

Yield and Oil Content of Mint under Different Nitrogen Fertilizer Treatments

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Abstract

Two species of mint *Mentha piperitha* (peppermint) and *M. arvensis* (Japanese mint) are widely cultivated in Iran, but their response to fertilizer regime has not been evaluated so far. A field experiment was conducted to investigate the effects of different organic and chemical fertilizer treatments [Control, 100% urea (95 kg N ha⁻¹), 75% urea (71.25 kg N ha⁻¹) + 25% vermicompost (3.3 t ha⁻¹), 50% urea (47.5 kg N ha⁻¹) + 50% vermicompost (6.75 t ha⁻¹), 25% urea (23.75 kg N ha⁻¹) + 75% vermicompost (10.1 t ha⁻¹) and 100% vermicompost (13.5 ton ha⁻¹)] on essential oil contents, yield and yield components of the two species of mint. Peppermint provided grater plant height, number of internodes, number of leaf and oil percentage compared with the Japanese mint under study. The results indicated that, irrespective of the mint species, plants treated with combined chemical and organic fertilizer presented taller plants, higher oil contents and oil yield compared with solo chemical or organic fertilizers. Oil percentage and essential oil yield of mint increased significantly under the treatment with 25% urea (23.75 kg N ha⁻¹) + 75% vermicompost (10.1 t ha⁻¹). Plant height and number of leaf increased along the replacement of organic fertilizer with chemical fertilizers. The results showed that there was a positive and significant correlation with leaf number and essential oil yield. Application of vermicompost in combination with chemical fertilizer increased plant height, oil percentage and essential oil in both species, suggesting that organic and chemical fertilizer combination improves performance and environmental sustainability.

Keywords: integrated fertilizer; japanese mint; nitrogen; peppermint; vermicompost

Introduction

In recent decades, efforts have been made to reduce the usage of synthetic N fertilizers and improve the efficiency of them so to reduce their impact on the environment. Integrate nutrient management approaches the controlled use of nutrient fertilizers. Fertilizers' management refers to application of suitable amounts of organic and inorganic fertilizers, in order to improve the health and productivity of soil and persuade plant growth (Ram *et al.*, 2012). Organic nutrients enhance the activity of soil organisms by providing organic matter for organisms such as fungal mycorrhiza which help plants in absorbing micro and macronutrients (Tilman *et al.*, 2002). On the other hand, chemical nutrients may have long-term adverse effects on soil properties and living organisms (García *et al.*, 2014). High application rate of nitrogen-based fertilizers can lead to increased leaching of nitrate into grand water (Yang *et al.*, 2015). To increase nitrogen use efficiency and decrease negative effects of them on the environment, the fertilizer

supply showed match during the growing season (Tilman *et al.*, 2002). Ammonium ions absorbed preferentially over nitrate ions, which means excess nitrate ions dissolved by irrigation or rain into grand water. However, organic fertilizer improved the physical and biological properties of soil and reduced the effect of over fertilization.

For organic fertilizers, nutrient release rate are much lower than mineral (inorganic) fertilizers (García *et al.*, 2014). Substitution of chemical fertilization with organic one or combining the two methods may be useful in many ecosystem processes such as nutrient cycling.

Vermicompost is one of the organic fertilizers, its nutrient profile being generally higher than other composts. Vermicompost can increase soil properties physically, chemically and biologically (Hussein *et al.*, 2006). Soil treated with vermicompost has better porosity, aeration bulk density and water retention. Also, chemical properties such as EC and pH improved. Vermicompost can increase the size, biodiversity and activity of microbial population in the soil. Application of vermicompost promotes root growth and makes easier nutrient absorption. Plants grown with vermicompost contain higher levels of the main

nutrients required for plant growth, like nitrogen, compared to untreated plants (Ram *et al.*, 2012). Increase in biological activity plant growth regulators or humic acid in the vermicompost are also responsible for enhancing plant growth. Roy *et al.* (2010) showed that plant height and shoot weight were the highest in the vermicompost treatment. The combination of vermicompost and chemical fertilizer also resulted in higher leaf area and shoot weight for common bean related to vermicompost alone (Suhane *et al.*, 2008). Chand *et al.* (2004) observed that organic fertilizer had great effects on the growth of mint in terms of root and shoot dry weight, number of leaves per plant, leaf area and the mean plant height.

The genus *Mentha* is one of the important members of Lamiaceae family, containing 25 species such as *M. piperita* and *M. arvensis*. These species are fast growing and generally tolerant to a wide range of climate, so that can be cultivated in many countries. Their essential oil is considered industrially and widely used in pharmaceuticals, confectionery and cosmetic.

The current investigation aimed to study the yield and oil yield of two mint species affected by various fertilizers.

Materials and Methods

Field experiments were carried out in 2016 at the field research station of the Agricultural Research Center of Tarbiat Modares University in Karaj.

The climate in this region is semi-arid, with warm and dry summers, a mean annual rainfall of 286.6 mm and a mean annual temperature of 12 °C. The soil is classified as a sandy loam, with 38% clay, 22% silt and 40% sand. The soil was air-dried and crushed before its pH and electrical conductivity (EC) were evaluated. Next there were determined the total organic carbon (using the Walkley and Black method, which involves sulfuric acid), total nitrogen (using the Kjeldahl method), available phosphorus (using the Olsen procedure), available potassium after extraction with ammonium acetate, and levels of the micronutrients iron, zinc, copper, and manganese after extraction with diethylene triamine pentaacetic acid (Tandon, 1995). Details of the properties of field soil and properties of the organic compost are shown in Table 1.

The experiment was conducted as factorial in a randomized complete block design with three replications. According to the recommended N requirements (120 kg/ha) and soil N content for peppermint, 65 kg ha⁻¹ N was supplied from fertilizer, using urea as N source, at two time points, before rhizome planting and remaining half of the urea was applied at fourth leaf stage respectively. Also, the crops were fertilized with 40 kg P₂O₅ ha⁻¹ phosphorous as triple superphosphate (TSP). The fertilizers were incorporated into the soil before bed formation.

The treatments included two species (S1: *M. piperita* and S2: *M. arvensis*), and six fertilization regimes: F1: Control, F2: 100% urea (95 kg N ha⁻¹); F3: 75% urea (71.25 kg N ha⁻¹) + 25% vermicompost (3.3 t ha⁻¹); F4: 50% urea (47.5 kg N ha⁻¹) + 50% vermicompost (6.75 t ha⁻¹); F5: 25% urea (23.75 kg N ha⁻¹) + 75% vermicompost (10.1 t ha⁻¹), and F6: 100% vermicompost (13.5 t ha⁻¹). The

physicochemical properties of the organic compost are shown in Table 3.

The vermicompost (which was obtained from KODSA organic products company, Iran) and half of the total urea applied were broadcast by hand and incorporated immediately into the soil using a rototiller, three days before planting. The remaining half of the urea was applied as top dressing when the mint seedlings were at the four-leaf stage.

Plots were 2.5 m long and consisted of six rows, with 25 cm spacing between the rows (plot size 3.125 m²). A two m alley was maintained between all plots to eliminate any influence of lateral water movement.

Mint seeds are available, but these species are natural hybrids (Tucker and Fairbrothers, 1990) and their future generation may produce seedling with different phenotype or oil percentage. Therefore, commercial mint species procreated only vegetatively (Lawrence, 2006) were used for the hereby study. To minimize disease and pest pressure within the research, certified virus free planting rhizomes were achieve from Medicine plant research, Inc, Karaj, Iran. The plants initiated from rhizome cuttings (7 cm long) and were planted directly by hand on 9 April 2016. The plants were thinned at the four leaf stage to achieve a density of approximately 20 plants m⁻². Weeds were controlled by hand-weeding using a hoe and/or a rototiller whenever necessary.

Because of the sandy soil, plots were irrigated frequently (at an interval of 1-2 days) with drip irrigation system, to avoid wilting at during the plant growth. The plants were harvested at lasting of August, at floral initiation, by cutting plants about 10 cm above the soil surface (Hussein *et al.*, 2006).

Data of growth parameters were measured: plant height (cm), fresh and dry weights of herbage (g). Fresh weight of all plots was taken as fresh herbage yield. In term of dry herbage yield, the dry weight of 1 kg fresh herb dried and weighted was recorded after being air-dried for 24 h to avoid loss of essential oils (Topalov, 1989). The leaf content of samples was calculated after separating the leaf of dry herb samples. An all-glass Clevenger-type apparatus was used to conduct 2.5 h of hydro-distillation on dried aerial parts (which were collected at floral initiation) of the two species under study and expressed as ml 50 g⁻¹ fresh herb. This method for the extraction of oils is recommended by the European Pharmacopoeia (European Pharmacopoeia, 1983).

Analysis of variance (ANOVA) of the data from each attribute was computed using the SAS package (SAS Institute, 2002). Significance of differences among species (S), various fertilizers (F) and their interaction (S×F) for variables were compared by LSD test (P≤0.05). Microsoft office Excel (2007) was used for figures drawing. The simple correlation among the parameters studied was also calculated to explore relationship between all characterizes.

Results and Discussion

Numerical data of yield for fresh and dry herb, essential oil and yield components with their statistical results are summarized in Table 2. Species treatment had significant

Table 1. General properties of top soil (0-30) and organic fertilizer (vermicompost)

	Soil	Vermicompost
pH	7.70	5.7
EC (ds m ⁻¹)	1.24	3.1
Organic carbon (%)	0.81	31.4
Total N (%)	0.08	3
Available P (mg kg ⁻¹)	4.00	1.51
Available K (mg kg ⁻¹)	150	1.3
Available Fe (mg kg ⁻¹)	8.40	0.5
Available Zn(mg kg ⁻¹)	1.14	112
Available Cu(mg kg ⁻¹)	2.60	43
Available Mn(mg kg ⁻¹)	7.40	992

Table 2. Mean square values from analysis of variance (ANOVA) of yield and yield components of two species of mint under effect of genotype and fertilizer

S.O.V	df	Plant height	Main branch	Stem diameter	No. of internode	No. of leaf	Oil percentage	Fresh herbage yield	Dry herbage yield	Essential oil yield	Harvest index
Repetition	2	*	**	ns	ns	ns	*	ns	ns	ns	ns
Species	1	**	Ns	ns	**	**	**	ns	ns	ns	ns
Fertilizer	5	**	Ns	ns	ns	ns	*	ns	ns	**	ns
Genotype × Fertilizer		ns	Ns	ns	ns	ns	ns	ns	ns	ns	ns
CV		4.98	10.1	7.9	6.2	7.2	10.9	12.6	17.2	18.1	23.1

Table 3. Main effects of genotype and fertilizer regimes on yield and yield components of mint species

Treatment	Parameters				
Genotype	Plant height	No. of internodes	No. of leaf	Oil percentage	Essential oil yield
Peppermint	75.4 a	30.6 a	59.7 a	0.57 a	20.1 a
Japanese mint	67.7 b	27.9 b	53.4 b	0.51 b	18.4 a
Fertilizer					
F1	65.1 c	27.8a	52.6b	0.50dc	20.0ab
F2	70.4 b	29.9a	56.9ab	0.54bc	21.7a
F3	75.3 a	28.4a	56.9ab	0.51dc	16.8bc
F4	75.2 a	29.6a	57.4ab	0.60ab	20.8ab
F5	72.4 ab	30.0a	58.2a	0.64a	23.4a
F6	70.90 ab	29.8a	57.6ab	0.45d	13.9c

For a given mean within each column of each section, the same letters are not significantly different ($p \leq 0.05$).

effect on plant height, number of internodes, number of leaf and oil percentage. Different doses of organic and inorganic fertilizer treatments resulted in significant differences of plant height, oil percentage and essential oil yield (Table 3). Although, interaction between species and fertilizer had no significant differences in any of parameters studied. However, main branch, stem diameter, fresh and dry herbage yield and harvest index were not affected by treatments. Mean effect of treatment are discussed below, in order of their statistical significance.

Plant height

Mean comparison showed that plant height were lower for Japanese mint. Among fertilizers' treatments, F3 showed the maximum plant height. However, F4 and F3 were not significantly different (Table 4). Actually, plant height was higher in all integrated fertilizer treatment compared with only chemical treatment.

Presumably, the application of vermicompost improved soil chemical, biological and physical properties and increased root activity, conducting to enhancement of plant absorption. Even more, unique mineral properties of vermicompost such as high association for NH₄ (Alidadi *et al.*, 2016) and high CEC, resulted in holding ammonium

in treated plants with vermicompost. When vermicompost was applied to the soil, the ammonium ions were slowly exchanged and oxidized by nitrifying bacteria. In this way, the soil N concentration and N uptake by plants significantly increased (Yang *et al.*, 2015).

Number of internodes and leaf number

Number of internodes and leaf number significantly responded to the two species under study. Results showed that peppermint significantly had more internodes and leafs per plant (Table 3). As show in Table 3, fertilizer treatments did not affect these two traits significantly, but numerically F₅ had the highest number of internodes and leaf per plant. In fact, combination of chemical organic fertilizer treatment performed better than chemical or solo organic fertilizer in regard to plant internodes and leaf number. Vermicompost has high microbial activity due to the presence of bacteria, fungi and actinomycetes that can product plant growth regulators (PGRs) such as auxin, gibberellins, cytokinins and abscisic acid (Frankenberger *et al.*, 1995). These results agreed with reports by other researchers who reported that co-application of organic fertilizers and chemical fertilizers improved crop production (Blaise *et al.*, 2005; Hassanzadeh *et al.*, 2011).

Table 4. Correlation analysis between traits of two species of mint exposed to various fertilizers' treatments

Traits	Plant height	Main branch	Stem diameter	No. of internodes	No. of leaf	Oil percentage	Fresh herbage yield	Dry herbage yield	Essential oil yield	Harvest index
Plant height	1	0.43**	0.15 ^{ns}	0.33*	0.55**	0.23 ^{ns}	0.01 ^{ns}	-0.06 ^{ns}	0.08 ^{ns}	-0.08 ^{ns}
Main branch		1	0.40*	0.08 ^{ns}	0.08 ^{ns}	0.10 ^{ns}	-0.14 ^{ns}	-0.17 ^{ns}	-0.05 ^{ns}	0.30 ^{ns}
Stem diameter			1	0.21 ^{ns}	0.02 ^{ns}	0.20 ^{ns}	0.30 ^{ns}	0.28 ^{ns}	0.30 ^{ns}	-0.02 ^{ns}
No. of internodes				1	0.80**	0.46**	0.19 ^{ns}	0.15 ^{ns}	0.39*	-0.16 ^{ns}
No. of leaf					1	0.48**	0.08 ^{ns}	0.05 ^{ns}	0.33*	-0.16 ^{ns}
Oil percentage						1	0.03 ^{ns}	0.04 ^{ns}	0.66**	0.01 ^{ns}
Fresh herbage yield							1	0.97**	0.72**	-0.71**
Dry herbage yield								1	0.76**	-0.71**
Essential oil yield									1	-0.51**
HI										1

Oil percentage and essential oil yield

Oil percentage and essential oil yield in mint were remarkably impressed by species and fertilizer treatments. Although, most of yield components of mint significantly responded to species and integrated fertilizers, fresh and dry herbage were not affected by treatments (Table 2). Similar to oil percentage, essential oil yield was also improved when integrated fertilizers were applied. Regardless of the species, 75% vermicompost + 20% urea (F5) outperformed than other fertilizers (Table 3). None of the species and fertilization affected fresh and dry weight and harvest index. Further, peppermint showed remarkably greater number of leaf, oil percentage and oil yield than Japanese mint (Table 3).

In the current experiment, it was found a positive and significant correlation between the number of internodes and the number of leaves with oil percentage and essential oil yield (Table 4). These results showed that the production of leaves was higher for peppermint. In mint, leaves are the site where oil synthesis and accumulation is taken place. Therefore, it has special significance for improving the oil percentage and yield. This could also be the reason for the higher oil percentage and essential oil yield in peppermint (Table 4). Under the F5 treatment, plants had the oil percentage and essential oil yield of 0.64% and 23.4% kg/ha respectively, which were significantly higher than plants treated with solo chemical or vermicompost. The advantage of oil percentage and oil yield following integrated fertilization could have raised from modification in soil chemical, physical and microbial population, which all of them can affect yield and yield components. Vermicompost has large particulate surface area that is suitable for the strong retention of nutrients. Yang *et al.* (2015) reported that vermicompost application could increase N uptake by plants. Consequently, plants which received organic fertilizers provide greater nutrients than those which received chemical fertilizers. Aziz *et al.* (2009) have found a positive correlation between vermicompost and oil yield of chamomile. Base on previews results, most nutrient are easily available in vermicompost such as soluble potassium, nitrate and phosphate, which can be responsible for plant growth increase and high crop yield

(Yang *et al.*, 2015). This result verifies again the advantage of integrated fertilizer over the other studies related essential oil yield production. Due to special properties of vermicompost, it provides good condition for microbial activity. Thus, it is not unexpected that soil treated with vermicompost is rich in plant growth regulation. Hormones like amylase, lipase, protease, pectinase and cellulose that cause decomposition of organic matter in the soil and provide the necessary nutrient for plant are present in higher concentrations. Improvement in soil hormones and available N, P and K due to integrated fertilizer have been reported in wheat by Suhane *et al.* (2008) and peppermint Ram *et al.* (2012).

Humic acid, and other plant regulator hormones, is another substance that can be found in vermicompost. It is demonstrated that soil bacteria can produce auxins, gibberellins and kinetin-like substances that had positive effect on plant growth directly (Barea *et al.*, 1976). It is concluded that plant regulators in the vermicompost could also have had influence on the plant growth and increase yield of mint. Some of the effects of humic acid on yield and yield component of plants have been shown (García *et al.*, 2014).

Conclusions

Yield of mint increased dramatically with the increase of vermicompost in fertilizer integrated treatments up to F5 scheme (10.1 t ha⁻¹). However, at greater vermicompost rate (F6: solo vermicompost) the oil percentage and oil yield decreased. Such a result was probably because of the characteristic of organic fertilizers used in the study. Due to continuous, but slow, nutrient release of the organic fertilizer, vermicompost alone is not likely responsible for supplying the plant in the short term. From the above results, it is clear that vermicompost in suitable proportion can significantly enhance oil content and oil yield of mint, by improving various chemical, physical and biological characteristic of the soil.

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