

Full Length Research Paper

Effects of nitrogen fertilizer rate on the essential oil content of *Mentha longifolia*, *Mentha microphyta* and *Ocimum basilicum* plants

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Nitrogen fertilizers are widely used in agricultural fields to improve crop production. The main objective of the present study was to investigate the effect of nitrogen levels on the quantity and quality of essential oils present in plants of *Mentha longifolia*, *Mentha microphyta* and *Ocimum basilicum*. It was found that these plants had a maximum content of essential oils when 200 kg nitrogen fertilizer was used per hectare. The composition of the essential oil in the tested plant species also differed significantly. The results suggest that the use of nitrogen fertilizers may increase the production of essential oils in plants. The results indicated that the use of nitrogen fertilizers also improves the quality of the essential oils produced in the plants.

Key words: Oil essential quality, essential oil composition, biosynthetic pathway, basil, mint, antimicrobial activity.

INTRODUCTION

Lamiaceae is a plant family with a worldwide distribution, from the temperate to the hot tropical regions, but is mainly distributed in the Mediterranean region. The family has more than 232 genera and about 7200 different species (Singh and Pandey, 2018). Plants of this family could be annuals or perennials, most of them are medicinal and aromatic plants. Many of the plants that belong to the Lamiaceae accumulate secondary metabolites such as terpenes/essential oils and other components in their leaves, stems and reproductive organs (Cherrat et al., 2014). These plant species have long been commercially cultivated for their essential oils and many cultivars of a single species exist (Diop et al., 2016). The essential oils of these plants have wide use in medicines, perfumes, as additives in cosmetics, and as flavouring agents in drinks and foodstuffs (Peixoto et al., 2009; Lawrence, 2007; Trease and Evans, 1983).

Mentha longifolia L. and *Mentha microphyta*, locally known as Habag and Na' N' aa, respectively, belonging to the family Lamiaceae, are well-known plant species in

Saudi Arabia and the Arab world. Leaves of *M. microphyta* are extensively used as tea-flavouring agent and as spices in cooking. Herbalists use the entire plant of *Mentha* in the treatment of gastric ailments, carminative and antispasmodic conditions (Diop et al., 2016; Akdogan et al., 2007; Deille, 2007; Banthorpe et al., 1981). The antimicrobial activity of essential oils of *Mentha* has also been reported (Arumugam et al., 2008; Di Stasi et al., 2002; Soliman et al., 1998). In addition, *Mentha* species have been used as a flavouring, pain relieving and carminative agent (Abbaszadeh et al., 2009; Kunnnmakkara et al., 2009; Mkaddem et al., 2009).

Extensive research work has been carried out around the world on the content and composition of essential oils obtained from different species and cultivars of wild and cultivated mint plants and other Lamiaceae species (Sutour, 2010; Abbaszadeh et al., 2009; Kunnnmakkara et al., 2009; Mkaddem et al., 2009; Lawrence, 1981, 1985). The essential oil content of the mint plant depends on the characteristics of the plant, environmental factors, and

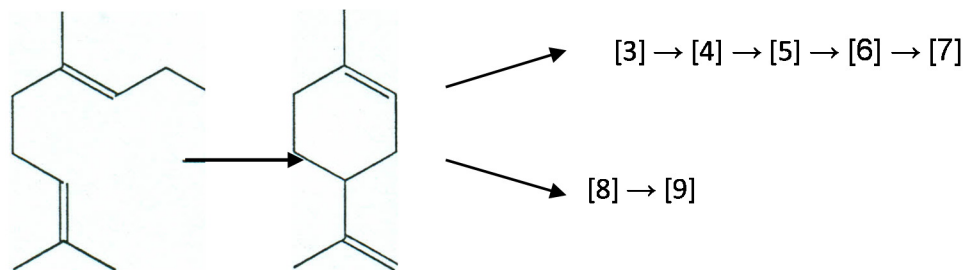


Figure 1. Biosynthetic pathway of C3- and C6-oxygenated monoterpenes from geranyl pyrophosphate

methods of isolation and analysis. The essential oil content also depends on the application of good agricultural practices (Kapp, 2015; Zhao et al., 2013; Mahboubi and Haghi, 2008; Sutour et al., 2008).

The characteristic components of the oils of mint and its various chemoforms are monoterpenes of either C3-oxygenated p-menthane type (e.g., pulegone, menthone, menthol) or C6-oxygenated p-menthane type (e.g., carveol, carvone). Chemogenetic evidence obtained from experiments has shown that a dominant gene is responsible for the formation of C3-oxygenated monoterpenes, such as carvone/dihydrocarvone, and a recessive genotype allows the formation of C6-oxygenated monoterpenes, such as pulegone, piperitone, piperitenone and menthone. Another dominant gene is responsible for the conversion of carvone into carveol and dihydrocarvone into dihydrocarveol (Lincoln and Murray, 1978; Murray et al., 1980; Karousou et al., 1998; Hefendehl and Murray, 1976; Lincoln et al., 1986; Croteau, 1991). Tracer experiments have demonstrated that limonene is the precursor of both C3- and C6-oxygenated p-menthane monoterpenes (Croteau, 1991; Kjonaas and Croteau, 1983; Figure 1).

Basil (*Ocimum basilicum*), belonging to the Lamiaceae family, is a perennial aromatic shrub that grows throughout the world. It is one of the main essential oil producing species and has been widely used as an insect repellent, in perfumery and cosmetics (Buchbauer, 2012). The leaf extract of *O. basilicum* was used as a mosquito repellent (Umeric et al., 1998). It has also been used as a flavouring agent in drinks and food stuffs. Herbalists have used the plant for prostate gland and kidney diseases (Zheljazkov et al., 2007; Kokkini et al., 1995). There are usually high differences in the concentrations of the main chemical constituents of this plant species (Hussain et al., 2008; Zheljazkov et al., 2008). It was found that two different biochemical pathways produce the different essential oil components of basil plants that grow in different parts of the world. These pathways are phenylpropanoids (methyl cinnamate, methyl chavicol, methyleugenol and eugenol), produced through the shikimic acid pathway, while terpenes (geraniol and linalool) are produced through the mevalonic acid pathway (Chalchat and Ozcan, 2008; Vina and Murillo, 2003).

Chemical variants in the genus *Ocimum* have been studied and the essential oil content, as well as the chemical composition of the oil have been found to vary according to the cultivar and the ecosystem (Purkayastha and Nath, 2006; Sajjadi, 2006; Kasali et al., 2005; Gangrade et al., 2000; Kokkini and Papageorgio, 1988). A correlation between atmospheric relative humidity and the composition of the essential oil was established for plant species growing at different altitudes. Other species showed that the chemical races are stable and not transitory phenotypes varying with the environment (Purkayastha and Nath, 2006; Sajjadi, 2006; Kasali et al., 2005; Trease and Evans, 1983). The essential oils of some chemical races are characterized by the presence of camphor, estragole, anethole, citral, cineole, limonene, linalool and traces of sesquiterpenoids. Other chemical races are characterized by a high content of linalool and were subdivided into three chemo types: methyl chavicol, linalool, and eugenol (Purkayastha and Nath, 2006; Sajjadi, 2006; Kokkini and Papageorgio, 1988). Other chemotypes, such as methyl cinnamate, have also been reported (Purkayastha and Nath, 2006; Sajjadi, 2006; Marotti et al., 1996).

Hence, the objective of this study was to investigate the effect of nitrogen fertilization level on the concentration and chemical composition of the essential oils obtained from the air-dried parts of *M. longifolia*, *M. microphyta* and *O. basilicum* plants that were grown in Al-Hassa, Saudi Arabia.

MATERIALS AND METHODS

Plant materials and experimental setup

M. longifolia, *M. microphyta* and *O. basilicum* plants were propagated by seeds obtained from the mother plants growing at King Faisal University research station, Al-Hassa, Kingdom of Saudi Arabia. The seeds were initially planted in pots containing autoclaved perlite in a greenhouse under a misting system. The seedlings were then transplanted in March 2016 in plots (2 x 3 m) in a greenhouse. The soil was a loamy sand of pH 6.5, EC 2.35 dS/m, organic matter 0.85%, CaCO₃ 4.61% with a

Table 1. Essential oil content of wild plant materials.

<i>Mentha longifolia</i>	1.15%	1.24%	1.25%	Mean 1.21% ^b
<i>Mentha microphylla</i>	0.65%	0.86%	0.88%	Mean 0.79% ^c
<i>Ocimum basilicum</i>	1.64%	1.66%	1.68%	Mean 1.66% ^a

Means followed by the same letter indicate no significant differences at $P < 0.05$ using LSD test.

Ca, Mg and P content at 65, 4.6, and 2.4 mg kg⁻¹, respectively. The recommended agricultural practices for the region were followed. 27 plots were used with the following combinations: Three (3) nitrogen fertilizer treatments (zero (control), 150 and 200 kg ha⁻¹) and three (3) plant species (*M. longifolia*, *M. microphylla* and *O. basilicum*) were replicated three times. N fertilizer was applied before transplanting as ammonium nitrate (26% N). 40 kg/ha of P and 160 kg/ha of K were also added to the soil prior to transplanting. The seedlings were transplanted in the greenhouse at a density of 10 plants/meter on March 5th. Weeds were controlled by hand weeding. After the establishment of the crop, the plants were irrigated every three days by drip irrigation. The plants were grown in a factorial design for three (3) months in the greenhouse. In June 2011, shoots of the plants were harvested from the central part of each plot and separated to analyze the essential oil composition in the shoots. The leaves were separated from the shoots and the essential oils were obtained.

Recovery of the essential oil

Plant (*M. longifolia*, *M. microphylla* and *O. basilicum*) materials, either in the pre-flowering stage or in the full-flowering stage, were air-dried under shade for 10 days at room temperature. The grossly pulverized material of each ontogenetic stage (100 g) was subjected to steam distillation for 3 h. The essential oil content was expressed in mL/100 g. The oil content values, mentioned elsewhere in this report, are an average of 3 determinations. The oil of *M. longifolia* was analyzed using a Varian 3380 gas chromatograph equipped with an FID-detector and a capillary column (30 m × 0.32 mm; 0.25 μm Carbowax film thickness). The injector and detector temperatures were 220°C and 250°C, respectively. The carrier gas was helium at a flow rate of 1.5 ml/min. The column temperature program was 50°C for 2 min and then 50°C - 2250°C with an increase of 10°C/min.

Identification of oil components

The essential oil components were identified by comparing their retention times with those of certified standards. Peak area data from the GC (FID) analyses were recorded in an integrator and analyzed using the appropriate software.

The GC-chromatogram revealed thirty-two components. Seven of these showed typical retention times from the available certified standards and were identified as carvone, carveol, lirnonene, menthone and isomenthone as major components. Alpha-pinene and myrcene were also estimated at low concentrations and, hence, were classified as minor components in *M. longifolia*. Piperitenone oxide, isopiperitenol and pulegone are major components, while alpha-pinene and myrcene have been identified as minor components in *M. microphylla*.

Data analysis

The experimental design was a factorial experiment in a randomised complete block with three replicates. The data of all the parameters were processed by analysis of variance and the treatment means were separated by least significant differences (LSD) (SAS, 2009).

RESULTS AND DISCUSSION

As there were no significant interactions between nitrogen and plant species treatments for any of the characteristics studied, their effects are discussed separately. In the present investigation, variations existed for both the essential oil yields of the wild *Mentha* and *Ocimum* species, and variations also exist for the yields obtained in the application of certain agricultural practices, namely nitrogen fertilization. In the control treatment, the oil content of *M. longifolia* (at full flowering stage) was 1.21% (Table 1). Similarly, oil content of *M. microphylla* for the same development stage was 0.79%. *M. longifolia* proved to be rich in essential oils, yielding twice as much essential oil as *M. microphylla*.

Mentha leaves were found to contain 1.2–3.9% (v/w) of essential oil, with more than 300 identified compounds (Zhao et al., 2013). However, the oil yields obtained from either *M. longifolia* or *M. microphylla* were not consistent with the reported values (Table 2). The yields were lower than those shown in several studies (Hefendehl and Murray, 1976; Umeric et al., 1998; Kokkini et al., 1995). On the other hand, essential oil yields obtained in the present study are remarkably higher than those reported (Trease and Evans, 1983; Banthorpe et al., 1981; Wan et al., 1998). It is a well-accepted fact that the essential oil content of a mint plant depends on the characteristics of

Table 2. Essential oil contents (%) of *Mentha* species during two ontogenetic stages.

Species	Oil content (%)		
	Pre-flowering	Full flowering	References
<i>M. longifolia</i> L	Not done	1.21	This study
<i>M. microphyla</i>	Not done	0.79	This study
<i>M. longifolia</i> L. subsp. schimperi	1.40	1.80	Hefendehl and Murray (1976)
<i>M. longifolia</i> L., Hudson ssp.	not stated	2.18	Umeric et al. (1998)
<i>M. longifolia</i> L., Hudson	not stated	0.30-1.40	Umeric et al. (1998)
<i>M. longifolia</i> subsp. petiolata	not stated	1.80-2.80	Kokkini et al. (1995).
<i>M. spicata</i> Huds.	0.50	0.90	Hefendehl and Murray (1976)

Table 3. Essential oil content of cultivated N-fertilized plant material at two ontogenetic stages [pre-flowering and full-flowering stages].

Pre-flowering stage	Control	N ₁₅₀ %	N ₂₀₀ %	Mean %
<i>Mentha longifolia</i>	1.08	1.53	1.15	1.25 ^a
<i>Mentha microphyla</i>	0.56	0.72	0.62	0.63 ^b
<i>Ocimum basilicum</i>	1.01	1.55	1.2	1.25 ^a
Mean	0.88 ^c	1.27 ^a	0.99 ^b	
Full-flowering stage	Control	N ₁₅₀	N ₂₀₀	
<i>Mentha longifolia</i>	1.21	2.40	1.30	1.63 ^b
<i>Mentha microphyla</i>	0.79	0.98	0.83	0.87 ^c
<i>Ocimum basilicum</i>	1.66	2.65	1.75	2.02 ^a
Mean	1.22 ^c	2.01 ^a	1.29 ^b	

Means followed by the same letter indicate no significant differences at P < 0.05 using LSD test.

the plant (ecotype and variety), environmental influences (climate, geographical location, and agricultural practices) and methods of analysis (extraction techniques and drying conditions). Variations in oil contents existed in the application of varying levels of N-fertilization (Table 3). Both species showed a change in essential oil content as a function of plant development stage. The yields were lower in the pre-flowering stage and increased gradually to a maximum at full-flowering (Table 3).

Increasing level of nitrogen fertilization was the most effective practice for increasing essential oil content in both species. Oil yields increased progressively with the increase in the nitrogen rate. A basal fertilizer (150 kg, N/ha) increased the oil yield of *M. longifolia* from 1.08% to 1.53% of the control, and for *M. microphyla* from 0.56% to 0.72% of the control (Table 3). The decreased yields obtained at the basal level of N-fertilization were obtained at 200 kg/ha (1.15%, 0.62%), but these levels were also higher than the control (essential oil yields obtained from the wild *Mentha* species).

The increase in essential oil yield due to N fertilizer application may depend not only on the increase of plant biomass, but also on the increase in essential oil concentrations in plant tissues, which indicates an

enhancement in the oil content.

Mentha essential oil has more than 300 identified constituents. The class of terpenes is the most common, since it consists of about 52% of monoterpenes and 9% of sesquiterpenes. Other groups, e.g. alcohols (6%), aromatic hydrocarbons (9%), aldehydes (9%), lactones (7%) and miscellaneous (8%) were recorded in smaller percentages (Abbaszadeh et al., 2009). Among the monoterpenes, menthol ranged between 35 and 60%, menthone between 2 and 44%, menthyl acetate between 0.7 and 23%, 1, 8-cineole between 1 and 13%, menthofuran between 0.3 and 14%, isomenthone between 2 and 5%, neomenthol between 3 and 4%, while limonene ranged between 0.1 and 6%. The β -caryophyllene was recorded as the main sesquiterpenes ranging between 1.6 and 1.8% (Hussain et al., 2010). The analysis of the oils by GC reveals that *M. longifolia* was rich in carvone (Table 4). Thirty-one compounds were detected in this oil, mainly identified as carvone (67.3%), limonene (13.5%), menthane (2.9%) and isomenthone (1.2%). The lack of authentic samples of essential oils has not allowed the identification of the other remaining 28 components that have been detected, in a lower percentage, in the oil of *M. longifolia* (Table 4).

Table 4 . Components (%) in the essential oil of *Mentha longifolia*.

Component	Retention Time Index	Level (%)
α -pinene	1032	0.2
Myrecence	1174	0.4
Limonene	1203	13.5
Menthone	1479	2.9
Isomenthone	1503	1.2
Carvone	1751	67.3

Conclusion

The mechanism behind changes in essential oil compositions is not known and may be related to better nutrition. It was concluded that nitrogen fertilization could improve the sustainability of the commercial cultivation of aromatic plants in low fertility, loamy, and sandy soils in Saudi Arabia.

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