Journal of Applied Research on Medicinal and Aromatic Plants xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Journal of Applied Research on Medicinal and Aromatic Plants

journal homepage: www.elsevier.com/locate/jarmap

Effect of shading on leaf yield, plant parameters, and essential oil content of lemon balm (*Melissa officinalis* L.)

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A R T I C L E I N F O

Keywords:
Medicinal and aromatic plants
Field trial
Light reduction
Biomass yield

A B S T R A C T

In the present study, the effects of a light reduction by plant protection nets on lemon balm (*Melissa officinalis* L.) have been investigated. The lemon balm plant stand was surveyed over three consecutive years under sandy soil conditions. Harvest took place in two cuts in each of the investigated years (2013–2015). Shaded plants tended to have a higher plant height. The moderate shading could not positively influence leaf yield, investigated plant parameters, and the essential oil content. It can therefore be concluded that the use of protection nets with a moderate light reduction of 10–15% is not advantageous for lemon balm cultivation under temperate climate conditions. However, their use, e.g. for the protection against insect pests, is not regarded as negative, as they were not impairing leaf yield or essential oil content of lemon balm.

1. Introduction

Lemon balm (*Melissa officinalis* L., Lamiaceae) is an important medicinal and spice plant. The contained essential oil gives the plant its typical lemon-like fragrance, which is appreciated as a flavoring ingredient for culinary uses.

Even more important is the wide use of lemon balm as a medicinal plant. For instance, it is used internally for treating tenseness, restlessness and irritability, nervous sleeping disorders, or functional gastrointestinal complaints (Blumenthal et al., 2000, 1998). Externally it is used against *Herpes labialis* (cold sores) (ESCOP, 2003). Furthermore, it is licensed as a standard medicinal tea in Germany for sleep disorders and disorders of the gastrointestinal tract (BfArM, 2015). The dried leaves (Melissae folium) are the parts of the plant that are used pharmaceutically (Ph. Eur. 7, 2011).

According to FNR (2014), there is a high demand for lemon balm in Germany, but the greatest part is imported from other countries. Therefore, increasing the cultivation area and productivity is of interest.

Lemon balm is a perennial plant, originating from an area between the Mediterranean region and the western Tien Shan (Hanelt, 2001). It has been grown in subtropical as well as temperate regions for a long time and is widely naturalized in those areas, including Germany. It can reach a height of about 1 m, and several harvests per year are possible. Normally, plant stands of lemon balm are cultivated for two to three years (Bomme et al., 2013).

Yield and quality of medicinal and aromatic plants can be influenced by genetic, phenological, and environmental factors, as well as by the cultivation management (Azizi et al., 2009; Mortensen, 2014; Mrlianová et al., 2002; Novak et al., 2000; Sellami et al., 2009). Therefore, it is of great interest to better understand the determining factors on the quality of lemon balm as well.

Generally, the use of different protection nets in the cultivation of a diverse range of plants is getting more and more popular. Nets are used, among others, for the improvement of the microclimate, for the protection of plants from excessive sunshine, hail, and pests, as well as for the modification of their morphology and quality (Ben-Yakir et al., 2012, 2008; Castellano et al., 2008; Oren-Shamir et al., 2001). It has been proposed to use protection nets for the prevention of leafhopper infestations in the cultivation of medicinal and aromatic plants (Meyer et al., 2010). Besides, it was shown that the use of agrotextile coverings for the improvement of the microclimate in lemon balm cultivation led to higher essential oil (EO) contents under Swiss conditions (Carron et al., 2008). The influence of differently colored shading nets on lemon balm has been investigated under Brazilian conditions, where to some extent higher leaf yields have been reported for the plants cultivated under the nets (Brant et al., 2009; Oliveira et al., 2016). However, it remains unclear how lemon balm plants react on shading nets under temperate climate conditions, as in Germany.

On the one hand, it is important to mention that any kind of covering reduces the light intensity, which may lead to a reduction of the energy supply for photosynthesis and a lower biomass production of

Abbreviations: a.i., active ingredient; DM, dry matter; EO, essential oil; FM, fresh matter; LAI, leaf area index; R:FR, red:far-red ratio; SPAD, soil & plant analyzer development

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http://dx.doi.org/10.1016/j.jarmap.2017.04.003
Received 12 July 2016; Received in revised form 6 April 2017; Accepted 12 April 2017
2214-7861/

the plants. On the other hand, plants receive far more light than they can use for photosynthetic processes even under temperate climate conditions, which puts them under light stress (Wilhelm and Selmar, 2011). Protective mechanisms, like the process of photorespiration, or the formation of secondary metabolites which diverts precursors from the primary metabolism, are costly for the plants and might affect the biomass yield (Logemann et al., 2000; Walker et al., 2016; Wilhelm and Selmar, 2011). On the other hand, some secondary metabolites, like the essential oil, are the valuable components of medicinal and aromatic plants like lemon balm. It is therefore of interest to find cultivation conditions that lead to an improved biomass yield, without impairing the content of valuable secondary metabolites. Therefore, it was the aim of the current study to clarify the effect of shading on growth, leaf yield, and essential oil content of lemon balm on a sandy soil site in Germany. It was of interest to know whether there is a shading effect in a lemon balm plant stand during a common cultivation period of three years.

2. Material and methods

2.1. Plant parameters

A field trial with lemon balm (*Melissa officinalis* L.) was established in 2012 at the experimental station Gross-Gerau (49° 56′ N, 8° 30′ E), Germany, on a sandy soil (soil type: arenosol; humus content: 1.1-1.5%; soil value: 20–25 points; pH: 6.5) after the pre-crop summer barley. The harvests took place in the years 2013, 2014, and 2015, with two cuts in each year.

The climate conditions (January to December) are characterized by a long-term air temperature of 9.9 °C and an annual precipitation of 606 mm (Table 1). During the experimental period, higher air temperatures compared to long-term average were observed in all three years. The annual precipitation differed between the experimental years, characterized by a high value in 2013 (739 mm), followed by lower precipitation in 2014 (662 mm) and 2015 (491 mm). However, the year 2014 was characterized by an exceptionally high precipitation in the summer, especially in August (Table 1).

A block design was established with the lemon balm genotypes 'Aufrechter Typ', 'Lemona', and 'NLC', to investigate the factor light with two levels (natural light and shade), with four replications. Due to severe winter losses of the genotypes 'Aufrechter Typ' and 'Lemona' in the first year, only 'NLC' was chosen for the investigations. The used genotype 'NLC' is characterized as an upright growing type, forming erect shoots in the first year of cultivation. This beneficial morphological parameter is combined with good yield characteristics and a good frost tolerance, which makes it interesting for a cultivation under German climate conditions. The plots (7 m × 1.5 m) were planted with 4 rows, each row consisting of 19 lemon balm plants. Taking account of a border effect, only the inner 2 rows with 17 plants each were considered as harvest plots, leading to 34 harvested plants per plot (4.70 m^2). To reach homogenous plant development and growth, the lemon balm plants of all plots were topped in spring after the first winter (May 6, 2013).

A basal dressing with P, K, Mg, and S was applied in spring according to the recommendations of VDLUFA (Association of German Agricultural Analytic and Research Institutes) to reach optimal nutrient contents in the soil (VDLUFA, 1991). Nitrogen was applied in the form of calcium ammonium nitrate. In the establishing year 2012, plants were fertilized in June with 50 kg N/ha, and at the end of August with 40 kg N/ha. In the harvest years, the plots were supplied with N in spring according to the mineral N content of the soil (2013: 90 kg N/ha; 2014: 60 kg N/ha; 2015: 50 kg N/ha), and additionally 50 kg N/ha after each cut.

Due to the sandy soil and weather conditions (cf. Table 1), plants had to be irrigated as needed, with 20–30 mm per irrigation time (in total 2012: 60 mm, 2013: 25 mm, 2014: 80 mm and 2015: 140 mm). Weeds were controlled manually, and with Fusilade Max (1 l/ha, a.i. fluazifop-P-butyl) or Basagran (2 l/ha, a.i. bentazon). For pest control, Karate Zeon (1 l/ha, a.i. lambda-cyhalothrin) and Calypso (0.25 l/ha, a.i. thiacloprid) were used against leafhoppers (*Eupteryx* sp.), and Askon (75 ml/ha, a.i. azoxystrobin and difenoconazole) against *Septoria* sp.

Light reduction for the shaded plots was realized with a light green polyethylene anti bird net with a mesh size of 18 mm (Novatec, Germany), installed at about 1.90 m height. In 2015, the net was used in a double layer. Light intensity was continuously registered for each of the two light variants with light sensors installed within the plant stand, at a height of about 1.20 m to make sure that they would not be overgrown by the plants during their development. Light sensors were a Silicon Pyranometer Smart Sensor for global radiation, and a Photosynthetically Active Radiation (PAR) Smart Sensor, both connected to a HOBO Micro Station (Onset Computer Corporation, Bourne, USA). Nets were set up on May 16, 2013, April 4, 2014, as well as April 4, 2015, and left above the plant stand until the last harvest.

Light intensity was registered in the periods August 2 to September 15, 2013, April 4 to September 9, 2014, as well as May 7 to June 6, 2015, and July 7 to August 18, 2015. The measurements of the sensors in each of the two light variants were regarded from 8:00 till 18:00 h, and the difference between the measurements from the shaded and from the unshaded plots were used for the calculation of an average light reduction.

Table 1
Air temperature and precipitation at the experimental station Gross-Gerau in 2013–2015.

	Temperature – Monthly average [°C]				Precipitation – Monthly sum [mm]			
	2013	2014	2015	Lt-av.[a]	2013	2014	2015	Lt-av.[a]
January	1.8	4.3	2.9	1.0	34.6	40.2	69.9	37.0
February	0.8	5.2	2.0	1.9	40.2	40.4	26.1	35.1
March	2.6	8.4	6.4	5.7	32.8	19.2	23.7	40.6
April	10.0	13.3	10.3	9.7	75.3	30.2	24.5	39.9
May	12.8	14.2	14.4	14.1	138.0	61.9	20.1	58.7
June	17.7	18.5	18.1	17.3	59.6	27.5	65.4	66.6
July	21.9	21.0	22.2	19.2	13.1	85.5	36.3	66.4
August	19.0	17.5	21.5	18.3	72.3	128.1	43.1	66.9
September	14.8	16.1	14.3	14.5	60.6	36.1	62.3	47.2
October	11.5	12.6	9.3	9.6	111.1	76.9	18.5	50.6
November	5.5	7.2	8.0	5.0	70.4	58.1	68.8	48.5
December	4.0	3.7	6.7	2.1	30.8	58.3	32.5	48.7
Sum					738.8	662.4	491.2	606.2
Average	10.2	11.8	11.3	9.9				

[a] Lt-av. = long-term average (1954–2015).