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Full Length Research Paper

Studying of essential oil variations in leaves of *Mentha* species

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To evaluate the beneficial impacts of different species on mint, some yield characters were investigated. Our objective in this research was the studying of different species effects on essential oil variations. In this respect, the experimental unit had designed by achieved treatment in completely randomized block design with three replicates. The factor including mint species (*Mentha longifolia var. amphilema* (from Qazvin and Ardabil localities), *Mentha spicata* (from Tehran and Yazd localities), Mentha piperita (from Tehran and Ardabil localities), *Mentha aquatica* (from Gheilan and Ardabil localities)) at Iran was studied. Our final statistical analysis was indicated that mint species significantly affected essential oil yield of leaves, essential oil percentage of leaves and leaf yield ($P < 0.01$) and leaf length ($P < 0.05$). *M. longifalia var. amphilema* from Ardabil locality were provided highest essential oil yield of leaves (25.13 kg/ha) and essential oil percentage of leaves (1.64%). Longest leaf length (4.01 cm) was achieved by *M. piperita* from Tehran locality and highest leaf yield (914.4 kg/ha) was obtained by *M. aquatica* from Tehran locality. Our findings indicated that were significant difference between essential oil variations of mint species. Therefore, the selection of species that performs well over a wide range of environments can increases essential oil yield of medicinal and aromatic plants.

Key words: Mentha species, essential oil yield of leaves, essential oil percentage of leaves, leaf yield.

INTRODUCTION

Medicinal and aromatic plants are an accessible, affordable and culturally appropriate source of primary health care for more than 80% of world's population. Plant secondary metabolites have been a fertile area of chemical investigation for many years, driving the development of both analytical chemistry and of new synthetic reactions and methodologies. Metabolites which are produced by routes other than the normal the species that make up the *Mentha* genus are widely distributed and can be found in many environments, most *Mentha* grow best in wet environments and moist soils. Mints will grow 10 - 120 cm tall and can spread over an indeter-minate sized area. Due to the tendency to spread unchecked, mints are considered invasive (Brickell and Trevor, 2002). All mints prefer, and thrive in, cool, moist spots in partial shade. In general, mints tolerate a wide range of conditions, and can also be grown in full sun. They are fast growing, extending their reach along surfaces through a network of runners. Due to their speedy growth, one plant of each desired mint, along with a little care, will provide more than enough mint for home use. Some mint species are more invasive than others. Even with the less invasive mints, care should be taken when mixing any mint with any other plants, lest the mint take over. To control mints in an open environment, mints should be planted in deep, bottomless containers sunk in the ground, or planted above ground in tubs and barrels (Bradley, 1992). Some mints can be propagated by seed. Growth from seed can be an unreliable method for raising mint for two reasons: mint seeds are highly variable - one might not end up with what one presupposed was planted and some mint varieties are sterile. It is more effective to take and plant cuttings from the runners of healthy mints. The most common and popular mints for cultivation are peppermint (*M. piperita*), spearmint (*M. spicata*) and (more recently) apple mint (*M. suaveolens*). Mints are supposed to make good companion plants, repelling pest insects and attracting beneficial ones. Mints are susceptible to whitefly and aphids. Harvesting of mint leaves can be done at anytime. Fresh mint leaves should be used

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immediately or stored up to a couple of days in plastic bags within a refrigerator. Optionally, mint can be frozen in ice cube trays. Dried mint leaves should be stored in an airtight container placed in a cool, dark, dry area (Ortiz, 1992). Mint was originally used as a medicinal herb to treat stomach ache and chest pains, and it is commonly used in the form of tea as a home remedy to help alleviate stomach pain. During the middle Ages, powdered mint leaves were used to whiten teeth. Mint tea is a strong diuretic. Mint also aids digestion, in a way that it breaks down the fats. In recent years, it has been often recommended for treating obesity (Quattrocchi, 1974). *Mentha aquatica* (Water Mint) is a perennial plant in the genus *Mentha* native throughout Europe, except for the extreme north, and also northwest Africa and southwest Asia (Huxley, 1992). It is a herbaceous rhizomatous perennial plant growing to 90 cm tall. The stems are square in cross section, green or purple, and variably hairy to almost hairless. The rhizomes are wide-spreading, fleshy, and bear fibrous roots. The leaves are ovate to ovate-lanceolate, 2–6 cm long and 1–4 cm broad, green (sometimes purplish), opposite, toothed, and vary from hairy to nearly hairless. The flowers are tiny, densely crowded, purple, tubular, and pinkish to lilac in color; flowering is from amidst to late summer. Water Mint is pollinated by insects, and also spreads by underground rhizomes, like other species of mint. All parts of the plant have a distinctly minty smell (Blamey and Grey-Wilson, 1989). *Mentha longifolia* (Horse Mint) is a species in the genus *Mentha* (mint) native to Europe, western and central Asia (east to Nepal and the far west of China), and northern and southern (but not tropical) Africa (Huxley, 1992) . It is a very variable herbaceous perennial plant with a peppermint-scented aroma. Like many mints, it has a creeping rhizome, with erect to creeping stems 40 - 120 cm tall. The leaves are oblong-elliptical to lanceolate, 5 - 10 cm long and 1.5 - 3 cm broad, thinly to densely tomentose, green to greyish-green above and white below. The flowers are 3 - 5 mm long, lilac, purplish, or white, produced in dense clusters (verticillasters) on tall, branched, tapering spikes; flowering in amidst to late summer. It spreads via rhizomes to form clonal colonies (Blamey and Grey-Wilson, 1989). *Mentha spicata* (Spear Mint or Spearmint) is a species of mint native to much of Europe and southwest Asia, though it’s exact natural range is uncertain due to extensive early cultivation. It grows in wet soils (Huxley, 1992) . It is an invasive species in the Great Lakes region where it was first sighted in 1843. It is a herbaceous rhizomatous perennial plant growing 30 - 100 cm tall, with variably hairless to hairy stems and foliage, and a wide-spreading fleshy underground rhizome. The leaves are 5 - 9 cm long and 1.5 - 3 cm broad, with a serrated margin. Spearmint produces flowers in slender spikes, each flower pink or white, 2.5 - 3 mm long and broad (Blamey and Grey-Wilson, 1989). *Mentha piperita* (Peppermint or brandy mint) is one of the most popular tonic herbs known to modern man. It is well known flavoring agent. Peppermint has been a popular home remedy for digestive ailments for two centuries in India. *M. piperita* is currently one of the most economically important aromatic-medicinal herbs produced in India. Scientific studies have not only confirmed the traditional uses of the plant but also encouraging the use of peppermint as a direct or adjunct therapy in modern practice. Peppermint is a sterile hybrid derived from a cross between *M. aquatica* and *M. spicata*. Peppermint is a perennial herb, growing up to 3 feet in height, and propagation is by underground stolons. The stems are usually reddish-purple and smooth. The leaves are fragrant, toothed and hairy on the underside. Flowers are bisexual and zygomorphic pinkish or purple color. *M. piperita* bloom from July through August in whorls and terminal spikes. The fruit consists of four 1-seeded nutlets (Blamey and Grey-Wilson, 1989). The genus *Mentha* includes aromatic herbs of difficult taxonomic classification due to a great variability in their morphological characters and frequent hybridisation. Previous investigations of their essential oils have revealed the existence of an important chemical polymorphism (Lawrence, 1978) and several varieties and chemotypes have been described for *M. spicata* (Kokkini and Vokou, 1989; Misra et al., 1989; Pino et al., 1998), *M. longifolia* (Maffei, 1988; Venskutonis, 1996), *M.* suaveolens (Hendriks et al., 1976) and *M. diemenica* (Brophy et al., 1996) among others. *M. pulegium* L., commonly known as pennyroyal, is traditionally used in the treatment of flatulent dyspepsia and intestinal colic due to its carminative and antispasmodic properties (Newall et al., 1996). Previous reports (Lawrence, 1998; Pino et al., 1996) on the composition of its essential oil showed that pulegone was the main constituent, and its percentage ranged from 25 - 92%. *M. rotundifolia* (L.) Huds. is a hybrid between *M. longifolia* (L.) L. and *M. suaveolens* Ehrh., whose essential oil has been the object of several studies (Kokkini and Vokou, 1989; Hendriks et al., 1976; Umemoto, 1998), and different chemotypes have been characterized. Some authors have considered *M. rotundifolia* (L.) metabolic pathways, mostly after the phase of active growth and under conditions of deficiency and the biological significance of many secondary metabolites is not exactly known. An essential oil is a concentrated, hydrophobic liquid containing volatile aroma compounds from some plants. Essential oils do not as a group needs to have any specific chemical properties in common, beyond conveying characteristic fragrances. They are not to be confused with essential fatty acids. Essential oils are generally extracted by distillation. Other processes include expression, or solvent extraction. They are used in perfumes, cosmetics and bath products, for flavoring food and drink, and for scenting incense and household cleaning products (Sellar, 2001) . Climate changes the essential oil percentage of more medicinal and aromatic plants, because in arid and semi-arid areas, more metabolites are produce in the plants and substances prevent from oxidization in the cells, but essential oil content reduce under drought stress, because the interaction between the amount of the essential oil percentage and shoot yield is consider important as two components of the essential oil content and by exerting stress, increases the essential oil percentage but shoot yield decreases by the drought stress, therefore essential oil content reduces (Aliabadi Farahani et al., 2009). *Mentha* (mint) is a genus of about 25 species (and many hundreds of varieties) of flowering plants in the family *Lamiaceae*. Species within *Mentha* have a sub cosmopolitan distribution across Europe, Africa, Asia, Australia, and North America. Several mint hybrids commonly occur (Davidson, 1999). Mints are aromatic, almost exclusively perennial, rarely annual, herbs. They have wide-spreading underground rhizomes and erect, branched stems. The leaves are arranged in opposite pairs, from simple oblong to lanceolate, often downy, and with a serrated margin. Leaf colors range from dark green and gray-green to purple, blue, and sometimes pale yellow (Brickell and Zuk, 1997). The flowers are produced in clusters ('verticils') on an erect spike, white to purple, the corolla two-lipped with four sub equal lobes, the upper lobe usually the largest. The fruit is a small, dry capsule containing one to four seeds. While Huds. as a synonym of *M. suaveolens* Ehrh (Hendriks et al., 1976). Biological yield obtained from *Mentha pulegium* L. and *Mentha rotundifolia* (L.) Huds. in Uruguay. Highest biological yield was provided from *M. pulegium* than *M. rotundifolia* (Lorenzo et al., 2002). In greenhouse study conducted in India, the morphological values of *M. arvensis, M. piperita, M. citrate, M. spicata* and *M. viridis* were evaluated. The results showed that highest development of larvae was achieved from *M. spicata* (Sagar and Sagar, 2006). The research objective was to evaluate changing of essential oil content in leaf of *Mentha* species.

MATERIALS AND METHODS

This experiment was carried out using a randomized complete block design (RCBD) with three replicates. The factor including mint species (*Mentha longifolia* var. amphilema (from Qazvin and Ardabil localities), *Mentha spicata* (from Tehran and Yazd localities), *Mentha piperita* (from Tehran and Ardabil localities), *Mentha aquatica* (from Gheilan and Ardabil localities at Iran was studied. To determine essential oil yield of leaves, essential oil percentage of leaves, leaf yield and leaf length, 10 plants were selected randomly from each plot at maturity. We selected 100gr dry matter of leaves from each plot for determination of essential oil percentage by Clevenger. Finally, essential oil yield was determined by the following formula (Aliabadi Farahani et al., 2008).

Essential oil yield = Essential oil percentage × Leaf yield

The data were subjected to analysis of variance (ANOVA) using Statistical Analysis System (SAS) computer software at $P<0.05$ (SAS institute Cary, USA 1988).

RESULTS AND DISCUSSION

Final results showed that mint species had significant effect on essential oil yield of leaves, essential oil percentage of leaves and leaf yield ($P < 0.01$) and leaf length [$P < 0.05$, Table 1 and 2]. *M. longifalia* var. amphilema from Ardabil locality was provided highest essential oil yield of leaves (25.13 kg/ha) and essential oil percentage of leaves (1.64%). Longest leaf length (4.01 cm) was achieved by *M. piperita* from Tehran locality and highest leaf yield (914.4 kg/ha) was achieved by *M. aquatica* from Tehran locality. The selection of compatible species with growth environment is important factor in controlling shoot dry matter SPECIES OF MINT. Countries need to perennial medicinal plants that furnish high-quality yields for farmers during all phases of the growing season. However, most medicinal plants used in this region have been developed and released based on values related to their establishment and production rather than their nutritive quality. SPECIES of mint were all released primarily because of high shoot yield. It is well established that maturation of shoot tissue has a detrimental effect on whole plant nutritive quality, although less is known about the effect of maturity on specific species that are widely grown in Iran. Essential oil yield in *M. longifalia* var. amphilema from Ardabil locality differed, with all species. Comparisons with *M. longifalia* var. amphilema from Qazvin locality and all species suggested that differences among SPECIES were related more to traits such as essential oil percentage and leaf yield. Other SPECIES evaluated with a view to diversifying the range of options available and reducing overdependence on a single SPECIES that may be prone to disease and pest attack.

For instance, *Mentha aquatica* is susceptible to root putrefaction and a devastating louse pest. Varieties of species are almost always required for complete control of SPECIES present and to provide leaf yield in the plants during the growing season. The introduction of genetically modified SPECIES OF mint resistant to drought, weed, diseases and pests will increase the potential of production into fields. Medicinal plants are composed of a community of plants that may differ in phenotypic plasticity, adaptation to grazing, and therefore, in persistence within the medicinal and aromatic plants community.

Conclusion

Our data indicated that was significant difference between essential oil yields in leaves of mint species. Therefore, the selection of species that perform well over a wide range of environments can increase quantity and quality yields of mint.

Table 1. Analysis of variance.

Source of variation	df	Mean squares			
		Essential oil yield	Essential oil percentage	Leaf yield	Leaf length
Replication	2	0.415	0.001	2399.268	0.457
Species of mint	7	59.813 **	0.578 **	91928.722 **	2.097*
Error	14	0.658	0.004	2183.423	0.278
CV (%)		8.38	5.38	6.25	18.96

** And *: Significant at 1 and 5% levels respectively.

Table 2. Means comparison.

Treatment	Essential oil content (kg/ha)	Essential oil percentage (%)	Leaf yield (kg/ha)	Leaf length (cm)
M. longifolia var. amphilema from Qazvin	13.69 b	1.55 b	882.7 b	3.17 abc
M. longifolia var. amphilema from Ardabil	25.13 a	1.64 a	807.6 bc	2.02 c
M. Spicnta from Tehran	12.37 b	1.52 b	815.8 bc	3.17 abc
M. Spicnta from Yazd	11.66 bc	1.51 b	767.8 c	2.47 bc
M. piperita from Tehran	10.01 c	1.09 c	914.4 a	4.01 a
M. piperita from Ardabil	10.99 bc	1.3 bc	844 b	3.3 abc
M. aquatica from Ardabil	2.38 d	0.5 d	460.4 d	2.93 abc
M. aquatica from Gheilan	3.06 d	0.63 d	484 d	3.57 ab

Means within the same column and factor, followed by the same letter are not significantly difference ($P < 0.05$).

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