Genetics and Plant Physiology – 2015, Volume 5(3–4), pp. 286–297

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EFFECT OF DRIP IRRIGATION METHOD ON GROWTH AND ESSENTIAL OIL COMPOSITION OF FIELD GROWN PEPPERMINT (*Mentha piperita* L.) AND SPEARMINT (*Mentha viridis* L.)

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Received: *03 November 2014* Accepted: *13 October 2015*

Summary: This study was carried out during two successive seasons (2010 and 2011) to assess the effect of two drip irrigation systems (surface and subsurface) on the growth and essential oil of *Mentha viridis* (spearmint) and *Mentha piperita* (pepper mint). For subsurface drip irrigation, a narrow furrow of about 10 cm depth was made using a pointed hand-hoe, and the irrigation pipe was laid down in the furrow. Each irrigation opening in the pipe was covered with a small clay plate to prevent clogging of the opening by soil, and then the pipe was buried by filling the furrow with soil. The results indicated that the subsurface drip irrigation system had a pronounced effect on the growth characters (plant height, fresh and dry weight of herb) compared with the surface drip irrigation system. Generally, data revealed that the subsurface drip irrigation system increased significantly essential oil percentage and essential oil yield compared with the surface system. The major components of spearmint essential oil were found to be carvon, menthyl acetate, α-pinene, 1,8- cineol and limonene. Menthol, menthone, menthofuran and menthyl acetate were the main components in the essential oil of peppermint.

Citation: Hendawy S. F., G. M. Ghazal, A. EL-Gohary, M. S. Aly , M. S. Hussein, 2015. Effect of drip irrigation method on growth and essential oil composition of field grown peppermint (*Mentha piperita* L.) and spearmint (*Mentha viridis* L.). *Genetics and Plant Physiology*, 5(3–4): 286–297.

Keywords: Drip irrigation; *Mentha viridis*; *Mentha piperita*; Essential oil.

Abbreviations: GC – gass chromatography; CEC – cation-exchange capacity; meq – milliequivalents.

INTRODUCTION

The scarcity of water in arid and semi-arid regions has increased the search for technology with improved water use efficiency. Drip irrigation is widely used in processing crops cultivation in areas with dry and warm summers and high evapotranspiration rates throughout the growing season. Drip irrigation or trickle irrigation is an irrigation method which minimizes the use of water and fertilizer by allowing

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water to drip slowly to the roots of plants, either onto the soil surface or directly into the root zone, through a network of valves, pipes, tubing, and emitters. Drip irrigation is believed to increase the yield production of some plants due to the efficient water uptake by the root zone (Cetin and Bilgel, 2002). This system also provides more frequent, precise and direct application of water in small quantities to the root zone (Sushil et al., 2011) and can avoid water contamination.

Subsurface drip irrigation has evolved into an irrigation method with high potential for efficient and economical productivity and its use has progressed from being a novelty employed by researchers to an accepted method of irrigation for both perennial and annual crops (Ayars et al., 1999). It has been found that subsurface drip irrigation reduces evaporation from the soil and increases the wetted soil volume and surface area more than surface systems allowing a deeper rooting pattern (Oliveira et al., 1996).

There are several species of common mints in Egypt such as spearmint (*Mentha viridis* L.), Japanese mint (*Mentha arvensis* L.) and peppermint (*Mentha piperita* L.). Mint is a medicinal and aromatic perennial herb belonging to family Lamiaceae (Labiateae) which includes about 25 species (Pandy, 1982; Bown, 1995).

Peppermint (*M. piperita*) is a perennial highly aromatic plant native to Europe which may grow as tall as three feet. The ancient Egyptians, Greeks and Romans knew it as a flavoring for food and as a medicine. It was first cultivated in England commercially around 1750 while its aerial parts have been widely used for their medicinal effects. Peppermint is widely known to relieve digestive ailments, being a popular remedy for at least two centuries. *Mentha piperita L.* is believed to be a hybrid of spearmint (*Mentha spicata L.*) and water mint (*Mentha aquatic L.*) (Murray et al, 1972), belonging to family Labiatae (Lamiaceae). The essential oil is obtained from the fresh leaves of *Mentha piperita L.* by steam distillation and it is widely used all over the world for flavoring as well as for cosmetic and medicinal purposes. Spearmint (*Mentha viridis*) herb and its volatile oil are used as flavoring agents for many kinds of food products and beverages, carminative, mouth preparations, gargles, tooth pastes and pharmaceuticals.

Therefore, it was of interest to study how two drip irrigation methods could influence the growth and essential oil composition of both species.

MATERIALS AND METHODS

In order to test the response of *Mentha spp* to the method of water delivery by drip-irrigation, a field plot experiment using a randomized complete block design was established in Eladlya Farm, Sharkia Government. Two spp (*Mentha piperita* and *Mentha virides*) in separate blocks, two drip delivery techniques (surface and sub-surface) and four replications of each treatment were used in the experiment. The main goal was to test the effect of water delivery technique, via surface or subsurface drip, on water use efficiency as reflected by the growth and yield of plants. Mint cuttings with roots were planted on inter row spacing of 20 cm and intra row spacing of 10 cm in the field in May in two seasons (2010 and 2011). Control of water delivery to each row of crops was

Table 1. Soil mechanical and chemical characteristics.

Mechanical analysis	Value
Sand %	81.5
Silt %	7.4
Clay %	11.1
Texture	Sandy Clay Loam
Chemical analysis	
PH 1:2.5ext.	7.85
Electrical Conductivity 1:2.5ext	1.12
Organic Carbon %	1.23
Organic Matter %	2.30
Total Nitrogen %	0.130
Total Phosphorus %	0.0128
Total Potassium %	0.020
$CaCO_3$ %	3.47
Soluble cations [meq/L]	
Na^+	6.11
K^+	0.21
Ca^{++}	2.38
Mg^{++}	1.10
Soluble anions [meq/L]	
CO_3^{--}	0.00
HCO_3^-	5.45
Cl^-	1.48
SO_4^-	4.39
CECmeq/100g	18.47

achieved by means of individual plastic buckets with lids fitted with taps and drip-irrigation pipes. Mechanical and chemical analysis of soil is shown in Table 1, while electrical conductivity and pH of water irrigation are presented in Table 2.

Each experimental plot had eight rows with surface and sub-surface drip delivery randomly assigned. The water application was maintained at a low single rate of 8 liters every 48 h (alternate days) for all treatments. For subsurface drip

Table 2. Electrical conductivity and pH of water.

Source	EC ppm	pH
Well (1)	1227.5	7.9

irrigation, a narrow furrow of about 10 cm depth was made using a pointed hand-hoe, and the irrigation pipe was laid down in the furrow. Each irrigation opening in the pipe was covered with a small clay plate to prevent clogging of the opening by soil, and then the pipe was buried by filling the furrow with soil. A single plant of *Mentha piperata* and *Mentha viridis* was manually planted 6-8 cm to one side of the irrigation pipe adjacent to a water opening. Concerning fertilizers application, all treatments received 15m^3 of compost (Table 3) + 300 kg superphosphate during preparing the soil. Also, 100 kg of ammonium nitrate + 50 kg potassium sulphate/acre were added after 30 days from sowing. After every harvest 75 kg ammonium nitrate + 25 kg potassium sulphate/acre were added. Two harvests were carried out; the 1st harvest was on 20 July and the second one on 20 September. The following parameters were recorded: plant height (cm), branches number/plant, fresh and dry weight of herb.

Determination of essential oils

Essential oils were determined in fresh herb of *Mentha viridis* and *Mentha piperita* plants. Leaves were separated from shoots, and the essential oils were obtained by hydro distillation of 100 gm of dried powder in 750 ml distilled water in Clevenger's apparatus for 3h (European pharmacopoeia, 1975). The composition of the volatile constituents was established using GC. The isolated pure oils were stored in tightly closed vials at 4°C until analysis.

GC conditions:

- GC analysis were performed on HP 6890 GC.
- Detector: FID (flame Ionization Detector).
- Column: HP5 fused silica Column (Size: 1=60m, Ø=0.25mm, stationary phase: macrogol 20 000 R [film thickness 0.25μm]).
- Oven program: The oven temperature program was initiated

Table 3. Physical and chemical analysis of compost.

	Tests	Unit	Results	Soil and more standard
		Physical analysis		
1	Bulk density	kg/m^3	916	600 : 1000
2	Moisture content	%	27.9	25 : 30
		Chemical analysis		
3	Electrical conductivity	dS/m	5.1	(1:5) < 10
4	pH_w (1:5)		7.6	7 : 8
5	Total organic carbon	13.2	%	> 10
6	Total organic matter	%	22.7	> 17
7	Ash 60 : 80	%	77.3	60 : 80
8	Total nitrogen	%	0.60	0.5 : 1.0
9	C/N ratio	----	22.1	1 : 15
10	Total potassium	%	1.42	----
11	Total phosphorus	%	0.30	----

at 60°C, held for 1 min then raised up to 240°C at a rate of 10°C/min.
– Carrier gas: Nitrogen was used as a carrier gas.
– Flow rate: 1.0 ml/min.
– Injector temperature250°C.
– Detector temperature 275°C.
– Injection volume: 1µl of diluted oil in hexane solution (10%).
– Splate ratio1:50.

Statistical analysis

The layout of the experiment was a complete randomized block design with three replicates, each replicate containing 10 plants. Data were subjected to analysis of variance using Costat Statistical Software (1986). Means of all data were compared by LSD method according to Snedecor and Cochran (1968). The simple correlation coefficient was determined according to Gomez and Gomez (1984).

RESULTS AND DISCUSSION

Vegetative growth and yield

Data tabulated in Table 4 show that subsurface irrigation had a well pronounced effect on growth characters compared with surface irrigation. In this respect, subsurface drip (SDI) is a low-pressure, high efficiency irrigation system that uses buried drip tubes or drip tape to meet crop water needs. Subsurface irrigation saves water and improves yield by eliminating surface water evaporation and reducing the incidence of disease and weeds. A subsurface drip system may require higher initial investment and cost will vary due to water source, quality, and filtration need, choice of material, soil characteristics and degree of automation desired. SDI technologies have been part of irrigated agriculture since 1960, with the technology advancing rapidly in the last two decades. The SDI system is flexible and can provide frequent light irrigations. This is especially suitable for arid, semi-arid, hot, and windy areas with limited water supply. Farm operations also become free of impediments that normally exist above ground with any other pressurized irrigation system.

Concerning the growth characters, the highest mean values were obtained in spearmint compared with peppermint.

Total essential oil yield

Our data presented in Table 5 show the effect of *Mentha spp* and/or irrigation systems on essential oil content (%) and essential oil yield (ml/plant). The results showed that the highest mean values for essential oil content were 1.585% in the 1st cut and 1.695% in the 2nd one while oil yield was 0.96 ml/plant in the 1st cut and 1.045 ml/plant in the 2nd one for peppermint.

Data indicated that subsurface drip irrigation increased significantly essential oil content (%) and yield (ml/plant) except essential oil content (%) during the 2nd cut. The mean values for essential oil content were 1.393 and 1.475% as a result of subsurface irrigation system against 1.15 and 1.265% as a result of surface drip irrigation system during the 1st and 2nd cuts, respectively. On the other hand, the mean values of oil yield were 1.37 and 1.81 ml/plant for subsurface irrigation system against 0.92 and 1.28 ml/plant for surface irrigation system during the 1st and 2nd cuts, respectively.

Concerning the effect of interaction between *Mentha spp* and drip irrigation systems, it can be concluded that the

Table 4. Effect of drip irrigation method on the growth parameters and total yield content of peppermint and spearmint.

Mint	Irrigation	1st cut				2nd cut			
		Plant height [cm]	Branches No/plant	Fresh Wt. [g/plant]	Dry Wt. [g/plant]	Plant height [cm]	Branches No	Fresh Wt. [g/plant]	Dry Wt. [g/plant]
M. piperita	Subsurface	20.3	9.3	76.7	7.6	29.7	8.7	118.3	11.7
	Surface	15.7	4.3	71.7	7.1	17.3	7.3	100.0	9.2
Mean value of *M. piperita*		18.0	6.8	74.2	7.4	23.5	8.0	109.2	10.5
M. viridis	Subsurface	29.0	7.7	135	17.8	24.7	9.7	129.3	12.8
	Surface	13.3	6.3	93.3	9.2	18.7	7.0	101.7	9.9
Mean value of *M. viridis*		21.2	7.0	114.2	13.5	21.7	8.4	115.5	11.4
Mean value of:	Subsurface	24.7	8.5	105.9	12.7	27.2	9.2	123.8	12.3
	Surface	14.5	5.3	82.5	8.2	18.0	7.2	100.9	9.6
LSD at 5% for	Mint	2.6	1.9	18.7	5.3	3.1	NS	4.7	NS
	Irrig.	1.5	1.2	18.5	0.9	1.2	NS	NS	0.9
	Interaction	3.4	2.1	NS	6.1	4.3	NS	4.9	NS

NS=non significant. Data are mean values of two successive seasons.

Table 5. Effect of drip irrigation method on the essential oil yield collected from two species - *Mentha viridis* and *Mentha piperita.*

Mint	Irrigation	1st cut		2nd cut	
		Essential oil [%]	Essential oil yield [ml/plant]	Essential oil [%]	Essential oil yield [ml/plant]
M.piperita	Subsurface	1.75	1.34	1.83	2.16
	Surface	1.42	1.02	1.56	1.56
Mean value of *M. piperita*		1.585	1.18	1.695	1.86
M. viridis	Subsurface	1.04	1.40	1.12	1.12
	Surface	0.88	0.82	0.97	0.97
Mean value of *M. viridis*		0.96	1.11	1.045	1.22
Mean value of:	Subsurface	1.393	1.37	1.475	1.81
	Surface	1.15	0.92	1.265	1.28
LSD at 5% for:	Mint	0.18	NS	0.113	0.113
	Irrig.	0.12	0.11	NS	0.248
	Interaction	NS	0.007	NS	NS

NS= non significant. Data are mean values of two successive seasons.

maximum mean values of essential oil content (%) were 1.75% and 1.83% as a result of the combination between peppermint and subsurface drip irrigation followed by 1.42% and 1.56% as a result of the combination between peppermint and surface drip irrigation during the 1st and 2nd cuts, respectively. On the other hand, the maximum mean values for essential oil yield in the 1st cut were 1.40 ml/plant followed by 1.34 ml/plant as a result of the combination between spearmint and subsurface drip irrigation and between peppermint and subsurface drip irrigation, respectively. During the 2nd cut, the mean values for essential oil yield were 2.16 ml/plant followed by 1.56 ml/plant as a result of peppermint in combination with subsurface and peppermint in combination with surface drip irrigation systems, respectively. These results may be due to the increment of essential oil content (%) and/or herb weights.

From the above results it can be concluded through determination of simple correlation coefficients that a positive relationship between herb fresh yield (g/plant) and essential oil percentage is highly significant where correlation was recorded (0.780 for *M. piperita* and 0.688 for *M. viridis*) and the simple regression equations were:

$$y = 69.32X - 22.01 \text{ for } M.\ piperita$$

and

$$y = 176.5X - 62.13 \text{ for } M.\ viridis.$$

This could be explained by the increase in biomass associated to plant modulation of photosynthetic carbon production into metabolic machinery of monoterpene biosynthesis (Khanuja et al., 2000). A positive correlation coefficient was noticed between essential oil (%) and essential oil yield (g/plant) (r = 0.780 and 0.688 for *Mentha piperita* and *M. viridis*, respectively) while simple regression equations were y = 2.025X – 1.801 for *Mentha piperita* and y = 1.616X – 0.593 for *M. viridis*. Also, essential oil yield (ml/plant) displayed positive and significant correlation coefficients with herb fresh weight where r = 0.964 and 0.928, while simple regression equations were y = 43.33 X + 25.80 and y = 76.63X + 32.25 for *M. piperita* and *M. viridis*, respectively.

Essential oil composition

Essential oil composition of both *Mentha viridis* and *Mentha piperita* under the two drip irrigation systems during both cuttings is shown in Table 6. Total identified compounds in *M. viridis* ranged from 91.20 to 97.05% against 88.25 - 91.20% in *M. piperita*. The data tabulated in Table 6 clearly show that total hydrocarbon compounds ranged from 14.03 to 17.10% in *M. viridis* while these compounds ranged from 4.77 to 6.74% in *M. piperita*. On the other hand, total oxygenated compounds ranged from 74.10 to 80.78% in *M. viridis* against 81.51 - 86.24% in *M. piperita*. It can be noticed that subsurface irrigation system gave the highest values for total oxygenated compounds compared with surface irrigation in both *Mentha spp.*

The major compounds in the essential oil of *M. viridis* were found to be carvon (5.50- 60.20%) followed by menthyl acetate (4.64 - 5.54%). α- pinene was identified as the third main constituent

Table 6. Chemical composition of essential oils of *Mentha viridis* and *Mentha piperita* (% of total oil content).

Constituents	*Mentha viridis*				*Mentha piperita*			
	Subsurface		Surface		Subsurface		Surface	
	1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut
α pinene	5.61	5.50	3.50	4.80	1.62	1.10	0.80	1.20
β pinene	3.60	3.70	7.40	3.50	1.42	2.00	2.17	2.50
limonene	3.70	3.60	3.10	3.00	3.70	1.80	1.80	2.10
1,8 Cineol	6.10	5.50	3.00	2.70	0.77	1.12	0.71	1.16
Menthofuran	1.12	1.45	1.60	2.14	5.90	5.90	6.20	5.46
Menthone	2.60	2.60	2.10	2.60	14.70	13.70	15.80	20.40
Menthol	2.90	2.61	2.70	2.50	45.10	44.82	45.40	42.80
Pulegone	1.57	1.81	2.07	2.44	4.50	5.90	4.59	5.40
Isomenthone	1.53	1.70	2.33	2.40	4.54	6.30	6.04	5.38
Menthyl acetate	5.46	5.19	4.80	4.46	5.00	6.30	6.00	5.10
Carvone	59.50	60.20	55.5	59.60	1.00	0.85	1.50	0.42
Eicosan	2.63	1.57	2.21	1.95	-	-	-	-
Docosane	0.73	0.93	0.89	0.78	-	-	-	-
Total identified compounds	97.05	96.36	91.20	92.87	88.25	89.79	91.01	91.92
Total hydrocarbon compounds	16.27	15.30	17.10	14.03	6.74	4.90	4.77	5.80
Total oxygenated compounds	80.78	80.06	74.10	78.84	81.51	84.89	86.24	86.12

(3.50 - 5.46%) and β- pinene was found to be the fourth main compound (3.50 - 7.40%) followed by 1,8- cineol (2.70 – 6.10%). Limonene was found to be the sixth main component which ranged between 3.00 and 3.70%. The effects of both drip irrigation systems on the main components are shown in Table 6. It is clear that subsurface drip irrigation increased carvon content by 7.21% and 1.01% compared with surface drip irrigation during the 1st and 2nd cuts, respectively. Also, subsurface drip irrigation increased menthyl acetate content by 13.75% and 11.85% compared with the other system during the 1st and 2nd cuts, respectively. The maximum values for α-pinene were obtained under subsurface drip irrigation which increased its content by 60.29% and 14.58% over the values obtained under surface drip irrigation during the 1st and 2nd seasons, respectively. Moreover, surface drip system increased β-pinene by 51.35% compared with subsurface drip irrigation during the 1st cut. On the other hand, subsurface system increased β-pinene by 5.71% compared with the other system during the 2nd cut. For 1,8-cineol , it is clear that sub surface drip irrigation increased this compound by 103.33% and 103.70% compared with the other system during the 1st and 2nd cuts , respectively. Surface drip irrigation increased limonene by 16.67% and 20.00% compared with the other system during the 1st and 2nd cuts, respectively. In this respect, Mkaddem et al., (2009) reported that *M. viridis* was rich in carvone (50.47%), 1, 8-cineole (9.14%), and limonene (4.87%). *R*-(–)-carvone is also the most abundant compound in the essential oil from several species of mint, particularly spearmint oil, which is composed of 50-80% *R*-(–)-carvone (Vollhardt and Neil, 2007). Spearmint is the major source of naturally produced *R*-(–)-carvone. However, the majority of *R*-(–)-carvone used for commercial applications is synthesized from *R*-(+)-limonene (Fahlbusch et. al., 2003). A non significant positive correlation coefficient was noticed between carvon and limonene (r = 0.502) while the simple regression equation was y = 0.081X – 1.450. In spearmint, limonene is hydroxylated exclusively at C6 position producing (-) - trans carveol and, following oxidation, (-) - carvone. This region-specific hydroxylation is mediated by two cytochrome P45 enzymes, limonene-6-hydroxylase (CYP71D18) and limonene -3-hydroxylase (CYP71D13) (Schalk and Croteau, 2000). In this respect, Carla and Manuela (2003) revealed that *Rhodococcus opacus* PW cells were found to produce *trans*- and *cis*- carveol and/or carvon as a result of limonene metabolism.

The main constituents of *Mentha piperita* affected by both drip irrigation systems are shown in Table 6. The main component in all treatments was menthol which ranged from 42.80 to 45.40% of the total identified compounds. The second main component was found to be menthone; it ranged from 13.70 to 20.40% from total constituents. Menthofuran was identified as the third main constituent in the essential oil of all different treatments (both drip irrigation systems and both cuttings); it ranged from 5.46 to 5.90%. Menthyl acetate was the next main component which ranged from 5.00 to 6.30%.

The mean values for the main compounds in *Mentha piperita* essential oil indicated that surface drip irrigation system increased menthol by 0.67%

compared with subsurface drip irrigation during the 1st cut while subsurface drip irrigation increased menthol by 4.72% compared with the other irrigation system during the 2nd cut. The second main compound, namely menthone was also identified in the essential oil of all treatments. It can be observed that surface drip irrigation system increased menthone by 7.48% and 48.91% compared with subsurface irrigation during the 1st and 2nd cuts, respectively. Menthofuran was identified as the third main constituents. It can be noticed that there was no difference between the 1st and 2nd cuts under subsurface drip irrigation. Surface drip irrigation increased menthofuran by 5.08% compared with subsurface irrigation during the 1st cut while subsurface irrigation increased menthofuran by 9.26% compared with the other irrigation system during the 2nd cut. Menthyl acetate was the next main compound. Our results showed that surface drip irrigation increased this component by 20% compared with subsurface drip irrigation during the 1st cut. On the other hand, it can be noticed that subsurface drip irrigation increased menthyl acetate by 23.53% compared with the other system during the 2nd cut. Farshbaf et al. (2004) reported that the main components of *M. piperita* oil were menthol (19.76%), menthan-3-one (19.31%), menthofuran + isomenthone (9.12%), 1,8-cineol + beta phellandren (8.8%) and menthol acetate (5.63%). Marine et al. (2009) found that in *M. piperita* oil menthol (37.4%), menthyl acetate (17.4%) and menthone (12.7%) were the main components. Schmidt et al. (2009) published also that the main components in *M. piperita* oil were menthol (40.70%) and menthone (23.40%). The composition is considerably influenced by environmental factors like temperature, photoperiod, nutrition, salinity, water stress, plant age, harvesting and planting time (Charles et al, 1990). From the above data it can be concluded that the negative relationship between menthol and menthone is highly significant (- 0.879) where the simple regression equation was $y = -2.210X + 114.5$. In Japanese mint, the highly demanded monoterpene menthol is produced by the conversion of pulegone into menthone and then into menthol which is further convertible into menthyl acetate similar to peppermint (Murray et al., 1972). A negative correlation between menthol and menthone on the one hand, and menthol and menthyl acetate on the other, was earlier reported (Kukreja et al., 1991; 1992).

CONCLUSION

The subsurface drip irrigation system is proved to be the more suitable technique for irrigation that can be applied to field growing plants.

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