

# The effects of cow manure vermicompost and municipal solid waste compost on peppermint (*Mentha piperita* L.) in Torbat-e-Jam and Rasht regions of Iran

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## Abstract

**Background** The utilization of organic fertilizer is an effective method in organic culture of medicinal plants because of its beneficial effects on soil structure, reduction of environmental problem and improvement in plant growth. The experiments were conducted in a research field at the University of Guilan at the Rasht and Torbat-e-Jam Razavi Khorasan Province, Iran. The experiment was conducted to determine the effects of 7 Mt ha<sup>-1</sup> of cow manure vermicompost, vermiwash prepared from 7 Mt ha<sup>-1</sup> of vermicompost, leachate vermicompost + vermiwash, 50 Mt ha<sup>-1</sup> municipal solid waste compost (MSWC), chemical fertilizer (50, 0, 300 NPK) and no fertilization as a control on peppermint yield and quality.

**Results** Organic fertilizers significantly affected all the measured characters except total phenols and antioxidant capacity compared to chemical fertilizers and the control. Plants treated with vermicompost, vermiwash or vermicompost leachate + vermiwash were the tallest and had the highest levels of chlorophyll a, chlorophyll b, total chlorophyll and carotenoids. Plants treated with vermicompost had the highest essential oil yield (24.21 ml m<sup>-2</sup>). Plant growth in the Rasht region had the highest number of lateral branches (18), fresh (10.51 g) and dry weight (4.64 g) of plant, fresh (2,102.9 kg ha<sup>-1</sup>) and dry (928.67 kg ha<sup>-1</sup>) yield, leaf area index (0.17) and carotenoids (531.82 mg 100 g<sup>-1</sup>), while the highest oil per plant (0.24 ml plant<sup>-1</sup>) and oil yield (18.49 ml m<sup>-2</sup>), chlorophyll a (7.37 mg 100 g<sup>-1</sup>), b (4.07 mg 100 g<sup>-1</sup>) and total

chlorophyll (11.44 mg 100 g<sup>-1</sup>), total phenol (7.48 mg gallic acid equivalent 100 g<sup>-1</sup>) and antioxidant capacity (71.89 (% of inhibition) were obtained from plants grown in the Torbat-e-Jam region.

**Conclusion** Organic fertilizers beneficially affect soil structure and nutrient availability. The use of sustainable organic materials can increase fertility without negative effects on human health and environment. We counsel farmers to use leachate vermicompost and vermiwash in organic cultivation of peppermint separately or mixed together.

**Keywords** Vermicompost · Medicinal plants · Organic culture · Essential oil

## Introduction

Peppermint (*Mentha piperita* L., lamiaceae.) is a high-demand aromatic and medicinal crop which is vegetatively propagated through stolon and rhizomes (Verma et al. 2013). Peppermint oil is used both as a medicinal and flavoring agent in foods and confectionery (Milovanović et al. 2009). In Iran, peppermint is a perennial crop established with transplants in the spring and has an average stand life of about 6 years (Ayyobi et al. 2014).

The commercial production of peppermint depends on the ecological conditions affecting the yield and quality. Locations with warmer climate have higher fresh herbage yield. It was also concluded that a temperate location was more suitable for peppermint oil production with high menthol contents (Telci et al. 2011). In each commercial production region, if appropriate amounts of fertilizers are not applied during production, physiological symptoms of deficiency can occur in plants (Olfati et al. 2012). Most

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producers use synthetic fertilizers because they are easy to transport, quickly available to plants and produce high yields. However, with succeeding crops, the quantities of chemical fertilizers must be increased because of low soil fertility. Organic fertilizers have beneficial effects on soil structure and nutrient availability, help to maintain the yield and quality, and are less costly than synthetic fertilizers (Thy and Buntha 2005).

Large amounts of organic wastes, i.e., biosolids, animal manures and household wastes, are produced in Iran. These wastes may be used in the production of herbs or to restore soil fertility (Benton and Wester 1998), as they contain large quantities of nitrogen (N), phosphorus (P) and potassium (K) (Elliot and Dempsey 1991). Compost is homogenous, retains most of its original nutrients and has reduced levels of organic contaminants, because it is degraded before use (Ndegwa et al. 2000). It can be applied to increase soil organic matter and nutrients which can be released upon decomposition, to improve soil structure and increase cation exchange capacity. The use of composts in agricultural soils is a widespread practice and positive effects on soil and vegetables are known from numerous studies (Gutierrez-Miceli et al. 2007; Peyvast Gh et al. 2007, 2008a, b, c, d; Olfati et al. 2009; Shabani et al. 2011; Ayyobi et al. 2013).

Certain species of earthworms can fragment organic material residuals into finer particles by passing them through a grinding gizzard (Ndegwa and Thompson 2001). Additionally, earthworms reduce populations of human pathogens, an effect obtained in traditional composting by increasing the temperature (Contreras-Ramos et al. 2004). There is evidence that earthworms produce plant hormones in their secretions (Suthar 2010b). Earthworms process materials 'casts' contain nutrients in forms easily available to plants (Suthar 2010a; Suthar and Singh 2008).

Greenhouse and field studies have examined the effects of vermicompost on cereals and legumes (Chan and Griffiths 1988), vegetables (Ayyobi et al. 2013, 2014; Peyvast et al. 2008a, b, c, d; Kochakinezhad et al. 2012), and field crops (Buckerfield and Webster 1998). Most of these investigations confirmed that vermicomposts have beneficial effects on plant growth (Atiyeh et al. 1999; Ayyobi et al. 2014; Chatterjee et al. 2014). The final vermicomposting product has a high electrical conductivity (EC) which increases soil salinity with continued usage. To reduce EC, vermicompost leachate and vermiwash have been developed (Suthar 2010b).

There is a demand for naturally derived agrochemicals for sustainable farming systems and organic production disallows the use of synthetic chemicals. There is no comprehensive study concerning the impact of organic fertilizers including vermiwash, leachate vermicompost or municipal solid waste compost on peppermint in different regions. This project was undertaken to determine the effects of these organic fertilizers in comparison to

chemical fertilizers on the yield and quality of peppermint in Rasht and Torbat-e-Jam regions of Iran which have different ecological conditions.

## Methods

The experiment was conducted in a research field at the University of Guilan Campus, Agriculture Faculty, Rasht, Iran (altitude 7 m below mean sea level, 37°16'N, 51°3'E), from April to August 2012 and at the Torbat-e-Jam Razavi Khorasan Province, Iran (35.°31'N, 60°48'E, 928 m elevation) during May to July 2013. Rasht features a humid subtropical climate with hot, humid summers and generally mild to cool winters with 1,355.5 mm of precipitation per year during average 109.4 rainy days. Rasht enjoys on average 1,524 h of sunshine per year, while Torbat-e-Jam features a semi-arid climate with hot summers and cool winters. The city only sees about 254 mm of precipitation per year. Torbat-e-Jam also has wetter and drier periods with the bulk of the annual precipitation falling between the months of December and May. Summers are typically hot and dry, with high temperatures sometimes exceeding 35 °C. Torbat-e-Jam enjoys on average 3,000 h of sunshine per year.

The soil at University of Guilan research field was loamy with pH 7.44, containing total N (1 %) and total C (1.08 %). There were 4,600, 1,700 and 4,000 mg kg<sup>-1</sup> of Ca, P and K, respectively, in soil dry matter (DM), with an EC of 0.1 ds cm<sup>-1</sup>. Torbat-e-Jam research field soil was clayey loam, pH 7.23, containing total N (3 %) and total C (1.5 %). There were 4,325, 1,95 and 3,86 mg kg<sup>-1</sup> of Ca, P and K, respectively, in soil DM, with an EC of 0.13 ds cm<sup>-1</sup>. The soil was prepared by plowing and disking. Local clones of peppermint were established by cutting on 15 April and 5 May in Rasht and Torbat-e-Jam, respectively. Each plot area was 4 m<sup>2</sup> containing 80 plants.

Earthworms (*Eisenia fetida*) (25 g earthworms•kg<sup>-1</sup> of cattle manure or 2.5 kg earthworms/m<sup>2</sup> per bed) were added and vermicomposted for 2 months (Peyvast et al. 2008a, b). The vermicompost had a water content of 380 g kg<sup>-1</sup>, pH 6.82, total C content of 23.8 % DM and a total N content of 1.5 % DM. The vermicompost (100 kg) was flushed with 50 L of water and the leachate (vermiwash) was collected. Leachate vermicompost (100 kg), after collecting vermiwash (50 L), was also stored for the next usage. The municipal solid waste compost had a pH of 6.82; containing total N (2.56 %) and total C (25 %). There were 5,320, 15,800 and 6,800 mg kg<sup>-1</sup> of Ca, P and K, respectively, in DM, with an EC of 4.9 ds cm<sup>-1</sup>.

A completely randomized block design with three replications was used in each place. Treatments included 7 Mt ha<sup>-1</sup> of cow manure vermicompost, vermiwash prepared from 7 Mt ha<sup>-1</sup> of vermicompost, leachate vermicompost +

**Table 1** GLM table effects of different places, fertilizers and their interactions on the vegetative characteristics

Source of variation	df	Mean square				
		Plant height	Number of lateral branches	Total plant fresh weight	Total plant dry weight	LAI
Place ( <i>P</i> )	1	13.32 <sup>ns</sup>	44.75**	22.12**	13.17**	0.002**
Replication ( <i>P</i> )	4	76.21	13.08	12.76	1.30	0.001
Treatments ( <i>T</i> )	5	430.20**	33.65**	23.92**	2.54**	0.005**
<i>P</i> × <i>T</i>	5	9.26 <sup>ns</sup>	0.01 <sup>ns</sup>	1.41 <sup>ns</sup>	0.01 <sup>ns</sup>	0.0007 <sup>ns</sup>
Error	20	16.26	1.42	0.53	0.06	0.0001
CV (%)		6.51	6.80	7.51	6.48	8.30

<sup>ns</sup> \*,\*\* non-significant or significant at  $P \leq 0.01$  and  $P \leq 0.05$ , respectively

vermiwash, 50 ha<sup>-1</sup> municipal solid waste compost (MSWC), chemical fertilizer (50, 0, 300 NPK) and no fertilization as a control. The vermicompost, MSWC and leachate vermicompost were spread over beds, and vermiwash was applied four times to plants at a 7-day interval with the first application 1 month after cutting.

At harvest, total leaf dry and fresh yield, oil yield, number of lateral branches per plant, plant fresh and dry weight, plant height, leaf area index, content of essential oil per plant, total carotenoids, total phenol, total chlorophyll, chlorophyll *a*, chlorophyll *b* and antioxidant capacity were determined.

Methanol extracts of the sample (1 g of sample in 10 ml of methanol) were used for the determination of total phenolics. The total phenolic content was evaluated by colorimetric analyses using Folin–Ciocalteu's phenol reagent (Singleton and Rossi 1965). The total phenolic content was expressed as mg gallic acid equivalent/100 g of sample. The free radical-scavenging activity against 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical was evaluated with the methods of Leong and Shui (2002) and Miliuskas et al. (2004) with minor modification. In the presence of an antioxidant, the purple color intensity of DPPH solution decays and the changes of absorbance are followed spectrophotometrically at 517 nm. Total carotenoids mg/100 g were determined by a modified method of Ranganna (1997) using acetone and petroleum ether as extracting solvents and measuring absorbance at 450 nm. Chopped stems, leaves and plants were placed in a forced air drying oven at 75 °C for 48 h to determine the DM. For the evaluation of the amount of essential oil, a sample of 100 g of drying matter mixed with 800 ml of water was distilled for 3 h using a Clevenger apparatus.

Data were subjected to GLM in SAS (ver. 9.1, SAS Institute, Inc., Cary, NC). If the interaction was significant, it was used to explain the results. If the interaction was not significant, the means were separated using Tukey's test.

## Results

Cultivation place affected all measured characteristics except plant height (Tables 1, 2, 3). Fertilizer type also

affected all measured characteristics except total phenol and antioxidant capacity (Tables 1, 2, 3). Interaction between cultivation place and fertilizer type affected total fresh yield and chlorophyll *a* content and antioxidant capacity (Table 1, 2, 3).

In both cultivation places, the highest fresh yield was related to the organic fertilizer (Fig. 1). The highest content of chlorophyll *a* in both cultivation places was obtained from vermicompost and its derivations (Fig. 2). Plants treated with different fertilizers did not have any significant differences in antioxidant capacity (Fig. 3).

The interaction between cultivation place and fertilizer did not show significant effects on other characteristics. In fact for both regions, we are able to recommend a type of fertilizer as a best type. In other words, different types of fertilizers did not modify the environmental condition in two places with different environmental conditions. The total phenol and antioxidant capacity averaged 6.40 mg gallic acid equivalent/100 g sample and 66.33 %, respectively.

Plant growth in the Rasht region had the highest number of lateral branches, fresh and dry weight of plant, fresh and dry yield, leaf area index and carotenoids, while the highest oil per plant and oil yield, chlorophyll *a*, *b* and total, total phenol and antioxidant capacity were obtained from plant growth in the Torbat-e-Jam region (Table 4).

Plants treated with vermicompost, vermiwash or leachate vermicompost + vermiwash were similar for all measured characteristics and of the highest vegetative and qualitative value (Tables 5). Differences between plants treated with organic fertilizers were not significant for leaf area index (Table 5) where all plants treated with organic fertilizer had higher leaf area index than plants treated with chemical fertilizer and the control.

## Discussion

The commercial production of peppermint depends on the ecological conditions affecting yield and quality (Telci et al. 2011). Locations with low sunshine, moderate

**Table 2** GLM table effects of different places, fertilizers and their interactions on total fresh and dry yield, content of oil per plant and oil yield

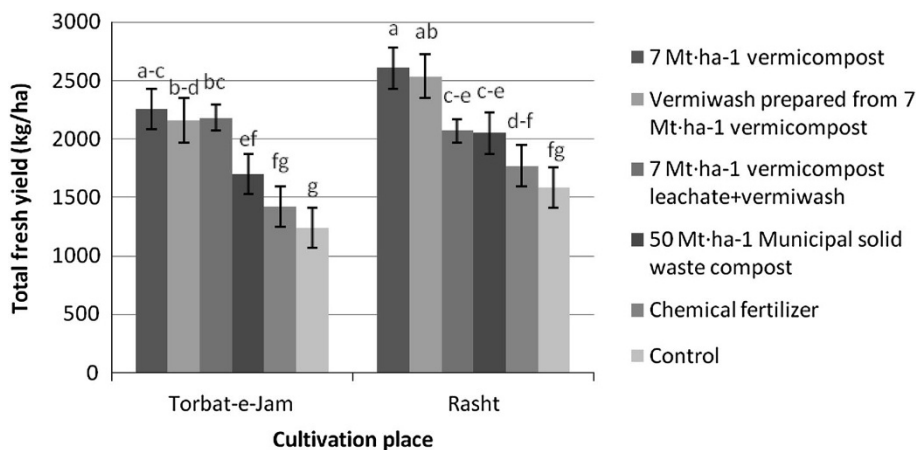
Source of variation	df	Mean square			
		Total fresh yield	Total dry yield	Content of oil per plant	Oil yield
Place ( <i>P</i> )	1	686,964.65**	232,324.00**	0.02**	20.73*
Replication ( <i>P</i> )	4	479,705.30	53,407.00	0.004	6.50
Treatments ( <i>T</i> )	5	1,003,607.71**	104,307.13**	0.003**	130.49**
<i>P</i> × <i>T</i>	5	54,779.09*	961.00 <sup>ns</sup>	0.0,002 <sup>ns</sup>	3.38 <sup>ns</sup>
Error	20	13,482.83	2,900.86	0.0,004	5.25
CV (%)		5.90	6.34	9.83	12.92

*ns* \*\*, \*\* non-significant or significant at  $P \leq 0.01$  and  $P \leq 0.05$ , respectively

**Table 3** GLM table effects of different places, fertilizers and their interactions on qualitative characteristics

Source of variation	df	Mean square					
		Chlorophyll <i>a</i>	Chlorophyll <i>b</i>	Total chlorophyll	Carotenoids	Total phenol	Antioxidant capacity
Place ( <i>P</i> )	1	45.85**	13.61**	109.44**	111,349.01**	40.65**	1,112.55**
Replication ( <i>P</i> )	4	0.73	0.55	1.83	6,702.05	0.20	0.55
Treatments ( <i>T</i> )	5	9.78**	2.01**	20.60**	22,074.98**	0.03 <sup>ns</sup>	0.38 <sup>ns</sup>
<i>P</i> × <i>T</i>	5	0.40**	0.01 <sup>ns</sup>	0.40 <sup>ns</sup>	0.01 <sup>ns</sup>	0.29 <sup>ns</sup>	0.09**
Error	20	0.08	0.03	0.18	501.74	0.37	2.10
CV (%)		4.80	5.74	4.39	4.70	9.49	3.18

*ns* \*\*, non-significant or significant at  $P \leq 0.01$ , respectively

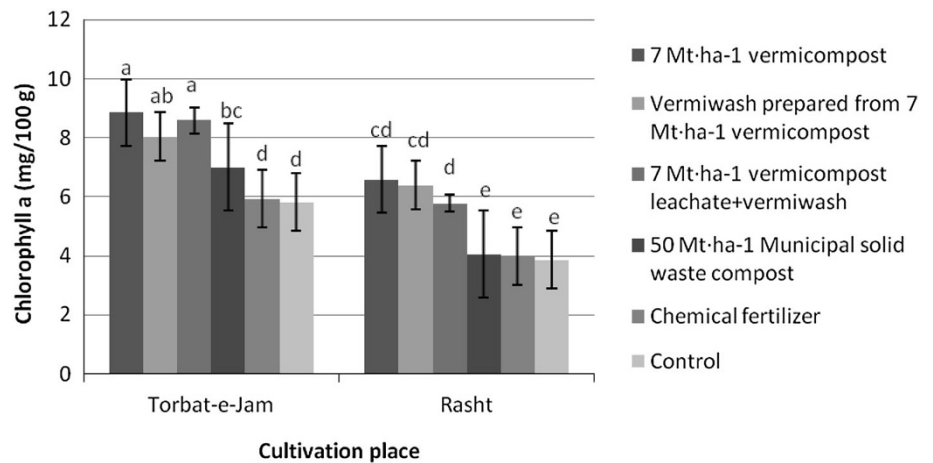
**Fig. 1** Interaction between cultivation place and fertilizer on total fresh yield of peppermint

temperature and high rainfall (Rasht) have higher fresh herbage yield, while locations with high sunshine, high temperature and low rainfall (Torbat-e-jam) have higher oil yield and antioxidant capacity. A previous research also concluded that temperate location was more suitable for peppermint oil production (Telci et al. 2011).

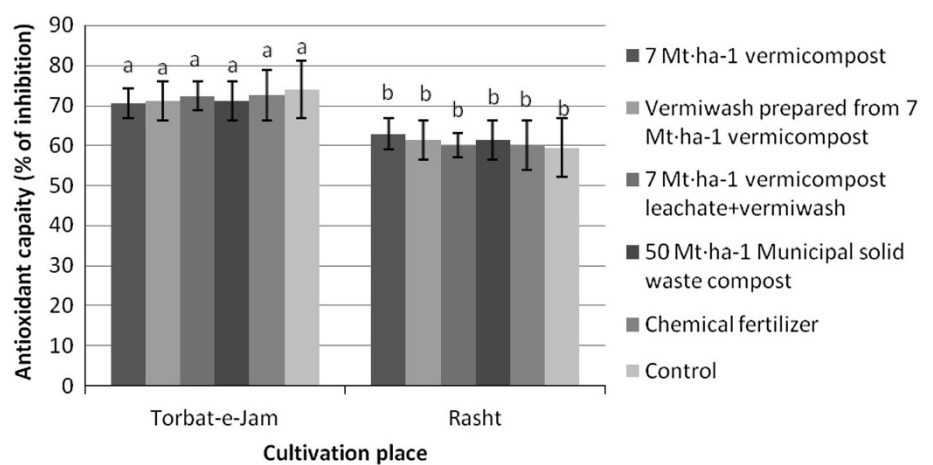
Municipal solid waste compost increased vegetative growth and yield of peppermint more than chemical fertilizer, although vermicompost had a significantly higher level than municipal solid waste compost (locations with low sunshine, moderate temperature and high rainfall (Rasht) have higher fresh herbage yield).

Organic fertilizers beneficially affect soil structure and nutrient availability; they maintain quantity and quality of yield and can be less costly than synthetic fertilizers (Olfati et al. 2012; Thy and Buntha 2005; Ayyobi et al. 2014). The use of sustainable organic materials can increase the fertility without negative effects on human health and environment. Vermicompost (Gutierrez-Miceli et al. 2007; Peyvast et al. 2008a, b; Ayyobi et al. 2013, 2014) and vermiwash (Suthar 2010a; Ayyobi et al. 2013, 2014) have been proposed as organic fertilizers previously. Most investigations confirmed that vermicomposts were beneficial to plant growth (Atiyeh et al. 2000; Peyvast et al. 2008a, d; Ayyobi et al. 2013, 2014).

**Fig. 2** Interaction between cultivation place and fertilizer on chlorophyll a of peppermint



**Fig. 3** Interaction between cultivation place and fertilizer on antioxidant capacity of peppermint



**Table 4** Effect of cultivation place on measured characteristics

Cultivation place	Plant height (cm)	Number of lateral branches	Total plant fresh weight (g)	Total plant dry weight (g)	LAI	
Torbat-e-Jam	61.28	16.40	8.95	3.43	0.15	
Rasht	62.5	18.63	10.51	4.64	0.17	
Cultivation place	Total fresh yield (kg/h)	Total dry yield (kg/h)	Content of oil per plant (ml/plant)	Oil yield (ml/m <sup>2</sup> )		
Torbat-e-Jam	1,826.6	768.00	0.24	18.49		
Rasht	2,102.9	928.67	0.18	16.97		
Cultivation place	Chlorophyll a (mg/100 g)	Chlorophyll b (mg/100 g)	Total chlorophyll (mg/100 g)	Carotenoids (mg/100 g)	Total phenol (mg gallic acid equivalent/100 g)	Antioxidant capacity (% of inhibition)
Torbat-e-Jam a	7.37	4.07	11.44	420.59	7.48	71.89
Rasht	5.11	2.84	7.95	531.82	5.36	60.78

Leachate vermicompost contains a slightly higher amount of sodium (Na); vermiwash contains a high level of potassium (K) which, as a primary nutrient, is needed in high amounts for plant growth.

The main problem that can arise from excessive vermicompost application is plant toxicity due to high salt content. With leaching, the negative effects of vermicompost related to high EC (Gutierrez-Miceli et al. 2007)

**Table 5** Effect of fertilizer treatment on measured characteristics

Treatment	Plant height (cm)	Number of lateral branches	Total plant fresh weight (g)	Total plant dry weight (g)	LAI
7 Mt ha <sup>-1</sup> vermicompost <sup>a</sup>	73.6 a	19.8 a	12.1 a	4.7 a	0.2 a
Vermiwash prepared from 7 Mt ha <sup>-1</sup> vermicompost	66.8 ab	19.4 ab	11.6 a	4.2 ab	0.2 a
7 Mt ha <sup>-1</sup> vermicompost leachate + vermiwash	65.5 ab	18.5 ab	10.5 ab	4.5 a	0.2 a
50 Mt ha <sup>-1</sup> municipal solid waste compost	58.3 bc	17.4 ab	9.0 bc	4.2 ab	0.2 a
Chemical fertilizer	57.8 bc	16.7 b	8.2 c	3.7 b	0.1 b
Control	49.5 c	13.3 c	7.0 d	2.9 c	0.1 b

Treatment	Total fresh yield (kg/h)	Total dry yield (kg/h)	Content of oil per plant (ml/plant)	Oil yield (ml/m <sup>2</sup> )
7 Mt ha <sup>-1</sup> vermicompost <sup>a</sup>	2,430 a	990 a	0.24 a	24.2 a
Vermiwash prepared from 7 Mt ha <sup>-1</sup> vermicompost	2,346 ab	874 ab	0.22 ab	19.2 abc
7 Mt ha <sup>-1</sup> vermicompost leachate + vermiwash	2,125 bc	949 a	0.21 abc	20.3 ab
50 Mt ha <sup>-1</sup> Municipal solid waste compost	1,876 c	870 ab	0.20 abc	17.5 bc
Chemical fertilizer	1,597 d	783 b	0.19 bc	15.0 dc
Control	1,412 d	621 c	0.17 c	10.4 d

Treatment	Chlorophyll a (mg/100 g)	Chlorophyll b (mg/100 g)	Total chlorophyll (mg/100 g)	Carotenoids (mg/100 g)	Total phenol (mg gallic acid equivalent/100 g)	Antioxidant capacity (% of inhibition)
e7 Mt ha <sup>-1</sup> vermicompost	7.72 a	4.03 a	11.75 a	549.94 a	6.42 a	66.65 a
Vermiwash prepared from 7 Mt ha <sup>-1</sup> vermicompost	7.22 a	3.95 a	11.17 a	523.55 a	6.25 a	66.20 a
7 Mt ha <sup>-1</sup> vermicompost leachate + vermiwash	7.18 a	3.91 a	11.09 a	518.42 a	6.48 a	66.14 a
50 Mt ha <sup>-1</sup> municipal solid waste compost	5.53 b	3.17 b	8.71 b	426.24 b	6.51 a	66.11 a
Chemical fertilizer	4.94 bc	2.89 b	7.84 bc	422.78 b	6.33 a	66.21 a
Control	4.84 c	2.76 b	7.61 c	416.33 b	6.40 a	66.68 a

<sup>a</sup> Values in columns followed by the same letter are not significantly different by Tukey's test at  $P < 0.01$

decreased and continuous application of this material may be possible. The leachate vermicompost, vermiwash and vermicompost can be used as organic fertilizers for sustainable peppermint cultivation.

## Conclusion

The results indicated that there were significant differences between cultivation places in all measured characteristics. Locations with low sunshine, moderate temperature and high rainfall (Rasht) have higher fresh herbage yield, while locations with high sunshine, high temperature and low rainfall (Torbat-e-jam) have higher oil yield and antioxidant capacity. Most differences between the two places were related to temperature and it seems that a temperate location is more suitable for peppermint oil production, while a humid location increases the vegetative growth of

peppermint. The results also showed that there were no significant differences between plants treated with vermicompost, vermiwash and vermicompost leachate + vermiwash in all measured characteristics. However, there are significant differences between organic fertilizers and chemical fertilizers in most measured characteristics. Organic fertilizers beneficially affect soil structure and nutrient availability; they maintain quantity and quality of yield and can be less costly than synthetic fertilizers. The use of sustainable organic materials can increase fertility without negative effects on human health and environment. We counsel farmers to use leachate vermicompost and vermiwash in organic cultivation of peppermint, separately or mixed together.

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