Manganese treatment effects on terpene compounds of Cuminum cyminum flowers

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**Abstract**

The present study was undertaken to identify terpene compounds in different organs i.e. root, shoot, leaf and flower of cumin (Cuminum cyminum L.) as well as the flowers of treated plants with different concentrations of manganese (Mn). The plants were sprayed with four different concentrations of Mn (0, 40, 80 and 160 ppm) at (1) both late vegetative and blooming stages, and (2) only at blooming stage. Later, \(\alpha\)-pinene, \(\beta\)-pinene, \(\alpha\)-phellandrene, m-cymene and \(\gamma\)-terpinene compounds were determined in different organs of untreated plants as well as in flowers of treated plants. Quantity and quality of the identified compounds were different in the various plant parts as well as in the flowers of treated plants. Terpene compounds of the flowers were the same as shoots, but had higher concentration in the flowers. Notably, \(\alpha\)-phellandrene was the only terpenoid compound in the leaves and \(\alpha\)-pinene and \(\beta\)-pinene were not detected in the roots. The highest concentration of \(\alpha\)-pinene was observed in the flowers of plants treated with 80 ppm concentration of Mn at blooming stage. According to the results, \(\alpha\)-phellandrene and m-cymene increased after treatment with the highest Mn concentration (160 ppm) both at vegetative and blooming stages. Also, \(\alpha\)-pinene and \(\alpha\)-phellandrene were reduced considerably in the flowers treated with 40 ppm concentration of Mn at blooming. Overall, the manganese treatments at both vegetative and blooming stages increased the monoterpene concentrations of the flowers more than treatment at the blooming stage alone.

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1. Introduction

Cumin (Cuminum cyminum L.) is a small, slender, glabrous herbaceous annual plant belonging to the family Apiaceae. The white or pink flowers bloom in small compound umbels (Vedamuthu et al., 1994). The seeds come as paired or separate carpel and are 3–6 mm long. In addition of medicinal properties (Aslam et al., 2003; Aruna et al., 2005), antiradical and antimicrobial properties of cumin seed oil have been reported (Ramadan et al., 2012). Cumin also has insecticidal (Ebadollahi et al., 2012), antifungal (Shahi et al., 2012) and larvicidal (Singha and Chandra, 2011) activities that can be exploited commercially as it is easily bio-degradable and less toxic to environment. Cumin seeds contain volatile oil (2–5%) that imparts the characteristic aroma to the seeds (Behera et al., 2004). The characteristic flavor of cumin is probably due to dihydrocuminaldehyde and monoterpenes (Weiss, 2002). Cuminaldehyde, \(\alpha\)-pinene, \(\beta\)-pinene, p-cymene and \(\gamma\)-terpinene have been usually reported as the main constituents (Bettaib Rebye et al., 2012). The terpenes are volatile compounds with strong flavor that are most often extracted from plant materials. These compounds are distributed in various parts of plants: in florets, fruits, leaves, bark, and roots (Sedlakova et al., 2002). El-Sawi and Mohamed (2002) reported that cumin herb had oil and terpenoid compounds. The composition of the volatile oil obtained from the cumin herb markedly differed from that of the seed (El-Sawi and Mohamed, 2002). Composition of the essential oil of cumin depends on many factors such as plant part, harvest-time, extraction method, type of cultivar, geographic origin, and storage conditions (Kan et al., 2007). The production of essential oils not only depends upon the metabolic state and preset development-differential differentiation program of the synthesizing tissue, but also is highly integrated with the physiology of the whole plant. In general, terpenoids are a predominant constituent of plant essential oils, but many of these oils are also composed of other chemicals like phenypropanoids (Sangwan et al., 2001). Application of micronutrients, as supplements to macronutrients, has been reported to have significant effects on herb yields and oil contents of ocimum, marjoram, mint, and geranium (Sharma et al., 1980; Wahab and Hornok, 1983). Manganese has been shown to be the most effective single micronutrient enhancing oil production (Nandi and

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This essential trace element plays an important role in several physiological processes as almost every compartment of the cell carries at least one enzyme whose activity is dependent on Mn. Manganese acts as cofactor for oxidases, dehydrogenases, and sugar transferases (Crowley et al., 2000; Culotta et al., 2005; Keen et al., 2000). Cumin herb and seed showed the differences in the oil constituents under different treatments of Mn. Application of the microelement spray increased the main constituents such as cuminaldehyde, p-cymene, α-terpineol, thymol, and acoradiene, but other constituents such as B-pinene decreased under this treatment (El-Sawi and Mohamed, 2002). To the best of our knowledge, there have been few reports on composition of the herb oil of cumin (El-Sawi and Mohamed, 2002). No report appears to have been published on the terpenoid components of cumin separated organs or on the effects of Mn treatments with different concentration on terpenoid compounds of cumin flowers with a length of 3–4 mm. However, physiological factors regulating terpene accumulation are poorly known. Given the economic importance of monoterpenes for different industries such as fragrance, flavor, and pharmaceutical industries, sufficient information concerning different aspects of essential oil (containing terpenoid compounds) metabolism in plants such as site of oil production, ontogeny, photosynthesis, and nutritional relationship is necessary. Also, knowledge of the processes that control monoterpenoid biosynthesis and accumulation in plants can be of value in increasing the yields of these commercially valuable natural products. The present study investigates terpene constituents of cumin root, shoot, leaf, and flower to illustrate their quantity and quality in each organ and also the changes of these compounds under different treatments of Mn in the flowers as the most important storage organs of the plant for human consumption.

2. Materials and methods

2.1. Plant material, growth conditions, and treatments

Seeds of cumin native to Neishabour region (Iran) were subjected on water current for 12–15 h. Then, they were surface sterilized by immersion in 2% (v/v) NaOCl for 3 min and sulfur 80% DF fungicide for 2 min, followed by three times rinsing in sterile water after each step. The seeds were next transferred to plastic pots containing Klaissmann-Deilmann potground H peat moss under equal greenhouse conditions (day/night cycle of 16/8 h, at 26 ± 3 °C) and irrigated three times per week. At late vegetative stage, the plants were subjected to different concentrations of Mn (as MnSO4.4H2O; 0, 40, 80 and 160 ppm) as foliar sprays at both late vegetative and blooming stages or at blooming stage only (Table 1). The terpene compounds of different organs were also investigated in the untreated plants. For terpene extraction analysis of different organs, samples of root, stem, leaf, and flower (with a length of 3–4 mm) were harvested (Fig. 1). To study the Mn content absorption, total aerial parts of the plants from each of the eight treatments (Table 1) were harvested. To investigate the effects of Mn on terpene constituents, flowers with a length 3–4 mm were selected from the different treatments 12 h after spraying. After washing with distilled water, the plant samples were dried at room temperature, protected from light, and stored in capped bottles.

2.2. Determination of Mn content in cumin

Aerial parts of the plants in different treatments were rinsed twice with distilled water. Samples of each treatment were dried for 24 h at 60 °C, weighed, and then ashed in a 480 °C oven for 16 h. After cooling, the ash was digested with 2 ml HNO3 (65%) and heated to dryness. The sample was then dissolved in 5 ml 3 N HCl and brought to volume in a 25 ml volumetric flask using 0.1 N HCl. The Mn contents were determined by atomic absorption system (GVC 902, Australia).

2.3. Terpene extraction from plant materials

Preparation of terpene extracts were performed as described previously (Crocoll et al., 2010) with slight modifications i.e. dried plant materials were ground to a fine powder with mortar and pestle. The powder (50–100 mg) was soaked in 2 ml ethyl acetate: pentane (2:1) for 24 h at room temperature with constant rotation. The solution was cleared with activated charcoal for 5 min and dried over a column of 50 mg water-free Na2SO4. The extracts were transferred into a brown capped bottle and stored at 4 °C.

2.4. GC–MS analysis of plant volatiles

Constituents of the extracted samples were identified by gas chromatography (Agilent 6890N, USA) coupled to a mass spectrometer (Agilent 5973, USA) or a flame ionization detector (FID). For analyses 1 μl of pentane or ethyl acetate: pentane (2:1) extracts were injected with an injector temperature of 230 °C. The terpenes were separated on a HP-5 column: 30 m length, 0.25 mm inner diameter, and 0.25 μm film thickness (J and W Scientific, Sanata Clara, CA, USA); GC–program: 40 °C for 2 min, first ramp

![Fig. 1. Cumin grown under greenhouse condition at vegetative (A) and flowering (B) stages. Bars: 10 mm.](image-url)
5 °C min⁻¹ to 175 °C, second ramp 90 °C min⁻¹ to 250 °C, final 3 min hold). GC–MS carrier gas: helium at 1 ml min⁻¹; GC-FID carrier gas: hydrogen at 2 ml min⁻¹.

2.5. Compound identification

The identity of the extract components was assigned by comparison of their retention indices relative to (C8–C22) n-alkanes with those of literature or with those of authentic compounds available in the laboratory. Further identification was made by matching their recorded mass spectra with those stored in the Wiley/NBS mass spectral library of the GC–MS data system and other published mass spectra (Adams, 2001). The percentage determination was based on peak area normalization without using correction factors.

2.6. Statistical analysis

Each sample was prepared by pooling material from at least ten individual plants to produce individual extract, and each experiment was replicated three times. The experimental layout was randomized block. Statistical analysis was performed using one-way ANOVA and significant differences among treatment means were calculated by Duncan’s multiple range test.

3. Results and discussion

3.1. Effect of Mn treatments on the Mn content of cumin

The results of this study showed that Mn uptake increased with increase in Mn concentration in treatments up to 80 ppm (Fig. 2). Manganese concentration in the plants that had been sprayed with different concentrations of Mn both at late vegetative and blooming stages showed 1.2–1.3 fold increase compared to the plants treated only at blooming stage. The highest concentration of Mn was observed in plants treated with 80 ppm concentration of Mn at both vegetative and blooming stages while the lowest was recorded for the controls. The rate by which a chemical substance passes through the cuticle and, more generally, the epidermal tissues of the plants depends on the following factors: (a) the concentration and the physical and chemical properties of the sprayed substance, (b) the plant species and its nutritional status, and (c) environmental conditions (Marschner, 1995; Schonherr, 2001; Schonherr and Luber, 2001). Increased accumulation of Mn in the treated plants are comparable with the results reported for orange (Papadakis et al., 2005), Trachyspermum ammi (Abd El-Wahab, 2008), and grape (Mou et al., 2011). On the other hand, the decrease of plants Mn concentration in the treatment with the highest concentration of Mn (160 ppm) revealed that there was no direct relation between concentration of Mn in this treatment compared with the plants tissues (Lidon and Teixeira, 2000). Plants did not exhibit Mn toxicity in any treatment. Toxicity symptoms of Mn in different plants occur at different levels of tissue Mn contents in the range of 400–2000 ppm (Singh and Steenberg, 1997). Since the plants tolerated the minimum concentration of Mn toxicity without any symptoms (403 μg g⁻¹ DW in BA 80 treatment), it is likely that cumin is relatively resistant to excess Mn.

3.2. Terpene composition of different organs

 Constituents of the extracted terpene were identified and quantified by GC–MS and GC-FID, respectively. The percentage composition of the constituents is presented in Table 2. The chromatographic analyses allowed the identification of different products representing 85.4–99.46% in the extracts (Table 2). Because of their importance in this research, the monoterpenes identified such as α-pinene, β-pinene, α-phellandrene, m-cymene, and γ-terpinene (constituting 27.5–99.2% of the total compounds) were studied (Table 2). The proportion of the aforementioned five monoterpenes was different in each plant part, with the major being β-pinene in flowers (61.39%), α-phellandrene in leaves (27.5%), β-pinene in shoots (21.17%), and γ-terpinene in roots (29.66%). The flowers and shoots were rich in all of the terpenoids with generally higher concentration in the flowers. Notably, α-phellandrene was the only terpenoid compound in the leaves and there were no α-pinene and β-pinene in the roots (Fig. 3).

The composition of the essential oil varies widely in various localities (Butkienė et al., 2008). Terpenoid is the most biologically “expensive” biosynthesis of all secondary compounds (Langenheim, 1994). Plants cannot maintain high levels of these defense substances in all the tissues and organs and at any given

Table 1

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>BA 0</td>
<td>Sprayed with distilled water at both late vegetative and blooming stages</td>
</tr>
<tr>
<td>A 0</td>
<td>Sprayed with distilled water at blooming stage</td>
</tr>
<tr>
<td>BA 40</td>
<td>Sprayed with 40 ppm concentration of Mn at both late vegetative and blooming stages</td>
</tr>
<tr>
<td>A 40</td>
<td>Sprayed with 40 ppm concentration of Mn at blooming stage</td>
</tr>
<tr>
<td>BA 80</td>
<td>Sprayed with 80 ppm concentration of Mn at both late vegetative and blooming stages</td>
</tr>
<tr>
<td>A 80</td>
<td>Sprayed with 80 ppm concentration of Mn at blooming stage</td>
</tr>
<tr>
<td>BA 160</td>
<td>Sprayed with 160 ppm concentration of Mn at both late vegetative and blooming stages</td>
</tr>
<tr>
<td>A 160</td>
<td>Sprayed with 160 ppm concentration of Mn at blooming stage</td>
</tr>
</tbody>
</table>

BA, sprayed before and after blooming; A, sprayed after blooming alone.
time. Therefore, particular mixtures with high content of toxic terpenoids are accumulated in the target tissues (Wahid et al., 1997). Accumulation of monoterpenes is confined to the early stages of fruit development so that young fruits contain high concentrations of them (Boumeester et al., 1998). Cumin fruits contain 2–5% volatile oil that imparts the characteristic aroma to the fruits (Kan et al., 2007). Most of the oil (containing terpene compounds) is produced in cumin fruits, although leaves and stems also contain oil (El-Sawi and Mohamed, 2002). We have reported high level of terpene (Limonene) synthase gene expression of cumin flowers and shoots (Ghannadnia et al., 2011). The close correlation of terpene synthase gene expression and terpene composition indicates that transcript regulation of terpene synthase gene is the most important regulatory mechanism controlling terpene composition (Crocoll et al., 2010) in flowers and shoot of cumin. The chemical composition of cumin volatile compound varies depending on the plant parts. In addition, α-pinene, α-phellandrene and m-cymene compounds were identified in the cumin flowers. These results are in agreement with that reported in the previous studies on Anethum graveolens (Radulescu et al., 2010). It seems likely that in flowers, the geranyl pyrophosphate (GPP) pool is large and is not limiting terpene production. This pool of GPP in young tissues may benefit the plant by allowing the rapid production of monoterpenes to repel herbivores or attract predators of the herbivores and thus protect new growth (Lucker et al., 2004). Among all of the major volatiles detected in cumin, only α-phellandrene was present in the leaves. Our result is consistent with previous studies on Anethum graveolens L. (Callan et al., 2007; Vokk et al., 2011). Also, our results revealed that although α-pinene and β-pinene were absent in the root components, the amount of α-phellandrene, m-cymene, and γ-terpinene were considerable in this organ. This result confirms the previous observations by El-Sawi and Mohamed (2002). Thus, flowers are not the only storage organs, but also vegetative parts of cumin contain terpenoid compounds that may serve as a valuable source for different usages.

3.3. Effects of manganese treatments on the terpene constituents of the cumin flowers

Table 3 shows the differences in the terpene constituents of cumin flowers after the different Mn treatments. All treatments showed the same constituents (Fig. 4). Extract from treated flowers with 80 ppm concentration of Mn at blooming stage (A 80 treatment) contained the highest proportion of α-pinene, while α-phellandrene and m-cymene concentrations were highest in flowers sprayed with 160 ppm concentration of Mn at both late vegetative and blooming stages (BA 160 treatment). Such increments were statistically significant. Also, treatment with 80 ppm concentration of Mn at both late vegetative and blooming stages (BA 80 treatment) significantly increased the concentrations of β-pinene and m-cymene compared to the treatment with the same concentration at blooming stage alone (A 80 treatment). On the other hand, application of 80 ppm concentration of Mn at blooming stage (A 80 treatment) significantly increased α-pinene concentration compared with all of the other treatments. Treatment with 40 ppm concentration of Mn at blooming (A 40 treatment) caused not only a reduction in β-pinene and γ-terpinene levels but also the absence of α-pinene and α-phellandrene compared

![Fig. 3. Composition of the terpenoids present in root, shoot, leaf, and flowers (3–4 mm in length) of cumin. Monoterpenes were extracted with ethyl acetate: pentane (2:1), identified by GC–MS and quantified by GC-FID (see Section 2 for details).](image)

![Fig. 4. Concentrations for the five main monoterpenic components of cumin flowers (3–4 mm) as affected by different treatments of Mn. Monoterpenes were extracted with ethyl acetate: pentane (2:1), identified by GC–MS, and quantified by GC-FID. Treatments: BA 0, BA 40, BA 80 and BA 160, the plants sprayed with distilled, water (0), 40, 80 and 160 ppm of Mn concentrations respectively at late vegetative and blooming stages; A 0, A 40, A 80 and A 160, the plants sprayed with the same concentrations of Mn only at blooming stage.](image)
with spraying with the same concentration at both vegetative and blooming stages (BA 40 treatment). These results are in concert with the previous reports (El-Sawi and Mohamed, 2002). Although the effects of nutrition on the constituents of essential oil have been reported by several investigators (Maximoos, 1985; Ibrahim, 1989; El-Sawi and Mohamed, 2002), little information is available regarding the effect of different Mn concentrations on terpenoid compounds of cumin different organs.

Overall, the results of this study showed that spraying with each concentration of Mn at both vegetative and blooming stages increased total terpene compounds compared with spraying with the same concentration at blooming stage alone (Table 3 and Fig. 4). Since α-pinene is a side product of limonene synthase gene (Colby et al., 1993; Dewick, 1999) the highest concentration of α-pinene observed in the A 80 treatment in this study is consistent with our previous molecular results based on maximum limonene synthase gene expression in treatment with 80 ppm concentration of Mn at blooming stage in cumin (Zarinkamar et al., 2012).

Essential oil biosynthesis is strongly influenced by several factors. Among the plant nutrients, Mn has important functions in plant metabolism, especially in chlorophyll synthesis, photosynthesis, nitrate reduction, amino acids and protein synthesis, activation of different enzymes, phytoc hormone regulation (Amberger, 1974) and also as cofactor for some monoterpenes synthase (such as limonene synthase) and cyclization of precursor (GPP) to the simplest monoterpenes (limonene) (Tabata, 2000). In a previous study, we reported that spraying cumin with 40, 80, and 160 ppm concentration of Mn at both vegetative and blooming stages significantly reduced terpene (limonene) synthase gene expression compared with applying the same concentrations at blooming stage alone (Zarinkamar et al., 2012). In contrast, the results of current study revealed that spray with the same concentrations at both stages enhanced the terpene compounds in the flowers compared with the treatment only at vegetative stage. Despite the low level of terpene synthase gene expression, high amounts of terpene compounds could suggest that terpene synthesis in the cumin flowers under different treatments of Mn concentration is regulated at postranscriptional (translational and/or post-translational) levels (Munoz Bertomeu et al., 2008). Decrease in gene transcript levels may result from either lowered gene expression or from a decreased stability of the transcripts or a combination of both (Fitzgerald et al., 2008). However, it seems likely that due to the effects of Mn on metabolism (Amberger, 1974), oil and terpene constituents (El-Sawi and Mohamed, 2002), and gradual application of Mn at both vegetative and blooming stages in this study, terpene compounds increased in the plant despite gene expression reduction (Zarinkamar et al., 2012). Production of essential oils and aromatics from plants is under diverse physiological, biochemical, metabolic and genetic regulations (Sangwan et al., 2001). Results of this study revealed that the chemical composition of cumin terpenoids varies depending on the plant parts. The young fruits as storage organs contain the highest concentration of the terpenoids. Although the maximum manganese absorption (approximately 404 μg g⁻¹ DW) was observed in the plants treated with 80 ppm concentration of Mn at both vegetative and blooming stages (BA 80 treatment), the flowers of the plants that were treated with 160 ppm at both stages (BA 160 treatment) had the greatest concentration of terpene compounds compared with the other treatments. It appears possible that the rate of Mn absorbed in the BA 160 treatment (approximately 340 μg g⁻¹ DW) was optimum for terpenoid biosynthesis.

4. Conclusion

Overall, our results revealed that not only flowers but also vegetative organs of cumin contained terpenoid compounds. Moreover, some of Mn treatments were found to influence the production of the terpenoids in the cumin flowers. Considering the economic importance of these valuable natural products, these results can be useful for application in different industries. According to the aim, further studies on quantity and quality of ripened seed extracts of the treated plants with Mn are needed.

References


