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Leaf and Essential oil yield response of Spearmint (*Mentha spicata* var. WG-SPM-Fran) under varying harvesting stages and row spacing

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The field experiment was conducted to determine the influence of harvesting stages and row spacing on leaf and essential oil yield response of spearmint var. WG-SPM-Fran at Wondo Genet and Hawassa during 2014/2015. The experiment comprised three levels of harvesting stages (flower bud initiation, 50% flowering and full blooming) and four levels of row spacing (30, 60, 90 and 120 cm) were used on a plot size of 3.6 cm length and 3.6 cm width. The treatments were arranged in a factorial Randomized Complete Block Design (RCBD) with three replications. SAS (version 9) software was used to compute the analysis of variance. The results revealed that the tested locations, treatments and interaction of both factors differed markedly on the response of leaf and oil yield response of spearmint var. WG-SPM-Fran. Of the two locations tested, location Hawassa showed the highest fresh leaf yield/row, dry leaf yield/row and essential oil yield/ha. In contrast, location Wondo Genet had greater percent essential oil content as compared to Hawassa. In case of treatment, the highest fresh leaf yield/row, dry leaf yield/row and fresh leaf yield/ha were obtained when it was planted at 120 cm row spacing and harvested at 50% flowering stage; whereas, the highest dry leaf yield and essential oil yield/ha were obtained when it was planted at 90 cm row spacing and harvested at full blooming stage. Moreover, the combined main effects showed that, the highest dry leaf yield/ha and essential oil yield/ha were obtained when it was planted at Wondo Genet at 90 cm row spacing and harvested at full blooming stage. Therefore, for greatest dry leaf and essential oil yield production it is better to plant this variety at Wondo Genet at 90 cm row spacing and harvested at full blooming stage.

Keywords: Mentha spicata var. WG-SPM-Fran, harvesting stages, row spacing, dry leaf yield, essential oil yield

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INTRODUCTION

The spearmint (*Mentha spicata)*, is a hybrid of *M. longifolia* and *M. rotundifolia*, morphological, cytological and biochemical data have shown that the tetraploid species of M. *spicata* 2n=48 (Lawrence, 2007). According to Gulluce *et al*. (2007) the essential oils of some Mentha species are potential candidates for exhibiting antimicrobial, antioxidant, antispasmodic, carminative, radical-scavenging and cytotoxic activities.

The fresh and dried plants and their essential oils are widely used in the food, cosmetic, confectionary, chewing gum, toothpaste, and pharmaceutical industries (Bensabah *et al*., 2013). The major compounds of *Mentha spicata* (L.) were carvone (59.40%), limonene (6.12%), germacrene-D (4.66%), β-caryophyllene (2.969 %), β-bourbonene (2.796 %), α-terpineol (1.986 %), Terpinene-4-ol (1.120 %) (Boukhebti *et al*., 2011). On the other studies the major constituents of the essential oil from the aerial of (*M. spicata*) were; carvone (42.74%), trans dihydrocarvone (21.58%), 1,8-cineole (8.41%), pulegone (6.83%), Limonene (6.1%) and β-Caryophyllene (3.05%) (Golparvar *et al*., 2015). The main compositions in Baghe-Bahadoran province were; carvone (54.34%), 1,8-cineole (21.78%), Linalool (5.82%), Limonene (5.2%) and trans dihydrocarvone (3.18%).

It is well known that yield and yield components of plants are determined by a series of factors including plant genetic (*Shafie et al*., 2009), climate, edaphic, elevation, topography and also an interaction of various factors (Rahimmalek *et al*., 2009). Among these factors crop management practices such as harvesting stage and row spacing are included. In Ethiopia, there is a limited finding regarding to this issue. Therefore, the objective of this study was to determine the influence of harvesting stages and row spacing on leaf and essential oil yield response of spearmint (*Mentha spicata* var. WG-SPM-Fran).

MATERIALS AND METHODS

The experiment was conducted at two locations such as Wondo Genet and Hawassa during 2014/2015. Their site descriptions were presented at table 1 below. This experiment consisted of three harvesting stages (flower bud initiation (FBI), 50% flowering (FPF) and full blooming (FB)) and four row spacing (30, 60, 90 and 120 cm) arranged in a factorial Randomized Complete Block Design (RCBD) with three replications. The size of each experimental unit was 3.6 cm length and 3.6 cm width. Respective spacing of 2 m and 1m was maintained between replication and plots. The spearmint variety WG-SPM-Fran which was used for this experiment was registered in 2014 by Wondo Genet Agricultural Research Center. Stolons were properly uprooted from plants grown at Wondo Genet Agricultural Research Center and cut in to 10 cm length to be used as a planting material. Then prepared stolens were planted in rows of a plot by covering the whole stolen with light soil.
During experimentation, all nursery and field horticultural practices were performed as required. Fertilizer 50 kg/ha P_2O_5 and 20 kg/ha N at the time of planting and additional 20 kg/ha N at 1 month after planting and after each harvest was applied properly.

Data collection

During the experiment data on fresh leaf weight/row (g), dry leaf weight/row (g), fresh leaf yield/ha (kg), dry leaf yield/ha (kg), essential oil content (%) and essential oil yield/ha (kg) from the randomly selected five samples from each experimental unit were measured and recorded critically for the different harvesting cycles at each harvesting stage.

Data analysis

Mean values of all data for all characters which were measured are subjected to analysis of variance (ANOVA) by using SAS (version 9) computer software programs (SAS inst., 2002). Least Significant Difference (LSD) was used to compare significant means at 5% probability level.

RESULT AND DISCUSSION

Fresh leaf yield per row (g)

Location had a significant ($p<0.05$) influence on fresh leaf yield/row (Table 2). The highest fresh leaf yield/row was obtained at Hawassa while the least value was obtained at Wondo Genet (Table 3). This indicated that fresh leaf yield/row was influenced by a change in environment.

Likewise, treatment has a very highly significant ($p<0.001$) influence on fresh leaf yield/row (Table 2). The highest fresh leaf yield/row was obtained when spearmint variety WG-SPM-Fran was planted at 120 cm row spacing and harvested at 50% flowering stage (Table 3). This could be due to the wider row spacing and optimum leaf production stage of the variety which reduces the competition within the crop and maximizes utilization of available soil moisture and nutrient which enables the variety to produce the highest fresh leaf yield/row. However, the interaction of location and treatment did not significantly ($p>0.05$) influence fresh leaf yield/row (Table 2). This might be due to the consistency of the values of fresh leaf yield/row when the location interacted with different treatments.

Dry leaf yield per row (g)

Location had a highly significant ($p<0.01$) influence on dry leaf yield/row (Table 2). The highest dry leaf yield/row was obtained at Hawassa while the least value was obtained at Wondo Genet (Table 3). This might be due to an increase in fresh leaf yield/row contributing to increase dry leaf yield/row.

Likewise, treatment has a very highly significant ($p<0.001$) influence on dry leaf yield/row (Table 2). The highest dry leaf yield/row was recorded when spearmint

Table 1. Summary of site descriptions for both locations in Ethiopia

Location	Latitude	Longitude	Soil pH	Soil type	Mean Annual Rainfall (mm)	Altitude (m.a.s.l)	Annual average temperature (°C)	
							Minimum	Maximum
Hawassa	7°05'N	39°29'E	7.2	Sandy loam (Andosol)	964	1700	12.94	27.34
Wondo Genet	7°19'N	38°38'E	6.4	Sandy clay loam (Nitosol)	1128	1780	11	26

Table 2. Analysis of variance for leaf and essential oil yield response of spearmint (*Mentha spicata* var. WG-SPM-Fran)

Statistics & parameters	Source of variance					R-Square	CV (%)
	Replication	Location (Loc)	Treatment (Trt)	Loc*Trt	Error		
DF	2	1	11	11	46	-	-
FLYR	3010978.36	4161942.55*	8022792.44***	658621.68ns	675911.9	0.77	16.59
DLYR	122670.69	798053.73**	1150084.37***	246550.69**	83603.14	0.81	18.14
FLYH	75546404	20408596ns	195815991***	31171396ns	16781030	0.78	17.42
DLYH	5023767.3	7933690.3ns	37426150.8***	16422817.0***	2203794.6	0.86	18.1
EOC	0.002	0.07***	0.006*	0.008**	0.003	0.63	9.89
EOYH	1500.53	14160.93***	3987.03***	1801.1**	610.25	0.74	22.4

Where, FLYR= Fresh leaf yield/row, DLYR= Dry leaf yield/row, FLYH= Fresh leaf yield/ha, DLYH= Dry leaf yield/ha, EOC= Essential oil content (%), EOYH= Essential oil yield/ha (kg), DF= Degree of freedom, CV= Coefficient of variance (%), *= significant at 0.05 probability level, **= significant at 0.01 probability level; ***= significant at 0.001 probability level.

variety WG-SPM-Fran was planted at 120 cm row spacing and harvested at 50% flowering, followed by planted at 120 and 90 cm row spacing and harvested at full blooming. In contrast, the least value was obtained at when it was planted at 30 cm row spacing and harvested at flower bud initiation stage (Table 4). This could be due to the wider row spacing and optimum leaf production stage of the variety which reduces the competition within the crop and maximizes utilization of available soil moisture and nutrient which enables the variety to produce the highest dry leaf yield/row. The interaction of location and treatment had a highly significant ($p<0.01$) influence on dry leaf yield/row (Table 2). The highest dry leaf yield/row was obtained when planted at Wondo Genet at 120 cm row spacing and harvested at full blooming; whereas, the least value was obtained when planted at Wondo Genet at 30 cm row spacing and harvested at flower bud initiation stage (Table 5). This could be due to the wider row spacing and optimum leaf production stage of the variety which reduces the competition within the crop and maximizes utilization of available soil moisture and nutrient which enables the variety to produce the highest dry leaf yield/row.

Table 3. Mean performance of locations on leaf and essential oil yield response of Spearmint (*Mentha spicata* var. WG-SPM-Fran)

Location	FLYR (g)	DLYR (g)	FLYH (kg)	DLYH (kg)	EOC (%)	EOYH (kg)
Hawassa	5195.3[a]	1699.56[a]	24052.3	7868.3	0.51[b]	124.32[a]
Wondo Genet	4714.5[b]	1488.99[b]	22987.5	8532.2	0.57[a]	96.27[b]
LSD@0.05	**390.06**	**137.18**	**1943.5ns**	**704.32ns**	**0.03**	**11.72**
CV (%)	**16.59**	**18.14**	**17.42**	**18.10**	**9.89**	**22.4**

Where, FLYR= Fresh leaf yield/row, DLYR= Dry leaf yield/row, FLYH= Fresh leaf yield/ha, DLYH= Dry leaf yield/ha, EOC= Essential oil content (%), EOYH= Essential oil yield/ha (kg), LSD= Least significant difference, CV= Coefficient of variance (%) and ns= not significant at $p<0.05$.

Table 4. Mean performance of treatments on leaf and essential oil yield response of Spearmint (*Mentha spicata* var. WG-SPM-Fran)

Treatments	FLYR (g)	DLYR (g)	FLYH (kg)	DLYH (kg)	EOC (%)	EOYH (kg)
30FBI	3349.4[f]	922.0[f]	14909[g]	4251.0[f]	0.52[bcd]	68.98[f]
30FPF	3785.9[f]	1092.1[ef]	17716[fg]	6081.9[de]	0.57[abc]	83.00[ef]
30FB	3724.5[f]	1172.2[def]	18223[efg]	6841.8[de]	0.57[ab]	92.91[def]
60FBI	4205.1[ef]	1285.4[cde]	19681[def]	6001.4[e]	0.53[abcd]	94.60[def]
60FPF	4204.1[ef]	1462.2[bcd]	19848[def]	7732.6[cd]	0.55[abc]	92.84[def]
60FB	4745.6[de]	1733.2[b]	24916[c]	10197.5[ab]	0.58[a]	134.09[abc]
90FBI	5241.8[cd]	1498.5[bcd]	23960[cd]	6786.4[de]	0.53[abcd]	106.24[cde]
90FPF	5046.1[cde]	1795.5[b]	22852[cde]	8703.1[bc]	0.57[ab]	112.58[bcd]
90FB	5978.3[bc]	2146.1[a]	30717[ab]	11892.3[a]	0.56[abc]	155.53[a]
120FBI	5736.1[bc]	1619.6[bc]	26072[bc]	7103.5[cde]	0.48[d]	110.79[cde]
120FPF	6938.9[a]	2205.0[a]	32394[a]	11448.0[a]	0.52[cd]	141.07[ab]
120FB	6502.7[ab]	2199.5[a]	30952[a]	11363.8[a]	0.51[bcd]	130.86[abc]
LSD@0.05	**955.44**	**336.03**	**4760.7**	**1725.2**	**0.06**	**28.71**
CV (%)	**16.59**	**18.14**	**17.42**	**18.10**	**9.89**	**22.4**

Where, the numerals in treatment represent row spacing in cm, FLYR= Fresh leaf yield/row, DLYR= Dry leaf yield/row, FLYH= Fresh leaf yield/ha, DLYH= Dry leaf yield/ha, EOC= Essential oil content (%), EOYH= Essential oil yield/ha (kg), LSD= Least significant difference and CV= Coefficient of variance (%). Means followed by the same letter with in the same column are not statistically significant a probability level of 0.05.

Fresh leaf yield per hectare (kg)

Fresh leaf yield/ha did not significantly ($p>0.05$) influenced by location. But, treatment had a very highly significant ($p<0.001$) influence on fresh leaf yield/ha (Table 2). This is supported by the finding of Beemnet *et al*. (2011). The highest fresh leaf yield/ha was obtained when spearmint variety WG-SPM-Fran was planted at 120 cm row spacing and harvested at 50% flowering, followed by planted at 120 cm row spacing and harvested at full blooming. In contrast, the least value was obtained when it was planted at 30 cm row spacing and harvested at flower bud initiation stage (Table 4). This could be due to the wider row spacing and optimum leaf production stage of the variety which reduces the competition within the crop and maximizes utilization of available soil moisture and nutrient which enables the variety to produce the highest fresh leaf yield/row.

However, the interaction of location and treatment did not significantly ($p>0.05$) influence fresh leaf yield/row (Table 2). This might be due to the consistency of the values of fresh leaf yield/row at the tested treatments when interacted with location.

Dry leaf yield per hectare (kg)

Dry leaf yield/ha did not significantly ($p>0.05$) influenced by location. But, treatment had a very highly significant ($p<0.001$) influence on dry leaf yield/ha (Table 2). This is supported by the finding of Beemnet *et al*. (2011).The highest dry leaf yield/ha was obtained when spearmint variety WG-SPM-Fran was planted at 120 cm row spacing and harvested at 50% flowering, followed by planted at 120 cm row spacing and harvested at full blooming. In contrast, the least value was obtained when it was planted at 30 cm row spacing and harvested at flower bud initiation stage (Table 4). As to fresh leaf yield/ha, the reason is true for dry leaf yield/ha.

The interaction of location and treatment has a very

Table 5. Interaction effect of Location with treatment on leaf and essential oil yield response of Spearmint (*Mentha spicata* var. WG-SPM-Fran)

Interaction	DLYR (g)	DLYH (kg)	EOC (%)	EOYH (kg)
Hw*30FBI	1151.1^{hij}	5329^{hij}	0.54^{bcdef}	94.48^{defghi}
Hw*30FPF	1299.6^{fghi}	6017^{ghi}	0.50^{fg}	89.68^{efghi}
Hw*30FB	1059.2^{ij}	4904^{ij}	0.51^{efg}	82.47^{ghi}
Hw*60FBI	1551.1^{efgh}	7181^{fghi}	0.56^{abcdef}	128.53^{abcde}
Hw*60FPF	1743.9^{cdefg}	8073^{efg}	0.49^{fg}	113.96^{bcdefg}
Hw*60FB	1551.1^{efgh}	7181^{fghi}	0.55^{abcdef}	123.68^{abcdef}
Hw*90FBI	1773.0^{cdef}	8208^{efg}	0.50^{fg}	131.34^{abcd}
Hw*90FPF	1984.4^{bcde}	9187^{def}	0.52^{defg}	141.30^{abc}
Hw*90FB	2107.6^{abc}	9757^{cde}	0.51^{efg}	149.24^{ab}
Hw*120FBI	1883.3^{cde}	8719^{def}	0.49^{fg}	150.06^{ab}
Hw*120FPF	2377.0^{ab}	11005^{bcd}	0.45^{g}	149.20^{ab}
Hw*120FB	1913.3^{bcde}	8858^{def}	0.48^{fg}	137.85^{abc}
WG*30FBI	692.9^{j}	3173^{j}	0.49^{fg}	43.48^{j}
WG*30FPF	884.5^{ij}	6147^{ghi}	0.63^{ab}	76.31^{ghij}
WG*30FB	1285.2^{ghi}	8780^{def}	0.64^{a}	103.36^{cdefgh}
WG*60FBI	1019.6^{ij}	4822^{ij}	0.50^{fg}	60.66^{ij}
WG*60FPF	1180.6^{hi}	7392^{efgf}	0.60^{abcd}	71.73^{hij}
WG*60FB	1915.3^{bcde}	13214^{ab}	0.62^{abc}	144.51^{ab}
WG*90FBI	1223.9^{hi}	5364^{hij}	0.56^{abcdef}	81.15^{ghij}
WG*90FPF	1606.7^{defgh}	8219^{efg}	0.63^{ab}	83.85^{fghij}
WG*90FB	2184.5^{abc}	14027^{a}	0.62^{abc}	161.82^{a}
WG*120FBI	1355.9^{fghi}	5488^{hij}	0.47^{fg}	71.51^{hij}
WG*120FPF	2032.9^{abcd}	11891^{abc}	0.59^{abcde}	132.95^{abcd}
WG*120FB	2485.8^{a}	13870^{a}	0.53^{cdefg}	123.86^{abcdef}
LSD@0.05	**475.21**	**2439.8**	**0.09**	**40.6**
CV (%)	**18.14**	**18.10**	**9.89**	**22.4**

Where, the numerals in treatment represent row spacing in cm, DLYR= Dry leaf yield/row, DLYH= Dry leaf yield/ha, EOC= Essential oil content (%), EOYH= Essential oil yield/ha (kg), Hw= Hawassa, WG= Wondo Genet, LSD= Least significant difference and CV= Coefficient of variance (%).Means followed by the same letter with in the same column are not statistically significant a probability level of 0.05.

highly significantly ($p<0.001$) influence on dry leaf yield/ha (Table 2). The highest dry leaf yield/ha was obtained when it was planted at Wondo Genet at 90 cm row spacing and harvested at full blooming followed by when it was planted at Wondo Gent at 120 cm row spacing and harvested at full blooming stage; whereas, the least value was obtained when it was planted at Wondo Genet at 30 cm row spacing and harvested at flower bud initiation stage (Table 5). This could be due to the wider row spacing and dalliance of harvesting stage enables to produce the highest dry matter accumulation thereby maximizes dry leaf yield/ha.

Essential oil content (%)

Location had a very highly significant ($p<0.001$) influence on Essential oil content (Table 2). The highest essential oil content was obtained at Wondo Genet; whereas, the least value was obtained at Hawassa (Table 3). This result is in line with the finding of Beemnet *et al*. (2014).

Treatment had a significant ($p<0.05$) influence on essential oil content (Table 2). The highest essential oil content was obtained when spearmint variety WG-SPM-Fran was planted at 60 cm row spacing and harvested at full blooming stage while the least value was obtained when it was planted at 120 cm row spacing and harvested at flower bud initiation stage (Table 4).

The interaction of location and treatment had a highly significant ($p<0.01$) influence on essential oil content (Table 2). The highest essential oil content was obtained when it was planted at Wondo Genet at 30 cm row spacing and harvested at full blooming stage; whereas, the least value was obtained when it was planted at Hawassa at 120 cm row spacing and harvested at flower bud initiation stage (Table 5).

Essential oil yield per hectare (kg)

Location had a very highly significant ($p<0.001$) influence on Essential oil yield/ha (Table 2). This result is in line with the finding of Beemnet *et al*. (2011; 2014). The highest essential oil yield/ha was obtained at Hawassa; whereas, the least value was obtained at Wondo Genet (Table 3).

Treatment had a very highly significant ($p<0.001$) influence on essential oil yield/ha (Table 2). The highest essential oil yield/ha was obtained when spearmint variety WG-SPM-Fran was planted at 90 cm row spacing and harvested at full blooming stage while the least value was obtained when it was planted at 30 cm row spacing and harvested at flower bud initiation stage (Table 4).

The interaction of location and treatment had a highly significantly ($p<0.01$) influence on essential oil content (Table 2). The highest essential oil content was obtained when the variety was planted at Wondo Genet at 90 cm row spacing and harvested at full blooming stage; whereas, the least value was obtained when it was planted at Wondo Genet at 30 cm row spacing and harvested at flower bud initiation stage (Table 5).

CONCLUSION

The result of this experiment revealed that, the main effect location had a significant influence on fresh leaf yield/row, dry leaf yield/row, percent essential oil content and essential oil yield; however, it did not significantly influence fresh and dry leaf yield/ha. Location Hawassa had showed a superior performance on fresh leaf yield/row, dry leaf yield/row and essential oil yield/ha as compared to Wondo Genet. The main effect treatment had a significant influence on all parameters which were studied. The highest fresh leaf yield/row, dry leaf yield/row and fresh leaf yield/ha were obtained when it was planted at 120 cm row spacing and harvested at 50% flowering stage; whereas the highest dry leaf yield/ha and essential oil yield were obtained when it was planted at 90 cm row stage. Moreover, the interaction effect of location and treatment exerted a significant influence on dry leaf yield/row, dry leaf yield/ha, percent essential oil content and essential oil yield/ha; however, it did not exerted a significant influence on fresh leaf yield per row and per hectare. The highest dry leaf yield/ha and essential oil yield/ha were obtained when it was planted at Wondo Genet at 90 cm row spacing and harvested at full blooming stage. Therefore, for the highest dry leaf and essential oil yield/ha production it is better to plant this variety at Wondo Genet at 90 cm row spacing and harvested at full blooming stage.

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