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EFFECT OF VERMICOMPOST AND VERMICOMPOST EXTRACT ON OIL YIELD AND QUALITY OF PEPPERMINT (*Mentha piperita* L.)

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Abstract: Organic fertilizers have beneficial effects on plants growth and quality. However, vermicompost increases electrical conductivity in soil due to increased salinity associated with continued usage. The experiment was conducted in a research field at the University of Guilan to determine effects of 7 Mt ha^{-1} of cow manure vermicompost, vermiwash prepared from 7 Mt ha^{-1} of vermicompost, leachate vermicompost + vermiwash, 50 Mt ha^{-1} municipal solid waste compost (MSWC), chemical fertilizer (NPK 50–0–300) and no fertilization as a control on peppermint yield and quality. Fertilizer type affected all measured characteristics except number of nodes, stem fresh weight, total phenols and antioxidant capacity. Plants treated with vermicompost, vermiwash or leachate vermicompost + vermiwash were the tallest and had the highest levels of chlorophyll a, chlorophyll b, total chlorophyll and carotenoids. Plants treated with vermicompost or vermiwash had the highest total plant fresh weight, leaf fresh weight and total fresh yield. The leachate vermicompost, vermiwash and vermicompost can be used as organic fertilizers for sustainable peppermint cultivation.

Key words: *Mentha piperita*, Iran, fertilizer, organic farming, sustainable agriculture.

Introduction

Peppermint (*Mentha piperita* L., *Lamiaceae*) oil has a long history of safe use both in medicinal preparations and as a flavoring agent in foods and confectionery (Milovanović et al., 2009). Peppermint oil is used externally and internally. In Iran, peppermint is a perennial crop established with transplants in the spring and has an average stand life of about six years. In Guilan province, the crop is harvested only once a season. For the highest oil yields, growers apply enormous amounts of nitrogen (N) fertilizer to maximize oil yield because stress such as insufficient nitrogen reduces yield (Grigoleit and Grigoleit, 2005; Jelačić et al., 2005).

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If appropriate amounts of fertilizers are not applied during production, physiological symptoms of deficiency can occur in plants (Takahashi, 1981; Olfati et al., 2012). Most producers use synthetic fertilizers because they are easy to transport, quickly available to plants, and produce high yields. However, with succeeding crops, quantities of chemical fertilizers must be increased because of low soil fertility (Thy and Buntha, 2005). Organic fertilizers have beneficial effects on soil structure and nutrient availability, help maintain yield and quality, and are less costly than synthetic fertilizers (Olfati et al., 2012; 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 production of herbs or to restore soil fertility (Benton and Wester, 1998) as they contain large quantities of nitrogen (N), phosphorous (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 they are 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 the positive effects on soil and vegetables are known from numerous studies (Gutierrez-Miceli et al., 2007; Peyvast et al., 2007, 2008a, 2008b, 2008c, 2008d; Olfati et al., 2009; Shabani et al., 2011). However, thermophilic composting is generally time-consuming, requiring frequent mixing with possible loss of nutrients.

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 temperature (Contreras-Ramos et al., 2004). There is evidence that earthworms produce plant hormones in their secretions (Suthar, 2010b). Earthworm processed materials ‘casts’ contain nutrients in forms easily available to plants (Suthar and Singh, 2008; Suthar, 2010a).

Greenhouse and field studies examined the effects of vermicompost on cereals and legumes (Chan and Griffiths, 1988), vegetables (Peyvast et al., 2008a, 2008b, 2008c, 2008d; Kochakinezhad et al., 2012; Ayyobi et al., 2013), and field crops (Buckerfield and Webster, 1998). Most of these investigations confirmed that vermicomposts have beneficial effects on plant growth (Atiyeh et al., 1999). Szczech (1999) suggested that chemical factors in vermicompost had no direct inhibiting effect on the soilborne fungus, but that bacteria and fungi in the vermicompost were antagonistic to *Fusarium oxysporum* Schlecht.

The final vermicomposting product has a high electrical conductivity (EC) which increases soil salinity with continued usage. To reduce EC, leachate vermicompost and vermiwash have been developed. Vermiwash may contain cytokinins, auxin, amino acid, vitamins, and enzymes possibly derived from microbes associated with earthworms (Suthar, 2010b). There is a demand for naturally derived agro-chemicals for sustainable farming systems, and organic production disallows the use of synthetic chemicals. There is no comprehensive study concerning the impact of organic fertilizer including vermiwash, leachate vermicompost or municipal solid waste compost on peppermint.

This study was undertaken to determine effects of these organic fertilizers in comparison with chemical fertilizer on oil yield and quality of peppermint.

Material and 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. The soil was a loam, pH 7.44, containing total N (1%), total C (1.08%), and there were 4,600, 1,700, and 4,000 mg·kg^{-1} of Ca, P, and K, respectively, in soil dry matter (DM), with an electrical conductivity (EC) of 0.1 dS cm^{-1}. The soil was prepared by plowing and disking. Local clones of peppermint were established by cutting on 15 April. Each plot area was 4 m^2 containing 80 plants.

Earthworms (*Eisenia fetida*) (25 g earthworms·kg^{-1} of cattle manure or 2.5 kg earthworms m^{-2} per bed) were added and vermicomposted for two months (Peyvast et al., 2008a, 2008b). The vermicompost had a water content of 380 g kg^{-1}, 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 leachate (vermiwash) was collected. Leachate vermicompost, after collecting vermiwash, was also stored for the next usage.

A completely randomized block design with three replications was used. Treatments included 7 Mt ha^{-1} of cow manure vermicompost, vermiwash prepared from 7 Mt ha^{-1} of vermicompost, leachate vermicompost + vermiwash, 50 Mt ha^{-1} municipal solid waste compost (MSWC), chemical fertilizer (NPK 50–0–300) and no fertilization as a control. Vermicompost, MSWC, and leachate vermicompost were spread over beds, and vermiwash was applied four times to plants at a seven-day interval with the first application one month after cutting.

At harvest, total leaf dry and fresh yield, oil yield, number of leaves, nodes and lateral branches per plant, leaves, stems and total plant fresh and dry weight, internode length, and plant height, leaf area index, content of essential oil per plant, total phenol, carotenoids and total chlorophyll, chlorophyll a, chlorophyll b, and antioxidant capacity were determined. Methanol extracts of sample (1 g of sample in 10 ml of methanol) were used for the determination of total phenolics. 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 galic 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 Miliauskas 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^{-1}) 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 hours to determine dry matter. 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 hours using a Clevenger apparatus.

Data were subjected to ANOVA in SAS (ver. 9.1, SAS Institute, Inc., Cary, NC). Means were separated using the Tukey test.

Results and Discussion

Fertilizer type affected all measured characteristics except number of nodes, stem fresh weight, total phenol and antioxidant capacity (Tables 1–4). The number of nodes, stem fresh weight, total phenol and antioxidant capacity averaged 11.48, 6.99 g, 5.35 mg galic acid equivalent/100 g sample and 60.78% respectively.

Table 1. ANOVA table effects of different fertilizers on vegetative characteristics.

Source of variation	df	Mean square				
		Plant height	Number of leaves	Number of nodes	Number of lateral branches	Internode length
Replication	2	77.35^{*}	10660.5^{**}	8.44^{*}	13.08^{**}	0.04^{ns}
Treatment	5	221.89^{**}	1571.56^{**}	0.41^{ns}	16.82^{**}	1.11^{*}
Error	10	14.38	267.71	1.31	1.42	0.19
C.V. (%)		6.06	17.05	9.98	6.39	7.28

^{ns}, *, **: non-significant or significant at P $\leq$ 0.01 and P $\leq$ 0.05, respectively.

Table 2. ANOVA table effects of different fertilizers on total plant, leaf and stem fresh and dry weight.

Source of variation	df	Mean square					
		Total plant fresh weight	Total plant dry weight	Leaf fresh weight	Leaf dry weight	Stem fresh weight	Stem dry weight
Replication	2	11.6^{**}	1.14^{**}	5.11^{**}	0.33^{**}	1.97^{ns}	0.58^{**}
Treatment	5	12.3^{**}	1.18^{**}	4.2^{**}	0.43^{**}	2.24^{ns}	0.31_{*}
Error	10	0.34	0.05	0.1	0.02	0.93	0.06
C.V. (%)		5.55	5.23	6.93	8.72	13.79	12.74

^{ns}, *,**: non-significant or significant at P $\leq$ 0.01 and P $\leq$ 0.05, respectively.

Table 3. ANOVA table effects of different fertilizers on leaf area index (LAI), total fresh and dry yield, oil yield, and content of oil per plant.

Source of variation	df	Mean square				
		LAI	Total fresh yield	Total dry yield	Oil yield	Content of oil per plant
Replication	2	0.001^{*}	463800.88^{**}	45662.0^{**}	6.87^{ns}	0.004^{**}
Treatment	5	0.002^{**}	491849.42^{**}	47357.86^{**}	52.57^{**}	0.001^{*}
Error	10	0.0002	13632.08	2359.86	5.25	0.0004
C.V. (%)		8.23	5.55	5.23	13.5	12.1

^{ns}, *, **: non-significant or significant at $P \leq 0.01$ and $P \leq 0.05$, respectively.

Table 4. ANOVA table effects of different fertilizers on total phenol, antioxidant capacity, chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids content.

Source of variation	df	Mean square					
		Total phenol	Antioxidant capacity	Chlorophyll a	Chlorophyll b	Total chlorophyll	Carotenoids
Replication	2	0.2^{ns}	0.56^{ns}	0.59^{**}	0.55^{**}	1.52^{**}	6702.05^{**}
Treatment	5	0.16^{ns}	4.73^{ns}	4.95^{**}	1.001^{**}	10.28^{**}	11037.49^{**}
Error	10	0.37	2.1	0.06	0.03	0.14	501.74
C.V. (%)		11.37	2.38	5.05	6.98	4.79	4.21

^{ns}, **: non-significant or significant at $P \leq 0.01$, respectively.

Plants treated with vermicompost, vermiwash or leachate vermicompost + vermiwash were the tallest plants and had the highest levels of chlorophyll a, chlorophyll b, total chlorophyll and carotenoids (Tables 5 and 8). Differences between plants treated with organic fertilizers were not significant for leaf area index (Table 7) where all plants treated with organic fertilizer had the higher leaf area index than the plants treated with chemical fertilizer and the control.

Table 5. Effects of different fertilizers on vegetative characteristics.

Treatment	Plant height (cm)	Number of leaves	Number of nodes	Number of lateral branches	Internode length (cm)
Vermicompost (7 Mt ha^{-1})	74.5a	131.07a	11.56a	20.91a	6.51a
Vermiwash prepared from 7 Mt ha^{-1} vermicompost	68.6ab	99.7ab	11.97a	20.50a	6.48a
Leachate vermicompost + vermiwash (7 Mt ha^{-1})	64.3ab	105ab	11.68a	19.59a	6.37a
Municipal solid waste compost (50 Mt ha^{-1})	60.8bc	98.33ab	11.6a	18.50ab	6.29a
Chemical fertilizer (NPK 50–0–300)	57.23bc	73.6ab	10.99a	17.82ab	4.90b
Control	50.16c	67.93b	11.1a	14.42b	6.18a

Values in columns followed by the same letter are not significantly different by Tukey test at $P < 0.05$.

There are no significant differences between different fertilizers for number of leaves, number of lateral branches, total plant dry weight, leaf dry weight, stem dry weight, total dry yield, oil yield and oil content per plant while the lowest values were related to plants cultivated without fertilization (Tables 5–7). Chemical fertilizer decreased plant internode length while there were no significant differences between other treatments. Plants treated with vermicompost or vermiwash had the highest total plant fresh weight, leaf fresh weight and total fresh yield (Tables 6–7).

Table 6. Effects of different fertilizers on total plant, leaf and stem fresh and dry weight.

Treatment	Total plant fresh weight (g)	Total plant dry weight (g)	Leaf fresh weight (g)	Leaf dry weight (g)	Stem fresh weight (g)	Stem dry weight (g)
Vermicompost (7 Mt ha^{-1})	13.02a	5.22a	5.96a	1.90a	7.36a	2.22a
Vermiwash prepared from 7 Mt ha^{-1} vermicompost	12.68a	4.80a	5.89a	1.87a	8.26a	2.45a
Leachate vermicompost + vermiwash (7 Mt ha^{-1})	10.34b	5.17a	4.48b	1.86a	6.53a	1.90ab
Municipal solid waste compost (50 Mt ha^{-1})	10.25b	4.78a	4.38b	1.88a	7.36a	1.90ab
Chemical fertilizer (NPK 50–0–300)	8.85bc	4.34ab	4.36b	1.40ab	6.68a	2.00ab
Control	7.93c	3.53b	2.77c	0.98b	5.75a	1.50b

Values in columns followed by the same letter are not significantly different by Tukey test at $P < 0.05$.

Table 7. Effects of different fertilizers on leaf area index (LAI), total fresh and dry yield, oil yield, and content of oil per plant.

Treatment	LAI	Total fresh yield (g)	Total dry yield (g)	Oil yield (ml/m^2)	Oil content per plant (ml/plant)
Vermicompost (7 Mt ha^{-1})	0.19a	2604.67a	1044.67a	22.90a	0.22a
Vermiwash prepared from 7 Mt ha^{-1} vermicompost	0.19a	2536.00a	960.00a	18.57ab	0.19ab
Leachate vermicompost + vermiwash (7 Mt ha^{-1})	0.19a	2068.67b	1034.67a	18.15ab	0.17ab
Municipal solid waste compost (50 Mt ha^{-1})	0.17ab	2050.00b	956.00a	17.07ab	0.18ab
Chemical fertilizer (NPK 50–0–300)	0.13b	1771.33bc	869.33ab	14.77ab	0.17ab
Control	0.13b	1586.67c	707.33b	10.36b	0.15b

Values in columns followed by the same letter are not significantly different by Tukey test at $P < 0.05$.

Organic fertilizers beneficially affect soil structure and nutrient availability, maintain quantity and quality of yield and can be less costly than synthetic fertilizers (Thy and Buntha, 2005; Olfati et al., 2012). The application of organic fertilizers may help alleviate soil erosion (Shahvali and Abedi, 2006) and saline and sodium problems as a result of excessive chemical fertilization and irrigation (Allahyari et al., 2008). The use of sustainable organic materials can increase fertility without negative effects on human health and environment. Vermicompost (Gutierrez-Miceli et al., 2007; Peyvast et al., 2008a, 2008b) and vermiwash (Suthar, 2010a) have been proposed as organic fertilizers previously. Most investigations confirmed that vermicomposts are beneficial to plant growth (Chan and Griffiths, 1988; Buckerfield and Webster, 1998; Atiyeh et al., 2000; Peyvast et al., 2008a, 2008d).

Table 8. Effects of different fertilizers on total phenol, antioxidant capacity, chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids content.

Treatment	Total phenol (mg GAE/ 100 g)	Antioxidant capacity (% of inhibition)	Chlorophyll a (mg/100 g)	Chlorophyll b (mg/100 g)	Total chlorophyll (mg/100 g)	Carotenoids (mg/100 g)
Vermicompost (7 Mt ha^{-1})	5.66a	62.79a	6.59a	3.42a	10.01a	605.55a
Vermiwash prepared from 7 Mt ha^{-1} vermicompost	5.36a	61.33a	6.40a	3.34a	9.74a	579.16a
Leachate vermicompost + vermiwash (7 Mt ha^{-1})	5.55a	59.92a	5.78a	3.29a	9.07a	574.03a
Municipal solid waste compost (50 Mt ha^{-1})	5.35a	61.24a	4.06b	2.55b	6.62b	481.85b
Chemical fertilizer (NPK 50–0–300)	5.18a	59.97a	3.97b	2.28b	6.25b	471.94b
Control	5.01a	59.40a	3.87b	2.14b	6.02b	478.40b

Values in columns followed by the same letter are not significantly different by Tukey test at $P < 0.05$.

Leachate vermicompost contains slightly higher amount of sodium (Na), while vermiwash contains a high level of potassium (K), which as a primary nutrient, needed in high amounts for plant growth (Atiyeh et al., 1999; 2000).

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) decreased and the 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 are no significant differences between plants treated with vermicompost, vermiwash and leachate vermicompost + vermiwash in most measured characteristics. However, there are significant differences between organic fertilizer and chemical fertilizer in most measured characteristics. Organic fertilizers beneficially affect soil structure and nutrient availability, 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 pippermint separately or mixed together.

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UTICAJ GLISTENJAKA I EKSTRAKTA GLISTENJAKA NA PRINOS ULJA I KVALITET NANE (*Mentha piperita* L.)

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R e z i m e

Organska đubriva pozitivno utiču na rast i kvalitet biljaka. Međutim, glistenjak povećava električnu provodljivost u zemljištu usled povećanog saliniteta povezanog sa stalnom upotrebom. Ogled je izveden na istraživačkom polju Univeziteta u Gilanu kako bi se utvrdili uticaji 7 t ha^{-1} glistenjaka kravljeg stajnjaka, tečnog đubriva dobijenog ekstrakcijom od 7 t ha^{-1} glistenjaka, ispranog glistenjaka + tečnog ekstakta glistenjaka, 50 t ha^{-1} komunalnog komposta od čvrstog otpada (MSWC), hemijskog đubriva (NPK 50–0–300) i bez đubrenja kao kontrole na prinos i kvalitet nane. Đubriva su ispoljila uticaj na sve merene osobine osim broja nodusa, težinu svežeg stabla, sadržaj ukupnih fenola i antioksidativni kapacitet. Biljke koje su tretirane glistenjakom, tečnim ekstraktom glisenjaka ili ispranim glistenjakom + tečnim ekstaktom glistenjaka su bile najviše i imale su najviše nivoe hlorofila a, hlorofila b, ukupnog hlorofila i karotenoida. Biljke tretirane glistenjakom ili tečnim ekstaktom glistenjaka su imale najvišu ukupnu svežu masu biljke, svežu masu lista i ukupan prinos sveže biljke. Isprani glistenjak, tečni ekstrakt glistenjaka, kao i glistenjak se mogu koristiti kao organska đubriva za održivo uzgajanje nane.

Ključne reči: *Mentha piperita*, Iran, đubrivo, organska poljoprivreda, održiva poljoprivreda.

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