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The effect of hand weeding and planting density on the yield, essential oil content and some morphological properties of peppermint (*MenthaPiperita*L.) in Hamadan

M. RAOOFI AND S. GITI

Department of Agronomy and Weed Science,
MohagheghArdebili University, Ardebil, Iran

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ABSTRACT

The effect of hand weeding and different densities of planting was investigated on the yield, essential oil content and some morphological properties of peppermint (Mentha piperita L.) in which a factorial experiment was carried out based on a completely randomized block design with three replications in two harvest in Hamadan region and in two cropping seasons of 2012 and 2013. The experimental factors include weed control in two levels (hand weeding and non-hand weeding), and plant density on four levels (6, 10, 14 and 18 plants m^{-2}). The identified weeds in the field, include Sorgum halepense, Convolvulus arvensis, Avena ludoviciana, Solanum nigrum, Mallow, Plantago major and Chenopodium album L. The results of both harvests showed that the presence of weeds led to a qualitative and quantitative reduction of peppermint. Peppermint had more favorable growth in the first harvest than the second one, so that this plant provided the maximum essential oil yield and wet/dry yield in the first harvest. The hand weeding increased wet weight, dry weight, yield of the essential oil, leaf area and green cover percentage; the increased density had a significant effect on wet/dry weight gain, essential oil yield and green cover percentage, so that maximum yield of the mentioned items was obtained in densities of 18 plants m^{-2}. The results showed that the adequate density of plants is required due to increase the quantity and quality of peppermint as a medicinal plant.

***Keywords*:** Density, essential oil, weeds, yield

Mentha piperita L. (peppermint) is one of the aromatic herbs which is planted to produce the essential oil. The essential oil of this plant has many medicinal uses (Zelko *et al.,* 2009). Peppermint belongs to the Lamiaceae family and has been created by crossing between *Mentha spicata* and *Mentha aquatic* (Omid Beigi, 1996). The United States of America and India are the largest producers of peppermint (Arabaci and Bayram, 2004). Although the production of active ingredients in medicinal plants is influenced by genetic factors, but their synthesis can be affected by environmental factors, so that environmental factors will cause changes in the quantity and quality of active ingredients (Omid Beigi, 1994). In addition to the environmental factors, farming techniques are effective in the growth and essential oil content in peppermint (Zelko *et al.,* 2009). Farming techniques, including planting density, date of planting and the harvest time, can affect the quantity and quality of the essential oil content in peppermint (ZehtabSalmasi *et al.,* 2008). Planting density is an important affecting factor in the yield of the peppermint (Delaluz *et al.,* 2002). Based on Izadi *et. al.,* experiment (2009), the most yield of the essential oil was obtained from 8 plants m^{-2}, among the densities in a range of 8, 12 and 16 plants m^{-2} in the first harvest.

Email: alvi.adnan@yahoo.com

Jabbar pour *et al.* (2012) also compared the densities of 8, 12, 16 and 20 plants m^{-2} and reached similar conclusions. Heydari *et al.* (2007) compared the densities of 8, 12, 16 and 20 plants m^{-2} and reported that the highest essential oil yield was obtained from the highest density of plant (20 plants m^{-2}). Weeds are one of the significant factors of yield loss (Raoofi *et al.,* 2012). Weeds compete with crops for limited environmental resources such as food, water and light, and thus, reduce significantly the yield and quality of crops (Zand *et al.,* 2007). Concerns about the economic value and environmental effects of herbicides, have prompted many researchers and crop producers to look for methods which can control the weeds (Gresel, 1992; Weiss, 1992). Herbicide is a valuable management tool in agriculture and are beneficial for farmers. But weeds management should be considered as an integrated science (Burnside, 1993), because it is more desirable to avoid herbicide in the fields of medicinal plants and it should be replaced with the alternative methods of farming and management such as determining the density. In general, with an increasing of the plant density up to a certain limit for that plant, the competition can be directed in a favor of crop plant (LA *et al.,* 2012). In regards to the importance of the peppermint and its medicinal value, the aim of this study was to determine the

most optimum plant density for maximum yield of the essential oil, as well as increasing the competitiveness power of the plant against the weeds in the area, to increase the quality of essential oil. By replacing of the agricultural and management methods, such as determining the optimal planting density, the cost of planting was reduced, whereas it was an environmentally benign since any herbicides were not used.

MATERIALS AND METHODS

The study was performed in two cropping seasons in 2012 and 2013 on a farm located at 7Km away from Hamadan-Tehran Road at latitude N 34° and 51′ and longitude E 48° and 32′ as factorial based on the completely randomized block design (CRBD) in 3 replication. The test agents included two levels of weed control (hand weeding and lack of hand weeding) and plant density on four levels (6, 10, 14 and 18 plants m^{-2}). In order to avoid the use of chemical fertilizers in the medicinal plants field, Nitroxin and Super Nitro Plus (8 kg ha^{-1}) were used (Jabbarpoor 2012). However, all samplings and research design were accomplished in the second year of growth peppermint, with the growth of peppermint on 09/04/2013, the needed and desired density of each treatment were applied in a specified number by secateurs. Each plot was created in four rows with a distance of 50 cm and a length of 3 m. The distance between each harvest was 60 cm and also, the distance between every two blocks was considered as 130 cm.

The wet and dry yield, essential oil yield, plant height, number of leaves on each plant, number of nodes on the main stem of the plant, leaf area and percentage of the coverage (were measured by the framework which was divided into 100 segments). In all treatments that must be controlled in terms of weed, weeds were weeded handy to the last sampling. After the treatments without weed control, all weeds remained along with the plants without any control until the end of sampling.

To increase the accuracy in weed control, hand weeding was averagely done every 3 days. Irrigation was averagely preformed about every 3 days and after each hand weeding to stopthe farmwilting. Sampling was carried out to investigate the mentioned properties in two harvests, and in each harvest, the plant was harvested in the early flowering from the soil surface with respect to 50 cm on both sides of the border of each plot. The first and the second harvesting was done in the second week of July and the second week of October, respectively. The harvested samples of each plot were weighed in the wet form, and its area was determined by the leaf area meter. Next, the harvested samples were took place for 48 hours in an oven at a temperature of 74 °C, and then, weighed to calculate the dry weight. The essential oil of peppermint was extracted using water distillation and by the use of Clevenger apparatus. The obtained data from sampling of both harvests as a factorial based on the CRBD and the obtained one year data as a split harvest in time was analyzed by SAS software and the comparison of averages was performed using the LSD test.

RESULTS AND DISCUSSION

Wet weight

The results showed that the effect of hand weeding and plant density on the yield of the wet plant (wet weight) in both harvests was significant at the 1% level (Tables 1 and 2). According to table-4, it was found that hand weeding has significantly impacted on the wet weight of peppermint. With an increasing of the plant density, the wet yield was increased since the greatest wet weight of peppermint was achieved in the density of 18 plants m^{-2} (Table 5). The results of combined analysis of hand weeding and plant density in both harvests on the wet weight of peppermint (Table 3) showed that the harvest has a significant effect on the level of 1%. In general, an increasing in the plant numbers per unit area was directly related to the wet weight. Observance of the proper density of plant leads to more successful performance in competition with weeds (Koochaki *et al.,* 2005). Heydari *et al.* (2007) published that the peppermint density of 20 plants m^{-2} (highest density) has the highest wet yield. In this regard, Giti *et al*. (2012) stated that with the increase of the plant density per unit area, the wet weight of plant will also increase. Hand weeding led to provide more space growth of peppermint. As a result, the plant could get a higher weight since weeds competes with the plant over resources and therefore, reduces the growth of peppermint Raoofi *et al*. (2014) proved that weeds lead to compete with plants and reduces their wet weight.

Beside this, a wide range of weeds in the field was observed that led to overcast on the peppermint and inhibition of its growth. It is obvious that the increase of the weed number reduces the yield (Rashid Mohassel *et al.,* 2006). The relation between the weight yield of peppermint and the density was very clear. At higher densities, peppermint was much

more successful in competition with weeds. Hand weeding, provided desirable conditions for peppermint growth that it was associated with a favorable growth. Generally, peppermint could better use resources and the conditions by hand weeding which caused the development of more canopy and therefore, the wet weight was increased.

Dry weight

The obtained results from dried weight yield were based on the wet weight. In the first and second harvests, the dry weight was influenced by hand weeding and the plant density (Tables 1 and 2). Hand weeding significantly increased the dry weight of peppermint and could have a direct impact on its dried yield. Generally, with an increasing of the density, dry weight of peppermint was increased, however, there was no significant difference between the density of 10 and 14 plants (Table 5) and an increasing trend was still governed. Enhancement of the plant number per unit area was most directly related to the dry weight, and the maximum dry weight yield of peppermint was achieved in density 18-plant m^{-2}. Thus, it is very important to determine the appropriate plant density. Manssori (2014) reported that the maximum dry weight yield of peppermint was achieved in 20-plant density (highest density). The results of combined analysis of data

Table 1: Analysis of variance of hand weeding and plant density of some morphological characteristics of *Mentha piperita* L. in the first plot

Sources of variations	Degree of freedom	Average of squares							
		Wet weight	Dry weight	Essence yield	Plant height	Number of nodes on main stem	Number of leaf plant^{-1}	Leaf area of plant	Green canopy cover (%)
Replication	2	13601012.2**	1585405.2^{ns}	54.7^{ns}	49.8^{ns}	0.502^{ns}	277^{ns}	329977^{ns}	302*
Hand weeding	1	21286854.6**	**841299.3	444.8*	37.9^{ns}	12.8^{ns}	2.7^{ns}	419044*	502*
Plant density	3	18949888.5**	789222.4**	88.8*	13.6^{ns}	8^{ns}	6.1*	39964^{ns}	298**
Hand weeding × plant density	3	307715.2^{ns}	14011.7^{ns}	5.5^{ns}	4.8^{ns}	4.2^{ns}	217^{ns}	80011^{ns}	26^{ns}
Tolerance	14	1278542.6	118991.0	25.2	34.0	10.5	237	15774	355
CV (%)		**23.6**	**24.6**	**25.2**	**17.2**	**13.6**	**10.7**	**26**	**13**

*Note: *,** are significant at 1% and 5% level, respectively*

Table 2: Analysing variance of hand weeding on some morphological characteristics of *M. piperita* in plot II

Sources of variations	Degree of freedom	Average of squares							
		Wet weight	Dry weight	Essence yield	Plant height	Number of nodes on main stem	Number of leaf plant^{-1}	Leaf area of plant	Green canopy cover (%)
Replication	2	5899744**	**60845707	*60.76	**23.08	1.7^{ns}	190^{ns}	68682.7^{ns}	404**
Hand weeding	1	9433441**	**598756407	**287.23	4.01^{ns}	3.8*	344*	1282201.8**	201**
Plant density	3	3174811**	**34116507	*9.77	11.2*	1.5^{ns}	980**	15965.9^{ns}	189*
Hand weeding × plant density	3	185002^{ns}	2276405^{ns}	8.96^{ns}	2.2^{ns}	4.3*	30^{ns}	1 .187^{ns}	1.1^{ns}
Tolerance	14	422541	50512	17.76	2.3	1.5	66	235011	3.3
CV (%)		**27**	**26087**	**27.52**	**6.4**	**7.7**	**6.4**	23	**6.5**

Table 3: Combined analysis of hand weeding, weeds and plant density on some morphological characteristics in both plots

Weed control	Wet weight (g m^{-2})	Dry weight (g m^{-2})	Essential yield (kg ha^{-1})	Leaf area of plant (cm)	Green canopy cover (%)
Lack of hand weeding	3239^{b}	933^{b}	14.45^{b}	1551^{b}	79^{b}
Hand weeding	4664^{a}	1428^{a}	24.21^{a}	2476^{a}	91^{a}

Note: Averages with common letters in each column, in each treatment based on the LSD test are not significantly different

Table 4: Comparison of hand weeding on some traits of *Mentha piperita* L in both plots

Sources of variations	Degree of freedom	Average of squares							
		Wet weight	Dry weight	Essence yield	Plant height	Number of nodes on main stem	Number of leaf plant^{-1}	Leaf area of plant	Green canopy cover (%)
Replication	2	18223263**	7731896**	133.652*	90.4^{ns}	85.6^{ns}	2.3^{ns}	345565.1^{ns}	645.5*
Hand weeding	1	33337112**	1774434**	848.655**	41.7^{ns}	191.5^{ns}	1.08^{ns}	5876779.3*	665.5*
Plant density	3	21799987**	1292232**	97.763^{ns}	26.5^{ns}	1465.4*	8.5^{ns}	4689.8^{ns}	554.7*
Hand weeding × plant density	3	233754^{ns}	37232^{ns}	6.448^{ns}	5.05^{ns}	71.7^{ns}	7.8^{ns}	256523.4^{ns}	2.8^{ns}
Tolerance	14	1234764	121152	21.987	21.8	206.6	5.8	384434.5	12.5
Time (plot)	1	1040011**	4645922*	6932.112**	6788.3*	683213.6^{ns}	778.6*	323354.7*	1823.4**
Reps × plots	2	3129923^{ns}	67215^{ns}	15.443^{ns}	4.4^{ns}	4431.5^{ns}	1.1^{ns}	709879.7^{ns}	50.4^{ns}
Plots × hand weeding	1	2799476^{ns}	219476^{ns}	25.923^{ns}	12.5^{ns}	133.7^{ns}	15.8^{ns}	722232.3^{ns}	38.6^{ns}
Hand weeding × plot	3	5554553^{ns}	90767^{ns}	31.043^{ns}	2.2^{ns}	125.9^{ns}	2.0^{ns}	21552.4^{ns}	65.6*
Plant density × hand weeding × plant density	3	342287^{ns}	5023^{ns}	3.032^{ns}	3.3^{ns}	225.6^{ns}	3.6^{ns}	156643.3^{ns}	4.9^{ns}
Tolerance	14	819887	93874	23.443	21.2	155.5	8.3	3788.4	18.4
CV (%)		**26**	**28**	**23.56**	**11.2**	**5.5**	**12.3**	**11.4**	**8.2**

Table 5: Comparing average density on some *M. piperita* traits in both plots

Density (Plants m^{-2})	Wet weight (g m^{-2})	Dry weight (g m^{-2})	Essential yield (kg ha^{-1})	Number of leaf	Green canopy cover (%)
6	2488.3^{d}	880.21^{d}	14.78^{d}	225^{b}	70.12^{d}
10	3311.7^{c}	1009.5^{bc}	19.5^{c}	219^{ab}	76.43^{c}
14	4921.2^{b}	1289.8^{b}	22.49^{b}	200^{a}	80.95^{b}
18	5940.4^{a}	1634.2^{a}	26.32^{a}	197^{a}	86.38^{a}

Table 6: Comparison of hand weeding on some traits of *M. piperita* in both plots

Weed control	Wet weight (g m^{-2})	Dry weight (g m^{-2})	Essential yield (kg ha^{-1})	Leaf area of plant (cm)	Height (cm)	Number of nodes on main stem	Number of leaf	Green canopy cover (%)
First	5266^{a}	1399^{a}	23.21^{a}	1679^{b}	62.9^{a}	31.12^{a}	265^{a}	86^{b}
Second	2856^{b}	877^{b}	17.43^{b}	1923^{a}	41.2^{b}	24.43^{b}	199^{b}	74^{a}

showed that the wet and dry weights were exposed to the harvest, hand weeding and the plant density (Table 3). Heidari *et al.* (2007) reported that the largest dry weight yield of peppermint was obtained in 20-plant density m^{-2}. The desirable conditions were provided for success of peppermint with hand weeds control, because it could take advantage of the resources and conditions more favorably and dominates weeds.

Essential oil

The results showed that the effect of hand weeding, and the plant density on the yield of essential oil was at a level of 5% in the first harvest. Moreover, in the second harvest it was at the levels of 1 and 5% (Tables 1 and 2). As shown in table-4, hand weeding has a significant effect on the enhancement of essential oil yield of peppermint. There is a direct relation between the density and the yield of the essential oil of peppermint, and with an increasing of the density, the essential oil yield also increases and the highest essential oil yield were observed in 18 plant density m^{-2} (Table 5). The combined analysis results of hand-weeding, and plant density in both harvests on the yield of peppermint essential oil (Table 3) showed that the harvest and hand-weeding have a significant effect at the level of 1%, and the mutual effects of hand-weeding and plant density was non-significant. Heydari *et al.,*

(2008) introduced the density as one of the most important factors of increasing the yield of peppermint essential oil and emphasized that 20-plant density m^{-2} has the highest essential oil yield. Even when weeds are close to peppermint, with an increasing of plant density, an increase of essential oil yield will be observed, since observing the proper plant density will cause the plant be more successful in competition with the weeds (Koochaki *et al.,* 2006). The weeds which are in the field, consume the nutrients (Mohammad Doost, 2010) and compete more successfully with the main plant and thus, lead to loss of the essential oil yield. In this regard, Malakooti (2000) also noted the impact of nutrients on the yield of medicinal plants and considered that their role is very important. In general, with the growth and spread of weeds (lack of hand weeding), the lower growth rates was observed in peppermint. Weeds can cause depletion of nutrients from the soil, which reduces the amount of essential oil yield. In this regard, Omid Beigi (1997) is also pointed to the important impact of the mentioned items on the essential oil yield of the medicinal herbs.

Height and number of nodes

The variance analysis of the hand weeding and the density on the height of peppermint showed that, except of the density in the second harvest, which has a significant effect on the yield at the level of 5%, the others did not have a significant effect on any of the two harvests (Table 1 and 2). Although, the peppermint height may have less importance than the other mentioned properties, hence, when the weeds are present, the main height of plant is very important, so if the weeds growth rate is higher than the original plant, they may have a negative effect on the plant and they can reduce the growth rate of the original plant with overcasting and absorbing nutrients from the soil. The combined analysis results of hand weeding, and plant density in both harvests on the height of peppermint showed that harvest has a significant effect on plant height at level of 5%, and the mutual effects of hand weeding, and plant density had no significant effect on this character (Table 3). Generally, the extensive presence of high weeds can reduce the useful space for the success of peppermint, when the weed growth is overcoming, it overcast on peppermint, and thus peppermint growth and thus, dry weight and essential oil yield were reduced. Weed interference with peppermint can significantly reduce their height. The effect of hand weeding, plant density and their mutual effects were insignificant on the number of nodes in the main stem of the plant in the first harvest (Table 1). In the second harvest, plant density effect was insignificant on the yield, but the effect of hand weeding and its mutual effect with the plant density were significant at 5% level (Table 2). The table of comparing averages showed that the number of nodes on the main stem in the first harvest was significantly more than second harvest (Table 6). In this experiment, peppermint had more growth in the first harvest than the second harvest because of the longer days and exposure to more light. This increases the number of nodes in the first harvest compared to the second. The results are consistent with that of Arabaci and Bayram (2004) and Heidari *et al*. (2008)

Number of leaves and leaf area

In the first harvest, hand weeding effect on the number of leaves per plant is insignificant but the effect of plant density on this trait was significant at the 5% level. Their mutual effect on the number of leaves per plant was also insignificant (Table 1). In the second harvest, the effect of hand weeding and plant density was significant at 5% and 1% levels on the number of leaves per plant, but their mutual effect was insignificant (Table 2). The effect of hand weeding on the surface of leaves in the first and second harvests is significant at 5% and 1% levels, but the effect of the density on the leaf area was insignificant in both harvests. The mutual effect of hand weeding and the density were insignificant on the leaf area in both harvests (Table 1 and 2). The number of leaves per plant was decreased with increasing of the plant density (Table 5)

The maximum number of leaves related to the 6-plant density m^{-2} was as many as 225 and the minimum number of leaves with 18-plant treatment m^{-2} was as many as 197. By reducing the density, competition between the plants reduces and the number of leaves per plant increases. Researchers (Delaluz *et al.* 2002; Zehtab Salmasi *et al.,* 2008) reported that the leaves decrease in high densities.

Although, the number of leaves in 18-plant density m^{-2} was less than the lower densities but the leaf area was higher and the percentage of the green coverage which has a direct relation was further. The high growth rate and a rapidly increasing in the density of weeds led to more early competition with the plants (Akey *et al*., 1990). In this experiment, with an increasing of the peppermint density, although caused to the decreasing of the leaves

number, the peppermint was better able to use the resources and conditions than weeds to its advantage. Hence, it could increase its leaf surfaces and compensated its fewer leaves. Comparison of the data average showed that hand weeding causes a significant increase in the level of peppermint leaves (Table 4). The combined analysis of the hand weeding and the plant density in both harvests showed that harvest and hand weeding had a significant effect on the level of 5% on the leaf area (Table 3). The wide range of weeds led to the absorption of the soil nutrients and this caused reducing of the available nutrients for peppermint. By reducing access to these nutrients, the growth rate of peppermint was more limited. This was compatible with the published paper results of Bazdirev *et al.* (2004). Due to the strong and rapid initial growth of the weeds root compared to peppermint, those acted more successful than the plant. Thus, in this experiment, weeds reduced the level of peppermint leaves.

Percentage of green cover

In the first harvest, the effect of hand weeding and the density was significant on the green cover percentage at 1% and 5% levels and in the second harvest, was at 5% and 1% levels. The mutual effect of hand weeding and the plant density was insignificant in both harvests (Table 1 and 2). The comparison table showed that the green cover percentage on the treatment of hand weeding is more than when weeding was not used (Table 4) and with an increasing of the plant density, the green cover percentage was increased (Table 5). Liebman (2001) and Bazdirev (2004) reported that in situations, where the position is favorable for plants such as weeding, the yield and the development of the plants will be much more than the competition mode with weeds and therefore, the green cover percentage will increase too. In this experiment, hand weeding led to better growth and development of peppermint and caused better formation of peppermint. Tolikov (1974) stated that with an increasing of the density per surface area, the more green cover is created. Delalu (2002), Aflatuni (2005) and Zehtab Salmasi (2008) proved that in higher densities of peppermint, the green cover percentage will increase.

The results showed that in Hamadan region, the density and hand weeding were significantly effective on the yield and essential oil content of peppermint and this plant in proper planting density in addition to producing efficient and high essential oil, uses more desirably resources and growth conditions than weeds. Accordingly, in order to the increasing the importance and the use of medicinal herbs in a scientific manner, it is recommended to use 18-plant density m^{-2} to achieve the maximum yield and essential oil content as well as reducing consumption of herbicides.

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