Journal of Medicinal Plants Research Vol. 4(25), pp. 2753-2759, 29 December Special Review, 2010
Available online at http://www.academicjournals.org/JMPR
ISSN 1996-0875 ©2010 Academic Journals

Review

Melissa officinalis L., a valuable medicine plant: A review

Moradkhani H.[1], Sargsyan E.[1], Bibak H.[2], Naseri B.[3], Sadat-Hosseini M.[2], Fayazi-Barjin A.[4] and Meftahizade H.[5]*

[1]Institute of Hydroponic Problems, National Academic of Sciences, Yerevan, Republic of Armenia.
[2]Department of plant production, faculty of Agriculture, university of Jiroft, Kerman, Iran.
[3]Faculty of Islamic Azad University, Ilam, Iran.
[4]Department of Plant Protection, University of Tehran, Iran.
[5]Researcher of ACECR Medicinal Plants Center, Ilam, Iran.

Accepted 6 December, 2010

***Melissa officinalis* L., a valuable medicinal plant in herbal medicine is native to the eastern Mediterranean Region and western Asia. The constituent of the essential oil of the plant in various climates is different, but citral (geranial and neral), citronellal, geraniol are main components. Many parameters influencing essential oil composition and yield, such as light intensity, nutrient, temperature, cultural practice genotype, plant part age, harvesting time. Lemon balm has been traditionally used for different medical purposes as tonic, antispasmodic, carminative, diaphoretic, surgical dressing for wounds, sedative-hypnotic strengthening the memory, and relief of stress induced headache, but in modern pharmacology is value in the management of mild to moderate Alzheimer's, against migraine and rheumatism, antitumel and antioxidant activities.**

Key words: *Melissa officinalis*, essential oil, pharmacology and antioxidant.

INTRODUCTION

Lemon balm, member of the family Lamiaceae (formerly Labiatae) is a perennial bushy plant and is upright, reaching a height of about 1 m. The soft, hairy leaves are 2 to 8 cm long and either heart-shaped (Zargari, 1991). The leaf surface is coarse and deeply veined, and the leaf edge is scalloped or toothed (Turhan, 2006). Flowers white or pale pink consisting of small clusters of 4 to 12 blossom in the summer. It is commonly referred to as 'lemon balm' because of its lemon-like flavor and fragrance (Anonymous, 2003). Lemon balm is a cross-pollinating species, and has complete perfect flowers with petals. Two stamens and four lobed ovaryes forming 1 to 4 nutlets. The seeds are very small about 1 to 1/5 mm long. Ovate dark brown or black in colour. The weight of seeds is 0.5 to 0.7 g a long storage periods causes a reduction in germination vigour. Seeds stored for five years may no longer germinate (Zargari, 1991).

Lemon balm has a hairy root system with many lateral roots, which makes the plant more adaptable to different environmental conditions; the upper parts of the plant die off at the start of winter. But new shoots re-emerge from roots at the beginning of spring (Turhan, 2006). *Melissa officinalis* is used in herbal medicine and is native to the eastern Mediterranean Region and western Asia (Meftahizade et al., 2010). Dried or fresh leaves and top aerial section of the plant are the parts which are used as medicine (Janina, 2003).

Cultivation

Lemon balm is widely cultivated in Europe and the United States, but also grows wild along paths and roadsides. The plants prefer sandy and loamy fertile soils, well drained and at pH range 5 to 7. It grows well in full sun, but it also grows in partial shade (Janina, 2003). When the plants grow in semi-shade, they produced larger

*Corresponding author. E-mail: Heidarmeftahi732@gmail.com, Hmeftahi@yahoo.com.

leaves and habitat than those grown in sunny condition. Lemon balm can rapidly grow at temperature range 15 to 35℃ and requires 500 to 600 mm precipitation well distributed throughout the growing season, otherwise it should be irrigated. It is sensitive especially to drought in the establishment year. Once it develops a deep root system, its water requirement lessens (Davis, 1982). There exist three other subspecies which are naturally expended in our wild flora; subsp. *officinalis*, subsp. altissima and subsp. inodora (Davis, 1982).

Disturbance

It is reported that the plant is mainly grown in Germany, France, Italy, Romania, Bulgaria, and North America. (Anonymous, 2003) Ceylan, (1987 reported that the subspecies of *M. officinalis* are evaluated in domestic markets and they are also on the list of the exported medicinal and aromatic plants. It is used in traditional medicine from ancient times. French monks and nuns, and Paracelsus (1493 - 1541), Swiss physician and chemist, prepared tonics, called as “life elixir”, contain lemon balm, and used. English writer John Evelyn (1620-1706), described this plant as “ruler of brain, strengthening to mental, and removing from melancholia”. Its essential oil was named “bal-smin” or “leader of the oils” in Hebrew. Avicenna recommends that lemon balm strengthened heart (Anonymous, 2003).

Chemical composition

Essential oil rate in drug herb changes between 0.02 to 0.30%, which is quite low compared to other member of the family labiatae. That is why the production cost and price of essential oil is very high in the market. Meftahizade et al. (2010), reported that the main constituent of the essential oil are citral (geranial and neral), citronellal, geraniol, beta-pinene, alpha- pinene, beta – caryophyllene, comprising 96% of the oil ingredients. Also carnat et al. (1998), reported the chemical composition of essential oil of lemon balm, and found that major components are citral representing 48% of the essential oil, followed by citronellal with 39.47% and caryophyllene with 2.37% in another investigation, the percentage of the main constitute found by Sarer and Kokdil, are followes: alpha-pinene (2.86%), beta-pinene (11.37%), linalool (2.74%), citronella (5.86%) borneol (0.62%), neral (12.22%), and geraniol (38.13%), in addition, fresh herb of lemon balm contains total phenolic (2253 /100 mg), L-Ascorbic acid (53.2 /100 mg) and carotenoids (46.3 /100 mg), to complete this review of composition it is important to mention that several subspeciers of Melissa are recognized which exhibit a very different chemical profile. The subspecies altissima produces oil mainly composed of beta- cubebene, terpinolene, gama-3-carene, terpinene, berta-caryophylene and muurolol (Dawson et al., 1988). The subspecies inodore, which had been used in Turkish folk medicine, contains beta-cubebena, beta-caryophyllene, alpha-cadinol and geranial and neral (7 and 6% respectively). Distinct chemotypes of *M. officinalis* have not been found. The quantitative composition of the essential oil can vary. But qualitatively it is relative constant. Masakova et al. (1979) reported that the citral isomers geranial and neral are the main component of *M. officinalis*. Caernat et al. (1998) reported that composition of essential oil of lemon balm are citral (neral + geranial), representing 48% of the essential oil. Neral and Beta-Caryophyllene was the main components in chemical analysis of essential oils from *M. officinalis* L. (meftahizade et al., 2010) Table 1.

Essential oils

In the lamiaceae family, essential oils are mainly produced in secretary structures known as glandular trachoma, of which there are two main kinds, peltate and capitates. In this respect, *M. officinalis* is no exception (Waker, 1993). And the effective materials are secreted into the cuticular apace where they accumulate. The amount of essential oils produced is directly connected with the number and physiology of these structures. In *M. officinalis*, the peltate glands are very sparsely distributed on the leaf and, furthermore, once the leaves have grown to 4 mm in size. The glandular pattern is fully developed and the number of peltate trichomes does not increase (Hose et al., 1997). This may be a physiological reason for low yield in *M. officinalis*.

Lemon balm essential oil, obtained from fresh or dried flower, leaf, and branches of this plant by water steam distillation or chemical extraction, is characteristic with fresh lemon odor, and light yellow colored. Its viscosity is lighter than that of water (Anonymous, 2003). It was desired that this value should not be lower than 0.05% (Baytop, 1984). The main components of the essential oil are 39% citronellal, 33% citral (citronellol, linalool) and 2% geranial. In addition, this oil contains three terpinene, phenol carbon-acid (rosmarinic acid), and flavonglychoside acids in low ratio. There are also caffeic acid, several flavonoids (luteolin-7-O-glucoside, isoquercitrin, apigenin-7-Oglucoside and rhamnocitrin), rosmarinic acid, ferulic acid, methyl carnosoate, hydroxycinnamic acid, and 2- (3', 4'-dihydroxyphenyl)-1,3-benzodioxole-5-aldehyde and some other aldehydes: beta-caryophyllene, neral, and geranyl acetate (Tagashira and Ohtake, 1998; Hohmann et al., 1999): essential oil content in the leaves at the stage just prior to blooming, or at the onset of blooming, ranged from 0.06 to 0.16 % (V/m), and the maximal essential oil content (0.09 to 0.45%) was in the plants from the second

Table 1. The components of essential oils obtained from *M. officinalis L.* leaves.

Components	Retention time	Mass percentage
Beta-ocimene Z	1020	0.2
Beta-ocimene E	1032	0.1
Citronellal	1086	0.01
Neral	1145	43.8
Geraniol	1221	5.3
Geranial	1246	5.2
Thymol	1258	7.9
Carvacrol	1274	0.8
Citronellyl formate	1276	0.2
Geranyl acetate	1362	2.3
Germacrene D	1375	0.3
Beta- caryophyllene	1424	13.5
Alpha-humulene	1448	0.7
Caryophyllene oxide	1575	0.3
Globulol	1581	6.8
Humulene epoxide	1617	0.3
5-cedranone	1629	0.2
Total		89.01

harvest in lemon balm from 17 European regions; the essential oil content in cultivar Citra from Bratislav was 0.13 and 0.23 to 0.27%, in 1st harvest and 2nd harvest, respectively were determined by Shultz et al., 1991. The essential oil content in the leaves of respective herbs was in the range 0.39 to 0.14% V/m (top third part-whole aerial part). Citrals (A and B) were 55.79, 48.46, and 59.74 and 56.87% in the top third part of the herb, the whole herb, and the leaves from those parts, respectively. Likewise, betacaryophyllene was 5.01, 3.87, 6.97 and 5.13%; beta-caryophyllene oxide was 17.19, 24.07, 15.64 and 17.82%; citronellal was 2.73, 5.51, 2.82 and 6.44% (Shamsi et al. (2005). Janina (2003) reported that the highest essential oil's ratio (0.14%) was obtained from the plants, cut in the beginning of blooming, grown in Çanakkale cological conditions. Essential oil obtained from a few different populations of *M. officinalis* L. cultivated in Poland had been investigated by Patora et al. (2003). In their study, the content of essential oil in the leaves and herb were recorded as 0.08 to 0.25 ml/100 g and 0.06 to 0.167 ml/100 g, respectively. In addition, essential oil was in the plant material from experimental patch then that from commercial cultivations, and essential oil of the fresh material was slightly higher than one of the dried one. Patora and Klimek (2003) have isolated six flavonoids from the leaves of lemon balm.

These flavonoids have been luteolin, luteolin 7-O-beta-D- glucuronopyranoside, apigenin 7-O-beta-D-glucopyranoside, luteolin 7-O-beta-D-glucuronopyranoside, luteolin 3′-O-beta-D-glucuronopyranoside and luteolin 7-O-beta-D-glucopyranoside- 3′-Obeta-D-glucuronopyranoside.

Effcet of environmental factors on essential oils content

If we look at differences found between genuine oils, we must consider the production parameters influencing essential oil composition and yield. These parameters can be dividing into intrinsic and extrinsic factor influencing on these effective material on the one hand, and processing and extracting methods used on the other hand (Janina, 2003). Generally, qualitative variation in oil composition is considered to be influenced by intrinsic genetic factors, whereas quantitative variation is influenced by extrinsic factors (Franz, 1993). As the essential oils show complex structures, essential oil rate or its chemical composition of lemon balm is strongly affected by several factors such as light intensity, nutrient, temperature, cultural practice genotype, plant part age, harvesting time, etc. for example, essential oil rate and tannin contents increase with increasing light intensity from 1000 to 1500 lux. Both essential oil content and its components depend upon the harvest cut height of lemon balm (Turhan et al., 2006). Average essential oil content in the top third part is 0.39%, whereas it is 0.14% in the whole aerial part. Thus there is an ontogenetic variation for essential oil in balm leaves (Hose et al., 1997). It is also important that the collection period of the plant material changes product quality criteria such as essential oil content and components.

Ozturk et al. (2004) reported that essential oil ratio affected positively by increasing water deficit while it was affected negatively by increasing salt concentration. Water deficiency increased essential oil from 0.12 to 0.16% and similarly Pitarevic et al. (1985) reported that a

long dry season should give a high oil production. Since the significant yield reduction starts at 25% water deficit, irrigation water deficiencies must not be applied over this level.

Hossein et al. (2009) reported that N fertilizer had significant effect on biological yield, essential oil percentage, essetial oil content, plant height and tiller number. Highest biological yield (6788 kg ha^{-1}) and plant height (61.63 cm) were produced by application of 90 kg N ha-1 and highest tiller number (32.6 tiller/plant), essetial oil percentage (0.2577%) and essetial oil content (16.05 kg ha^{-1}) were obtained under application of 60 kg N ha^{-1}. Consequently, optimal application of N fertilizer increased quantity and quality values of balm, but application of inordinate N fertilizer reduced all plant values.

The developmental stage of the plant is of considerable importance. Both the developmental stage of the entire plant and the maturity or age of single leaves on the same plant can influence the quantitative composition of the volatile oils produced. The harvesting time can have significant influence as well, since essential oils can vary considerably owing to diurnal and seasonal variation (Lawrence, 1986). The time of harvest during the day influenced oil content, and morning harvest after spraying the crop with water to prevent volatile terpene loss gave the best yield. Terminal leaves of *M. officinalis* contained 30% more essential oil compared with middle stem or basal leaves (Franz, 1993). However, the researchers found little difference in composition according to leaf position, whereas others (Hefendehl, 1970; Hose et al., 1997) reported more drastic differences. Buds and flowers contained the least essential oil, followed by the basal leaves, whereas terminal leaves contain the maximum. In respect to the oil composition, buds, flowers as well as the terminal leaves showed high concentrations of the citrals (90%), whereas middle stem and basal leaves show a decrease of these compounds in favour of citronellal and caryophyllene. In a valuable research by Turha et al. (2004), reported, the recommended plant density was 40*20 cm with plants propagated from seedlings. Lemon balm yield was affected by plant density, propagation method, and age. Essential oil content varied from 0.20 to 0.28%, which was similar to the content found by Holla et al. (2000), and it was not significantly affected by any treatment. In another experiment conducted by Ceylan et al. (1994) in Izmir-Turkey, they found that lemon balm herb yield changes depending upon amount of N application and plant density. The highest herb yield (11204 kg/ha) was obtained from the highest amount of N (180 kg/ha) at 30 * 30 cm plant density. On the other hand, at 60 kg/ha N application the maximum lemon balm herb yield was 8547 kg/ha at 40*20 plant density. In an experiment, with the same level of N application (60 kg/ha) and plant density, maximum yield was 11167 kg/ha in the second year. Therefore, this variation in the yield can be attributed to different climatic conditions and applications, such as fertilization and frequency of irrigation. In addition, propagation from seedlings seemed better than from cuttings with roots. It is difficult to interpret the reason for this compared with the present results. But it can be speculated that the lower production of cuttings obtained from 3-year-old plants may be because of age of plant source.

Farahani et al. (2009) in a research about effects of irrigation levels on essential oil of balm, reported that, highest shoot yield and height plant were relate to non stress treatment, highest essential oil yield was achieved under 40% FC and essential oil percent related to 20% FC. Highest stem diameter was related to 20% FC. It could be concluded that moderate drought stress is beneficial for balm essential oil. Substantial quantitative differences were reported in composition according to whether dried or fresh herb of *M. officinalis* was distilled (Shalaby et al., 1995). The oil from dried plant material was darker in colour, exhibited relatively higher concentrations of citronellal, pcaryophyllene and caryophyllene oxide, and lower concentrations of neral and geranial compared with the oil from fresh herb. Linalol, citronellal and geranyl acetate were reported to be elevated in the oil distilled from dried herb, whereas neral, geranial, nerol, geraniol, caryophyllene and its oxide were elevated in the oil distilled from fresh herb (Enjalbert et al., 1983).

The pharmacological activity of *M. officinalis*

Lemon balm has been traditionally used for different medical purposes as tonic, antispasmodic, carminative, diaphoretic, surgical dressing for wounds, sedative-hypnotic strengthening the memory, and relief of stress induced headache (Blumenthal et al., 2000). It is currently used for the relief of stress-induced headache, as a mild sedative-hypnotic, and as an antiviral to improve healing of herpes simplex cold sores (Blumenthal et al., 2000). The distillate (or hydrosol) of lemon balm (known as Arrack) is commonly used as an antidepressant, Ibn Sina (Avicenna), the well-known Iranian scientist, recommended *M. officinalis* for above indications. In addition, other traditional medicines have indicated that lemon balm is useful for seasonal effective disorder when mixed with St. John's wort (Kuhn et al., 2000). Furthermore, it is stated that the essential oil of lemon balm which is, used in aromatherapy, may be beneficial for mild depression. Despite of all this reports no pharmacological study showing antidepressant effect of this plant has been reported. Native to northern Mediterranean Regions (Tavares et al., 1996). Average essential content in the top third part is 0.39% (shultze et al., 1992). Major components are citral (neral+geranial) representing 48% of the essential oil, followed by citronellal with 39.47% and -caryohyllene with 2.37%

(Tavares et al., 1996). Essential oils of lemon balm are used as an anti-tumeral agent as a potential for cancer remedy or prevention (Janina, 2003). The volatile oils of lemon balm may also be used as an anti-virus agent and contains as anti- herpes simplex virus type 2 (HSV-2) substances (Turhan, 2006). Akhondzadeh et al. (2003), carried out the investigation to asses the efficacy and safety of lemon balm extract using a fixed dose (60 drops/day) in patients with mild to moderate Alzheimer's disease. Patients with mild to moderate Alzheimer's disease aged between 65 and 80 years were treated for four months, and divided two groups randomly to placebo or fixed dose of lemon balm extracts. At four months, Melissa extract produced a significantly better outcome on cognitive function than placebo. Besides, there were no significant differences in the two groups in terms of observed side effects except agitation, which was more common in the placebo group. With respect to conclusions, *M. officinalis* extract is value in the management of mild to moderate Alzheimer's disease and has a positive effect on agitation in such patients. Valnet (1990) have suggested that the potential for lemon balm to mitigate the effects of stress for example indicates Melissa for internal use for migraine, indigestion, neuralgic problems, insomnia, and spasms amongst others. However, in the application section he refers to the infusion, hydrolat, aromatic wine and tincture. The essential oil is mentioned only for external oil frictions against migraine and rheumatism (Valnet, 1990). In many cases, indications listed refer to the herb, not the essential oil, and practical experiences are extremely rare (Sheppard-Hanger, 1995).

Bolkent et al. (2005), reported that the administration of *M. officinalis* L. extract reduced total cholesterol and total lipid liver tissue, moreover increased glutathione levels in the tissue. As a result, it was suggested that *M. officinalis* L. extract exerted an hypolipidemic effect and showed a protective effect on the liver of hyperlipidemic rats. Probably the most interesting aspect of *M. officinalis* is its reported antiviral activity. A double blind study in Germany with 231 patients compared the antiviral activity of a concentrated dried extract of *M. officinalis* incorporated in a cream against a placebo. Results showed that Melissa was conclusively superior to the placebo against Herpes Already in 1964, activity of the extract on several viruses including Hwpes simplex, myxovirus, Semliki Forrest virus and Vaccinia virus were reported.

De Sousa et al. (2004) performed the study on antitumoral and antioxidant activities of lemon balm essential oil. The chemical composition and the biological activities of lemon balm essential oil obtained under controlled harvesting and drying conditions. Obtained findings showed that this oil was very effective against a series of human cancer cell lines and mouse cell line. Also, this oil possessed antioxidant activity, as evidence by reduction of 1, 1- diphenyl-2-picryl-hydrazyl (DPPH). These results pointed out to the potential use of lemon balm essential oil as an antitumoral agent. Sedative properties of the aqueous alcoholic extract (in the mouse) at low doses have been reported, as well as peripheral analgesic activity with high dosage (Soulimani et al., 1991). Sedative properties were also reported for the essential oil in the mouse and, furthermore, seemed to be dose-dependent (1 and 3 mg/kg), in that lower doses were more active. The authors proposed caryophyllene as the component responsible for the sedative activity (Wreker et al., 1993).

In food

Essential oil of lemon balm and extracts (*M. officinalis* subsp. *officinalis* and of *M. officinalis* subsp. inodora) can be used as antioxidant (Marangui et al., 2004; Mimica et al., 2003; Meftahizade et al., 2010) have described antimicrobial and free radical scavenging capacity (RSC) together with the effects on lipid preoxidation (LP) of lemon balm essential oil in their study. The antimicrobial activity was tested against 13 bacterial strains and six fungi. The examined essential oil exhibited very strong RSC; the most powerful scavenging compounds were monoterpene aldehydes and ketons (neral/geranial, citronellal, isomenthone, and menthone) and mono- and sesquiterpene hydrocarbons (E-caryophyllene). The most effective antibacterial activity was expressed on a multiresistant strain of Shigella sonei. A significant rate of antifungal activity was exhibited on Trichophyton species (Marangui et al., 2004). Meftahizade et al. (2010) reported that extract of *M. officinalis* can effect on shelf life of cofectionary, as well as on odor of Bezhi Bersagh, as a local confectionary in Iran.

In perfume and cosmetic industry

Due to hydrosol its essential oil and the strongly scented leaves are also used in perfumes and natural cosmetics. Hydrosol is the watery solution of the distillation that contains both the water-soluble plant components and micro-drops of essential oil (Janina, 2003). Because of they acidify the water or the product which is beneficial to the skin or in the body. Thus the hydrosol acts as a healing anti-inflammatory and mild but therapeutic antiseptic. Bacteria do not live well an in acidic environment which is why acids such as vinegar make good preservatives for such food items as pickles Chilé peppers and Olives. Acidic environments are astringent and so the hydrosols are useful in skin care products as astringents that constrict and contract the tissues. The hydrosols can be used externally in skin care products as a douche or internally diluted and taken as a tonic drink (Rose, 2000).

Micropropagation

Tavares et al. (1996) increase of BAP (Banzyl adenin pourin) concentration up to 1 mgl/l gave greatest efficiency in shoot number. Sato et al. (2005) reported that 8.8 µmol BAP in 11.42 µmol caused increase proliferation rate in shoot tip explants in *M. officinalis*. On the media containing 3 mgl^{-1} BAP, (82 to 90%) of explants showed shoot proliferation with 3.2 to 4.1 shoots per explant average (Meftahizade et al., 2010). The multiplication rate was highest (3.44 ± 0.25 cm) when 3 mgl^{-1} BAP in combination with 1 mgl/l NAA (naphtalic acetic acid) was used. Presence of NAA promotes shoot elongation as reported by Barrueto et al. (1999). Tavares et al. (1996) and meftahizade et al, (2010) also reported that, higher concentration of BAP induced more but smaller shoots, suggesting an inverse relation between the number of shoots and their elongation. The best results with respect to shoot regeneration in *M. officinalis* were also obtained on medium supplemented with indole acetic acid (IAA) and banzyl adenine (BA) (George, 1993). Murashique and Skoog (MS) medium can produce more shoot per explants and regeneration rate as well as B5. This result can be attributed to the No3/NH4 on MS and B5 media (66:34 and 50:50, respectively for MS and B5). This ratio is an important parameter on nitrogen uptake and pH regulation during plant tissue culture (George, 1993). It can relate to the interaction between these growth regulators. MS medium supplemented with 2 to 3 mg/L BAP in combination with 1 mg/l NAA is recommended for *M. officinalis* shoot initiation from shoot tip explants (Gogu et al., 2005). The addition of BAP and GA to the nutritive medium favoured multiple shooting and inhibited root formation of the new shoots that originate in nodes and shoot tips. Internode, leaf and root fragments provided callus on media comprising 2,4-D. Callus formation process was more intense if 2,4-D was associated with BAP.

The callus was compact, green in the case of internodes and leaves and cream for roots. Its proliferation from internode fragments was more intense. The callus provided by internodes and leaves on MS supplemented with BAP or with BAP and GA maintained its characteristics, assured its proliferation and sporadically induced root formation on its surface. The addition of 8.87 µmol L^{-1} BA or 11.42 µmol L^{-1} of IAA plus 8.87 µmolL^{-1} of BA to the MS medium resulted in a significant increase of shoot number per explants (Sato, 2005).

Shoot induction from hypocotyls

Tavares et al. (1996) reported that, cotyledonary nodes and leaves from 10 days-old seedlings were more suitable for regeneration than others, such as hypocotyledons. But meftahizade et al. (2010) reported, hypocotyls excised from 20 days-old seedlings had appropriate response to callus induction. Morphogenesis response of

M. officinalis was depending on explants and type of growth regulators used in culture media (Bajaj, 1986; Kool et al., 1999). Callus induction was obtained when IAA = 1.5 mgl/l, NAA = 1.5 mgl/l and kinetin = 0.5 mgl/l were used. This callus was a pale yellow, friable, with or without small green globules (meftahizade et al., 2010).

Root induction in regenerated shoots

Root formation required the presence of NAA in the culture medium (Tavares et al., 1996). The rooting ability of *M. officinalis* was inhibited in medium containing activated charcoal. 96% rooting were obtained after 25 days with 1 mg/l NAA, while IBA at the same time and concentration induced rooting in 64% of shoots (Meftahizade et al., 2010).

Disease

Sequence data (GenBank Accession Nos AY842508–AY842510) revealed that the plant was infected with Tulip virus X (TVX).

Drying of *M. officinalis* leaf

Hensel et al. (2008) used the stepwise process control to dry the *M. officinalis* L variety citronella. They found that the lowest energy consumption and shortest drying time was observed for change point 50%. But in terms of quality, the change point 50% shows high color change and the lowest content of essential oil. The combination 40 /50°C, for change point 20%, shows nearly no color change and the same essential oil content of standard drying, the stepwise drying strategy 40/50°C with change point at moisture content 20% is recommended for lemon balm. A decrease of 27% in drying time can be reached together with a reduction of 11% in consumed energy compared with conventional drying at no changes in quality. Further investigations are done with other temperature combinations (e.g. 30/60, 30/50, 30/40°C).

REFERENCES

Akhondzadeh S, Nooroonzian M, Mohammadi M, Ohadinia S, Jamshidi AH, Khani M (2003). *Melissa officinalis* extract in the treatment of patient with mild to moderate Alzheimer's disease: a double blind, randomised, placebo controlled trial. Food Protuguense Apr., 6 (4): 625-632.

Anonymous (2003). Microsoft Encarta Encyclopedia, 1993- 2003 Microsoft Corporatio, 6 (4):625-632.

Barrueto CLP Adriane CMG, Silvia BRC, Ana CM (1999). Plant regeneration from seedling explants of Eucalyptus grandis E.urophylla. Plant Cell, Tissue and Organ Cult., 56: 17-23.

Baytop T (1984). Treatment with Plants in Turkey. Ist. Arc University, 3255: 75-89
Blumenthal M, Goldberg A, Brinckmann J (2000). Herbal Medicine-Expanded Commission E Mongraphs. Newton, MA: Integrative Medicine Communications, 123: 230-232.
Bolkent S, Yanardag R, Karabulut-Bulan O, Yesilyaprak B (2005). Protective role of *Melissa officinalis* L. extract on liver of hyperlipidemic rats: a morphological and biochemical study. Ethnopharmacol., 14: 391-8.
Carnat A, Fraisse D, Lamaison JL (1998) the aromatic and polyphenolic composition of lemon balm (*Melissa officinalis* L. subsp. officinalis) tea. Pharmaceutics Acta Helvetiae, 72: 301-305.
Ceylan A, Bayram E, Ozay N (1994). Investigations on agronomic and technological characteristics of lemon balm (*Melissa officinalis* L.). Turkish J. Agric. For., 18: 125-130.
Davis P (1988). Aromatherapy- an AZ: C.W. Daniels, Saffron Walden, UK, citing Naves Natural Perfume Materials. NY Reinhold Publ., 14 (6): 452-456
De Sousa AC, Alviano DS, Blank AF, Alves PB, Alviano CS, Gattas CR (2004). *Melissa officinalis* L. Essential oil: Antitumoral and antioxidant activities. J. Agric. Food Chem., 52 (9):2485-2489.
Drozd J, Anuszewska E (2003). The effect of the *Melissa officinalis* extract on immune responce in mice. Acta Pol. Pharm. Sep-Oct., 60(5):395- 400.
Enjalbert A, Bessette AE, Bessette AR, Fisher DW (1983). Analysis of Oils, Melisse. Fitoterapia, 54(2): 59-63.
Farahani HA, Abbaszadeh B, Valadabadi SA, Darvishi HH (2009). Nitrogenous fertilizer influence on quantity and quality values of balm (*Melissa officinalis* L.). J. Agric. Ext. Rural Dev., 1(1): 031-033.
Franz Ch (1993) Genetics. In Hay, R.K.M. and Waterman, P.G. (eds). Volatile Oil Crops: London: Longman Group UK Limited.
George EF (1993). Plant propagation by tissue culture. Part1. The technology. UK: Butlerand Tammer, pp: 574
Bajaj YPS (1986) Biotechnology in agriculture and foresty. Tress I. Berlin: Springer-Verlag, 1: 1-23.
Gogu GH, Diana E, MaftieI D, Nicu N (2005). Investigations on the invitro morphogenetic reaction of *Melissa officinalis* L. Species. Analele Mtiinifice ale Universitii , Alexandru Ioan Cuza. Genetic Biol.. Mol., pp:103
Hefendehl (1970) Zusammensetzung des Aetherischen Oels von *Melissa officinalis* L. sekundire and changes of decomposition. Arch.
Pharm., 303: 345- 357.
Hensel O, Cuerveo S (2008). Drying of Lemon Balm (*Melissa officinalis* L.) using stepwise process control. Conference on International Research on Food Security. Nat. Resour. Manage. Rural Dev., 123-129.
Hohmann J, Zupko I, Redei D (1999). Protective effects of the aerial parts of Salvia officinalis, *Melissa officinalis* and *Lavandula angustifolia* and their onstituents against enzyme-dependent and enzymeindependent lipid peroxidation. Planta Med., 1999; 65: 576-578.
Holla M, Vaverkova S, Tekel J, Havranek E(2000). Content and composition of the oil from *Melissa officinalis* L. after application of Ridomil 72 WP. J. Essen. Oil Res., 12(4): 496- 498.
Hose M, Erman F and Helski G (1997). Ontogenic variation of the essential leaf oil of *Melissa officinalis* L. Pharmazie 52: 247-253.
Janina MS (2003) *Melissa officinalis*. The Int. J. Aromather., 10: 132-139.
Kennedy DO, Little WSAB (2004). Attenuation of laboratory-induced stres in humans after acute administration of *Melissa officinalis* (lemon balm). J. Pharm. Pharmacol., 56(5): 677-681.
Kool LT, Keng CL, Hoe CTK (1999). *In vitro* rooting of sentag shoot (Azadiracta excelsa L.) and acclimatization of the plantlet. *In vitro* Cell Dev. Plant, 35: 396- 400.
Kuhn MA, Winston D (2000). Herbal therapy and supplements: a scientific and traditional approach. Philadelphia, PA, USA: Lippincott Williams and Wilkins, pp. 210-212.
Lawrence BM (1986). Essential Oil Production. A Discussion of Influencing Factors. In: Biogeneration of Aromas. Am. Chem. Soc., 27: 363-369.
Marongiu B, Porcedda S, Piras A, Rosa A, Deiana M (2004). Antioxidant activity of supercritical extract of *Melissa officinalis* subsp. officinalis and *Melissa officinalis* subsp. inodora. Phytother. Res., 18(10): 789-792.
Masakova S, Holedin M, Ginberg A (1979) Chemical composition of volatile oil in lemon balm. inodora. Phytother. Res., 8: 89-94.
Meftahizade H, Lotfi M, Moradkhani H (2010). Optimization of Micropropagation and establishment of cell suspension culture in *Melissa officinalis* L. Afr. J. Biotechnol., 9(28), pp. 4314-4321.
Meftahizade H, Moradkhani H, Naseri B, Lotfi M, Naseri A (2010). Improved *in vitro* culture and micropropagation of different *Melissa officinalis* L. genotypes. J. Med. Plant Res., 4(3). 240-246.
Meftahizade H, Sargsyan E, Moradkhani H (2010). Investigation of antioxidant capacity of *Melissa officinalis* L. essential oils. J. Med. Plant Res., 4(14): 1391-1395.
Mimica-Dukic N, Bozin B, Sokovic M, Simin, N (2003). Antimicrobial and antioxidant activities of *Melissa officinalis* L. (Lamiaceae) essential oil. Acta Pol. Pharm. Nov.-Dec., 60(6): 467-470.
Ozturk A, Unlukara A, Ipek A, Gurbuz B (2004). Effects of salt stress and water deficet on plant growth and essential oil content of Lemon Balm (*Melissa officinalis* L.). Pak. J. Bot., 36(4): 787-792.
Patora J, Majda T, Gora J, Klimek B (2003).Variability in the content and composition of essential oil from lemon balm (*Melissa officinalis* L.) cultivatedin Poland. J. Endocrinol. Invest. Oct., 26(10): 950-955.
Pitarevic ID, Kustrak J, Kuftinec U, Blazevic N (1985). Influence of ecological factors on the content and composition of the essential oil in Salvia officinalis. Proc. 15th International Symposium on Essential Oils, July 19-21, 1984, The Netherlands, 203-207.
Rose J (2000). An Herb for Skin Care. Aromatic Plant Project Pres Release, 8: 897-901.
Saglam C. Atakisi I, Turhan H, Arsalanoglu F, Onemli F (2004). Effect of propagation method, plant density, and age on lemon balm (Melissa officinalis) herb and oil yield. N. Z. J. Crop Hortic. Sci., 32: 419-423.
Sandra PC, Oliver H (2008). Drying of Lemon Balm (*Melissa officinalis* L.) using stepwise process control. Conference on International Research on Food Security, Natural Resource Management and Rural Development.
Sarer E, Kokdil G (1991). Constituents of the essential oil from *Melissa offcinalis*. Planta. Medicus, 57: 89- 95.
Sato A, Dasilva S, Celso LSL, Rosane ASSG, Maria AE (2005). Essential Oil Composition of *Melissa officinalis* L. *in vitro* Produced under the Influence of Growth Regulators. J. Braz. Chem., 16: 1387-1390.
Schultze W, Zaglein S, Hose KH, Kubeczka FC (1993). Volatiles in flowers of balm (*Melissa officinalis* L.). In: Advances in Labiate Science. (Eds.): R.M. Harley and T. Reynolds. The Royal Botanic Gardens, UK, pp. 357-366.
Shalaby AS, Arker S (1995). Oil of *Melissa officinalis* L., as Affected by Storage and indicator of therapeutic use. Planta Medicus, 36: 274.
Shamsi AM, Amnzade Y, Jahanshir F, Jamshidi A (2005). Production of suspension cell culture in *Melissa officinalis* and comparison of produced secondary metabolites in callus versuswhole plant. J. Med. plant, 13: 68-71.
Sheppard-Hanger S (1995). The Aromatherapy Practitioner Reference as indicator of therapeutic use. Planta Medicus, 36: 274.
Tagashira M, Ohtake Y (1998). A new antioxidative 1,3- benzodioxole from Melissa officinalis. Planta Med., 64: 555-558.
Tavares AC, pimento MC, Goncalves MT (1996) Micropropagation of *Melissa officinalis* L. through proliferation of axillary shoots. Plant cell Rep., 15: 441-444.
Turhan M (2006) Hand book of herbal plants, chapter 4. *Melissa officinalis*, 3: 184-245.
Tzanetakis IE, Mackey IC, Martin RR (2005). Plant Pathol. 54: 562-568.
Valnet, J (1990). Aromatherapy. 11th Edn. France: Maloine, Paris, 11: 242-246
Wagner H, Sprinkmeyer L (1973). Pharmacological Aspect of Balm Spirit, 113:1159–1166
Werker J (1993) Function of Essential Oil Secreting Glandular Hairs in Aromatic Plants of the Lamiaceae – A Review. Flavor and Fragrance J. 8: 249-255.
Zargari A (1991). Medicinal plants. Tehran: Tehran University Publications, pp. 77-83.