in prescriptions for cold remedies, cough drops, dentifrices, mouth washes; in scenting cigarettes; flavoring tobacco, chewing pan (rolled betel leaf containing menthol, lime paste, betel nut

and other products), bakery products and in cosmetic products. In aromatherapy, the essential oil is prized for its cooling effect on the skin; for pain relieving properties; for treating digestive problems, migraine, heartburn, aching feet, travel sickness, sinus and catarrh problems.

Materials and Methods

An investigation was carried out to study the effect of row spacing and nitrogen levels on growth and yield of Japanese mint variety

Kosi at the Department of Plantation, Spices, Medicinal and Aromatic Crops, Kittur Rani Channamma College of Horticulture, Arabhavi, University of Horticultural Sciences, Bagalkot during 2015. The experiment was laid out in split plot design with twelve treatments and three replications, considering spacing as main plot and nitrogen levels as sub plot.

Japanese mint is propagated through stolons of uniform thickness and standard size of 3.0 to 4.0 cm in length was separated from the underground part of the plant.

Phosphorous and potassium fertilizers at the rate of 50 and 40 kg per ha in the form of single super phosphate and muriate of potash respectively were uniformly applied to all the plots as basal dose. The crop was harvested 100 days after planting.

Estimation of chlorophyll and carotenoid content

Total chlorophyll, chlorophyll a and chlorophyll b content were determined by following DMSO method at 45 and 90 days after planting (DAP). Third fully expanded leaf from the top was brought in polyethylene bags from the field and was cut in to small pieces; known weight of leaves was kept in test tube containing 7.0 ml of dimethyl sulphoxide (DMSO).

The test tube was incubated at 65°C for 30 minutes, later leaf residue was removed by decanting the solution and final volume was made upto 10ml with DMSO. The absorbance of the extract was measured at 645, 652 and 663 nm in a UV- Vis Spectrophotometer (Elico, SL – 159) and a blank was run using DMSO. The total chlorophyll, chlorophyll a and chlorophyll b contents were calculated by using the following formula and expressed in mg per gram fresh weight.

Calculation

Calculate the amount of chlorophyll present in the extract mg chlorophyll per gram tissue using the equations given by Sadasivam and Manicham⁹.

$$\text{mg chlorophyll a / g tissue} = 12.7 (A663) - 2.69 (A645) \times \frac{v}{1000 \times w \times a}$$

$$\text{mg chlorophyll b / g tissue} = 22.9 (A645) - 4.68 (A663) \times \frac{v}{1000 \times w \times a}$$

$$\text{mg total chlorophyll / g tissue} = 20.2 (A645) + 8.02 (A663) \times \frac{v}{1000 \times w \times a}$$

Where

A = absorbance at specific wavelengths,

V = final volume of chlorophyll extracted in 80% acetone

W = fresh weight of tissue extracted

a = path length

Carotenoid (mg/g) can be calculated using the formula given by Bajracharya².

$$\text{Carotenoid} = 4.69(A440) - 0.268(20.2(A645) + 8.02(A663))$$

Quality parameters

Menthol content

Menthol content was estimated by using gas chromatography and expressed in percentage (%).

Results and Discussion

Among the row spacing at 45 DAP, the row spacing of 60 cm (S₃) recorded the highest chlorophyll – a content (1.83 mg/g of tissue) which was found to be on par with 45 cm (1.63 mg/100 mg of tissue).

Table.1 Effect of row spacing and nitrogen levels on chlorophyll “a” content (mg per gram fresh weight) in Japanese mint (*Mentha arvensis* L.)

Treatments	Chlorophyll “a” content									
	45 DAP					90 DAP				
	N ₁	N ₂	N ₃	N ₄	Mean	N ₁	N ₂	N ₃	N ₄	Mean
S ₁	1.34	1.43	1.51	1.48	1.44	1.44	1.99	2.27	1.97	1.91
S ₂	1.58	1.62	1.68	1.67	1.63	1.74	1.83	1.93	1.85	1.83
S ₃	1.73	1.79	1.98	1.82	1.83	2.19	2.33	2.44	2.32	2.32
Mean	1.55	1.61	1.72	1.65		1.79	2.05	2.21	2.04	
For comparison of mean										
	S.Em ±			CD @ 5 %		S.Em ±			CD @ 5 %	
Row spacing(S)	0.057			0.224		0.056			0.218	
Nitrogen (N)	0.035			0.104		0.052			0.154	
S at same level of N	0.077			NS		0.095			0.287	
N at same or different level of S	0.060			NS		0.090			0.267	

Main plot treatments (S)

S₁: 30 cm

S₂: 45 cm

S₃: 60 cm

Sub plot treatments (N)

N₁: 50 kg/ha

N₂: 100 kg/ha

N₃: 150 kg/ha

N₄: 200 kg/ha

DAP: Days after planting

Table.2 Effect of row spacing and nitrogen levels on chlorophyll “b” content (mg per gram fresh weight) in Japanese mint (*Mentha arvensis* L.)

Treatments	Chlorophyll “b” content									
	45 DAP					90 DAP				
	N ₁	N ₂	N ₃	N ₄	Mean	N ₁	N ₂	N ₃	N ₄	Mean
S ₁	0.54	0.66	0.63	0.58	0.60	0.41	0.49	0.47	0.56	0.48
S ₂	0.63	0.66	0.75	0.67	0.67	0.71	0.73	0.73	0.77	0.73
S ₃	0.80	0.75	0.86	0.81	0.80	0.75	0.72	0.83	0.78	0.77
Mean	0.65	0.69	0.74	0.68		0.62	0.64	0.67	0.70	
For comparison of mean										
	S.Em ±			CD @ 5 %		S.Em ±			CD @ 5 %	
Row spacing(S)	0.031			0.121		0.020			0.078	
Nitrogen (N)	0.023			NS		0.023			NS	
S at same level of N	0.045			NS		0.039			NS	
N at same or different level of S	0.039			NS		0.039			NS	

Main plot treatments (S)

S₁: 30 cm

S₂: 45 cm

S₃: 60 cm

Sub plot treatments (N)

N₁: 50 kg/ha

N₂: 100 kg/ha

N₃: 150 kg/ha

N₄: 200 kg/ha

DAP: Days after planting

Table.3 Effect of row spacing and nitrogen levels on total chlorophyll content (mg per gram fresh weight) in Japanese mint (*Mentha arvensis* L.)

Treatments	Total chlorophyll content									
	45 DAP					90 DAP				
	N ₁	N ₂	N ₃	N ₄	Mean	N ₁	N ₂	N ₃	N ₄	Mean
S ₁	1.87	2.08	2.14	2.06	2.03	1.84	2.47	2.74	2.53	2.39
S ₂	2.21	2.27	2.43	2.34	2.31	2.44	2.56	2.66	2.62	2.57
S ₃	2.52	2.54	2.83	2.64	2.63	2.94	3.05	3.27	3.10	3.09
Mean	2.20	2.29	2.40	2.34		2.40	2.69	2.89	2.75	
For comparison of mean										
	S.Em ±			CD @ 5 %		S.Em ±			CD @ 5 %	
Row spacing(S)	0.088			0.344		0.049			0.193	
Nitrogen (N)	0.035			0.104		0.039			0.115	
S at same level of N	0.102			NS		0.075			0.227	
N at same or different level of S	0.060			NS		0.066			0.198	

Main plot treatments (S)

S₁: 30 cm
S₂: 45 cm
S₃: 60 cm

Sub plot treatments (N)

N₁: 50 kg/ha
N₂: 100 kg/ha
N₃: 150 kg/ha
N₄: 200 kg/ha

DAP: Days after planting

Table.4 Effect of row spacing and nitrogen levels on carotenoid content (mg per gram fresh weight) in Japanese mint (*Mentha arvensis* L.)

Treatments	Carotenoid content									
	45 DAP					90 DAP				
	N ₁	N ₂	N ₃	N ₄	Mean	N ₁	N ₂	N ₃	N ₄	Mean
S ₁	2.18	2.33	2.46	2.45	2.35	2.23	2.46	2.65	2.51	2.46
S ₂	2.43	2.53	2.69	2.61	2.56	2.40	2.57	2.66	2.61	2.56
S ₃	2.67	2.71	2.85	2.74	2.74	2.63	2.69	2.84	2.77	2.73
Mean	2.42	2.52	2.66	2.60		2.42	2.57	2.71	2.63	
For comparison of mean										
	S.Em ±			CD @ 5 %		S.Em ±			CD @ 5 %	
Row spacing(S)	0.025			0.097		0.036			0.139	
Nitrogen (N)	0.026			0.078		0.022			0.065	
S at same level of N	0.046			NS		0.048			NS	
N at same or different level of S	0.045			NS		0.037			NS	

Main plot treatments (S)

S₁: 30 cm
S₂: 45 cm
S₃: 60 cm

Sub plot treatments (N)

N₁: 50 kg/ha
N₂: 100 kg/ha
N₃: 150 kg/ha
N₄: 200 kg/ha

DAP: Days after planting

Table.5 Effect of row spacing and nitrogen levels on menthol content in Japanese mint (*Mentha arvensis* L.)

Menthol content (%)					
Treatments	N ₁	N ₂	N ₃	N ₄	Mean
S ₁	59.04	60.77	63.53	62.45	61.44
S ₂	60.44	61.05	64.14	63.50	62.28
S ₃	62.67	64.51	66.08	65.02	64.57
Mean	60.71	62.11	64.58	63.65	
For comparison of mean					
	S.Em ±		CD @ 5 %		
Row spacing(S)	1.109		NS		
Nitrogen (N)	1.300		NS		
S at same level of N	2.243		NS		
N at same or different level of S	2.252		NS		

Main plot treatments (S)

S₁: 30 cm
 S₂: 45 cm
 S₃: 60 cm

Sub plot treatments (N)

N₁: 50 kg/ha
 N₂: 100 kg/ha
 N₃: 150 kg/ha
 N₄: 200 kg/ha

The minimum chlorophyll – a content (1.44 mg/g of tissue) was noticed at 30 cm (S₁). At 90 DAP also, the row spacing of 60 cm (S₃) recorded the highest chlorophyll – a content (2.32 mg/g of tissue) and minimum chlorophyll – a content (1.83 mg/g of tissue) was noticed at 45cm (S₂) (Table 1).

Among the row spacing levels, the row spacing of 60 cm (S₃) recorded the highest (0.80 and 0.77 mg/100 mg of tissue) chlorophyll – b content and was found to be on par with 45 cm (0.67 and 0.73 mg/g of tissue). The minimum chlorophyll – b content (0.60 and 0.48 mg/g of tissue) was noticed in 30 cm (S₁) at 45 and 90 DAP, respectively (Table 2).

Row spacing had significantly influenced the chlorophyll and carotenoid content at 45 and 90 DAP. At 90 DAP, the higher chlorophyll (3.09 mg/g fresh weight of tissue) and carotenoid (2.73 mg/g fresh weight of tissue) content was observed at 60 cm row spacing. The least (2.39 and 2.46 mg/g fresh weight of tissue, respectively) was noticed at closer row spacing of 30 cm. This might be due to wider spacing which provides more penetration of light resulting to proper photosynthetic activity in the plants (Table 3 and 4).

Among the nitrogen level at 45 DAP, N₃ (150 kg N/ha) recorded the highest chlorophyll-a (1.72 mg/g of tissue) which was on par with N₄ (1.65 mg/g of tissue) and N₂ (1.61 mg/100 mg of tissue). The minimum chlorophyll –a content (1.55 mg/g of tissue) was noticed in N₁ (50 kg N/ha). Similar trend was noticed at 90 DAP. Application of nitrogen at 150 kg per ha (N₃) recorded the highest chlorophyll-a (2.21 mg/g of tissue) which was on par with N₂ (2.05 mg/g of tissue). While, the minimum (1.79 mg/g of tissue) chlorophyll –a content was noticed in N₁ (50 kg N/ha).

The effect of nitrogen level did not differ significantly for chlorophyll – b content at 45

and 90 DAP. However, maximum (0.74 mg/g of tissue) and minimum (0.65 mg/100 mg of tissue) chlorophyll-b content were observed in N₃ (150 kg N/ha) and N₁ (50 kg N/ha), respectively at 45 DAP. At 90 DAP, application of nitrogen at 200 kg per ha (N₄) recorded highest (0.70 mg/g of tissue) chlorophyll – b content and minimum (0.62 mg/g of tissue) was noticed in N₁ (50 kg N/ha).

Cholorophyll and carotenoid content in leaves differed significantly due to different levels of nitrogen at 45 and 90 DAP. At 90 DAP, the application of 150 Kg N per hectare (N₃) resulted in higher chlorophyll (2.89 mg/g fresh weight of tissue) and carotenoid (2.71 mg/g fresh weight of tissue) content. While the minimum (2.40 and 2.42 mg/g fresh weight of tissue) chlorophyll and carotenoid content was observed in plants supplied with 50 kg N per hectare (N₁). This might be due to increased nitrogen supply which would retard the leaf senescence and improve photosynthate. The results are in conformity with the results of Khanom, *et al.*, (2008); Angelinia *et al.*, (2015); Probir, *et al.*, (2015) in stevia.

Quality parameters

Menthol content (%)

No significant difference was noticed in menthol content of herb per plant due to row spacing. However, row spacing of 60 cm (S₃) recorded highest (64.57 %) menthol content (Table 5). This is due to better penetration of light which created congenial temperature regime favourable for accumulation of menthol in the leaves at wider row spacing. These results are in line with the findings of Duhan *et al.*, (1975) in *Mentha arvensis*.

Menthol content did not differ significantly due to nitrogen. However, treatment N₃ (150 kg N/ha) recorded the highest (64.58 %)

menthol content and the lowest (60.71%) was found in N₁ (50 kg N/ha). The results are in accordance with the findings of Muniram *et al.*, (1987) Kumar *et al.*, (2010) and Izhar *et al.*, (2015) in *Mentha arvensis*.

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