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Reflectance evolution due to effect of manganese to the peppermint antioxidant capacity activity and monoterpene contents

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1. Introduction, Knowledge, Objectives

Essential oil produced by peppermint (*Mentha* × *piperita* L.) is mainly composed of several monoterpene compounds and known for its antioxidant capacity. From an economic point of view, peppermint essential oil is one of the most popular and widely used oils in food, cosmetic and medicinal products (Herro and Jacobs, 2010). Monoterpene biosynthesis is strongly affected by several intrinsic (genotype, ontogeny etc.) and extrinsic (environmental) factors. Among others, manganese (Mn) plays an important role in various enzymatic and non-enzymatic reactions related to antioxidant activity, monoterpene biosynthesis and utilization of other mineral elements (Ca, Mg, Fe and P) (Marschner, 1995). However, excessive Mn concentrations in plant tissues can cause oxidative stress (Lei et al, 2007).

Consequently, in intensive mint cultivation systems it is crucial to detect plant Mn stress at an early stage, in order to minimize loss of oil productivity. To achieve this, it is advantageous to develop a real-time, non-contact and non-destructive sensing methodology. Current computational intelligence techniques have allowed the development of hyperspectral optic systems that supply information from a targeted object (Story and Kacira, 2015). In addition, reflectance indices (RI) as the result from the combination of two or more spectral bands recorded by the hyperspectral camera could give detailed information about plant Mn status.

The present study aims to provide more insight into optimization of peppermint Mn threshold in order to achieve maximum oil production and quality. For this purpose, peppermint cuttings were grown in quartz sand under greenhouse conditions. Additional Mn was applied to the root zone at four levels (0.0, 0.5, 1.0, and 2.0 mg L^{-1}). Monoterpene content, antioxidant capacity and nutrient content of the leaves were determined by chemical analyses. The results were correlated with leaf spectral properties recorded by hyperspectral camera.

2. Material and Methods

The experiment was conducted during September-December 2017 in a greenhouse at Hochschule Geisenheim University, Geisenheim, Germany. Uniform sized cuttings of *Mentha x piperita* L. plants were raised and transplanted into pots (1 L) filled with quartz sand (particle size 0.1 - 0.4 mm). Plants were cultivated under four different Mn supply levels (0.0, 0.5, 1.0, and 2.0 mg L^{-1}). Nutrient solutions were prepared by mixing Kristalon and Calcinit (both YARA, Oslo, Norway) with rainwater at a ratio of 70:30 with a set EC of 1.5 dS m^{-1}. Target-Mn concentration was adjusted by adding the respective quantity of $Mn(NO_3)_2$ to the nutrient solutions. Each treatment consisted of 20 pots. Individual pots were regarded as replicates. The plants were irrigated with 50 ml nutrient solution every three days. Each 10 plants from every treatment were harvested 48 and 76 days after 27 and 55 days after Mn treatments started (DAMS), respectively. The leaves were stored in liquid nitrogen immediately after sampling. The frozen leaf material was ground to fine powder and used for determination of the antioxidative capacity (photoluminescence method, protocol of Analytik Jena AG, Jena, Germany) and the monoterpene content (extraction in methyl chloride and quantification by GC-MS, modified procedure of Gershenzon et al. 2000). An amount of the grounded powder was dried for determination of leaf nutrients using the Kjeldahl extraction and quantification by ICP-OES. The hyperspectral camera Imspec V10 (Spectral Imaging Ltd, Finland) was used to obtain reflectance measurements of the intact mint plants. Katsoulas et al. (2014; 2016) and Elvanidi et al. (2016) give info about camera's set up and the calibration procedures followed. Reflectance indices (RI) that combine the information resulted from near-infrared (750 nm), red-edge (650 nm) and green spectrum (550 nm) were correlated with Mn and monoterpene factors. Comparison of means was performed by applying one-way ANOVA at a confidence level of 95 % ($p \leq 0.05$) using SPSS (IBM, New York, USA). Additionally, linear regression analysis in SPSS was performed between RIs or Mn concentration imposed to the plants and some abiotic parameters.

3. Results

Table 1 presents the nutrient content, antioxidative capacity and monoterpene compounds in the mint leaves at two harvests (27 DAMS and 55 DAMS) in plants cultivated with different concentrations of additional Mn. The plants supplied with 1 and 2 mg L^{-1} Mn had significantly higher nitrogen concentrations at the 2nd harvest. In fact, the difference observed between the values of plants with 0, 1 and 2 mg L^{-1} Mn supply was more than 4 % ($p<0.05$). Significantly lower concentrations of phosphorus were recorded in leaves of plants that were fertilized with very low additional Mn concentrations. Additionally, linear regression was observed between the Mn content accumulated in the root zone with the Mn concentrations measured in the leaf area. On the other hand, no variation was observed in potassium, calcium and copper values.

The antioxidative capacity of the plants increased with increasing Mn supply at the 1st harvest. Particularly, the plants supplied with 2 mg L^{-1} Mn presented more than 12 % higher antioxidative activity ($p<0.05$) compared to plants with no additional Mn. However, until the 2nd harvest, the extra Mn supply reduced the antioxidative capacity. The concentration of the leaf monoterpenes Beta-Pinene, Beta-Mycrene, Eycalyptol, Menthone, and Pulegone were increased by 8% in plants with 0.5 mg L-1 additional Mn supply at the 1st harvest ($p<0.05$). However, the extra Mn applied to the plants the following days affected negatively their concentration. On the other hand, Methylocetate, Neomenthol and Levomenthol concentration increased with increasing Mn supply. Particularly, at the 2nd harvest, the difference in Neomenthol and Levomenthol content between the control and 2 mg L-1 Mn supplied plants was more than 30 % ($p<0.05$).

Table 1. Leaf nutrient content, antioxidant capacity and monoterpene compounds in oil produced in mint leaves supplied with different Mn doses in the root zone.

Nevertheless, the mean values of $RI=R_{750\ nm}/R_{650\ nm}$ and $RI=R_{750\ nm}/R_{550\ nm}$ were linearly correlated with leaf Mn and Menthone content (Fig. 1). As the Mn and Menthone values decreased, both RI increased, while the determination coefficient of their linear regression was significant and equal to 0.81 and 0.64, respectively ($p<0.05$).

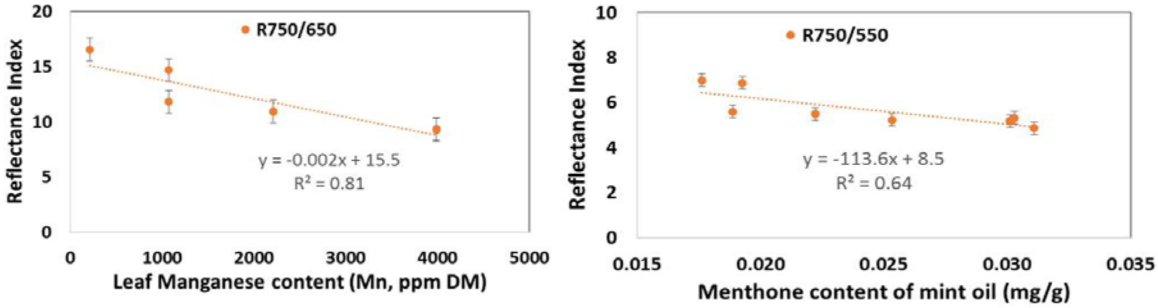

Figure 1. Linear regression between (a) RI= R750 nm/R650 nm and Mn concentration (mg L-1 DM) in the mint leaves and (b) RI= R750 nm/R550 nm and Menthone concentration (mg/g) in the mint oil.

4. Discussion

Recently, it has been reported that the expression levels of Mn transporter genes in shoots of ryegrass genotypes were increased by increasing supply of Mn (Reyes-Díaz et al., 2017). Accordingly, there was a strong positive relationship between the Mn accumulation in leaves and the applied concentration of Mn in nutrient solution (Table 1). These results are in line with those of Nazari et al. (2017, 2018). Due to this significant relationship, the Mn concentration in the leaf area may be predicted according to the Mn concentration applied through the irrigation system. Moreover, it is known that the concentration of other nutrient elements affecting the quality and quantity of essential oils such as nitrogen (Abdelmajeed et al, 2013) might be influenced by Mn supply.

Additionally, Mn has a profound role as a cofactor for the antioxidant activity (Zelko et al., 2002). According to the literature (Nazari et al., 2017, 2018), antioxidative capacity was expected to be elevated with the external supply of Mn. Since Mn has a profound role in plant metabolism it can affect the content and chemical composition of essential oils in plants. The study of Ghannadnia et al. (2014) showed also differences in the terpene compounds among different Mn concentrations. In the current research, the antioxidant capacity due to Mn supply, was elevated clearly for 27 DAMS, while for 55 DAMS no systematic variation was observed. In this case, the long-period of Mn supply affected negatively the antioxidant levels of the leaves.

Finally, the results of the statistical analysis illustrated that RI750/650 and RI750/550 can effectively estimate Mn and Menthol status. Similar findings are presented by Adams et al. (1993). Therefore, the results of RI that combine the information in near-infrared region, red-edge and green spectral region could potentially be used in quantifying crop Mn and Menthol status, however this relationships need to be further evaluated.

5. Conclusions

The additional manganese applied in the plants through irrigation system plays an important role in the antioxidant capacity and monoterpene concentration of mint essential oil. However, different Mn supplies in the root zone affect differently the aforementioned factors. Particularly, the Mn concentration varied from 0.5 to 1 mg L^{-1} is indicated to increase the antioxidant capacity in the mint plants and produce optimum amount of most of the monoterpene compounds in the mint plants. However, excess Mn supply for more than 27 days could provide negative results. Meanwhile, the optimum Mn threshold to produce maximum amount of Menthylacetate, Neomenthol and Levomenthol varied from 1 to 2 mg L^{-1}. Additionally, significant correlation was observed between the Mn and Menthol plant status with RI measured in near-infrared and red-edge or green spectral region.

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