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Effects on growth, essential oil content and composition of the volatile fraction of *Achillea millefolium* L. cultivated in hydroponic systems deficient in macro- and microelements

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ABSTRACT

The aim of the study was to determine which elements effect the growth, essential oil content and composition of the volatile fraction of *Achillea millefolium* L. Acclimated scions were transferred into pots containing either complete nutrient solution or a solution of equivalent compositionbut lacking one of the elements N, P, K, Ca, Mg, S, Fe, B, Zn, B, Mo or Cu. The experiment was of completely randomized design, and each of the thirteen treatments was replicated four times with each replicate comprising two plants. Plants were cultured in the greenhouse under natural light for 55 days, under hydroponic conditions. Visual symptoms of element deficiency were assessed daily. Plants were harvested at the end of the culture period and growth parameters (number of leaves; stem diameter; root size; shoot, root and total dry matter), (levels of chlorophylls a; b; total chlorophyll and carotenoids), content of essential oil, and compositions of leaf headspace volatile fractions were evaluated. The results showed that the composition of the nutrient solution exerted a significant effect on all of the growth parameters, although visual symptoms of nutrient deficiency were more marked following omission of macroelements compared with microelements. With regard to total dry matter, the order of limiting nutrients was Ca = K = N > P > S > Mg for macroelements and Zn > Fe > B > Cu > Mn > Mo for microelements. Omission of P, S, B or Mn induced increases in essential oil content. The major components of the volatile fractions were identified as sabinene, 1,8-cineol, borneol, β-caryophyllene and β-cubebene, and the proportions of these compounds were affected substantially by the omission of macro-or microelements.

1. Introduction

*Achillea millefolium*L. (Asteraceae), commonly known as yarrow, milfoil or thousand-leaf, is an erect herbaceous perennial that grows to a height of between 20 and 60 cm. The plant has a spreading rhizomatous growth form, the leaves are arranged spirally on 5–20 cm long stems, and the white to pink inflorescences are composed of many florets arranged in a formation resembling a single flower (Lorenzi and Matos, 2002). Yarrow has a long history of use in folk medicine and is traditionally employed in the treatment of wounds, cuts, abrasions, headache, inflammation, pain, flatulence, spasmodic diseases and gastrointestinal disorders. The species is reported to possess anti-inflammatory, analgesic, anti-tumor, antioxidant, anti-microbial and choleretic properties (Applequist and Moerman, 2011), and the essential oil and the flavonoids luteolin, quercetin and riboflavin have been characterized as the principal bioactive components of the plant (Kindovlits and Németh, 2012).

The demand for medicinal and aromatic plants as raw materials in the production of pharmaceuticals, natural cosmetics, perfumes and agro-foods has been increasing in recent years. However, the rate of growth and the yield of natural products can be affected significantly by the environmental conditions under which plants are cultivated (Gil et al., 2002). Nutrient availability represents a key factor in determining the level of biosynthesis and accumulation of secondary metabolites (Verpoorte et al., 2002). It is well known that macro- and microelements play essential roles in plant growth and development through their association with, or involvement in, numerous physiological processes including respiration, pho-

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Table 1
Growth parameters and oil content of *Achillea millefolium* L. plants cultured in complete nutrient solution and in solutions deficient in individual macro- and microelements.

Treatment	Stem diameter (mm)	Root size (cm)	Number of leaves	Shoot dry matter (SDM) (g per plant)	Root dry matter (RDM) (g per plant)	SDM/RDM	Total dry matter (g per plant)	Oil content (%)	Relative dry matter (%)	Relative oil content (%)
Complete	1.30a	36.28b	9.80a	14.66b	11.43b	1.28c	26.10a	0.07e	100	100
- N	0.45b	40.81a	5.33b	2.60c	5.10c	0.51c	7.70c	–	29	–
- P	0.70b	37.33b	6.50b	7.20c	7.10c	1.01c	14.30b	0.12a	55	171
- K	0.60b	27.73d	6.83b	5.23c	1.43d	3.66b	6.66c	–	25	–
- Ca	0.25c	12.00e	5.33b	1.87c	0.32d	5.84a	2.20c	–	8	–
- Mg	1.08a	37.66b	8.83a	21.56a	10.76b	2.00c	32.32a	0.06f	124	86
- S	1.38a	31.93c	9.83a	17.43b	10.26b	1.70c	27.70a	0.08d	106	114
- Fe	1.08a	42.93a	7.20a	12.20b	7.13c	1.71c	19.33b	0.06f	74	86
- Zn	0.95a	27.90d	6.83b	13.60b	4.13c	3.29b	17.73b	0.06f	68	86
- B	1.26a	35.40b	8.77a	18.00b	10.07b	1.79c	28.07a	0.08c	107	114
- Mn	1.46a	35.41b	9.66a	18.93b	15.02a	1.26c	33.95a	0.10b	130	143
- Mo	1.20a	35.46b	9.00a	25.84a	9.80b	2.64c	35.67a	0.06f	137	86
- Cu	1.53a	36.76b	10.50a	14.35b	15.93a	0.90c	30.28a	0.05g	116	71

Within each column, mean values followed by the same letterbelong to the same group according to the Scott–Knott test ($P = 0.05$).

tosynthesis and cell wall formation. However, these nutrients also provide the cofactors required by numerous enzymes of primary and secondary metabolism, and the limited availability or absence of an element can induce alterations in the biosynthetic and/or catabolic capacity of a plant (Figueiredo et al., 2008).

The symptoms associated with mineral deficiency are more or less characteristic of each element, although the severity of expression varies with species/cultivar and the environmental conditions. Full understanding of mineral nutrition would allow an adequate level of each essential element to be established for a particular plant species and, thereby, assist in determining the conditions required for maximal growth and secondary compound production. However, despite the large body of literature relating to the effects of organic fertilizers, macronutrients and salinity on the production of essential oils by medicinal plants, there is limited information on the effect of macro-or microelement omission on the growth and essential oil composition of *A. millefolium*. The aim of this study was, therefore, to assess plant growth, photosynthetic pigment and essential oil content, and composition of the volatile fraction of *A. millefolium* cultured in hydroponic system, with complete nutrient solution and omitting the elements N, P, K, S, Ca, Mg, Fe, B, Zn, Mn, Mo and Cu individually.

2. Materials and methods

2.1. Effect of macro- and microelement omission on growth and oil content

The scions employed in all of the experiments were derived from rhizome cuttings that had been cultured in the greenhouse for 60 days. Scions were acclimated and adapted in a quarter- and half-strength Hoagland's solution for one week each, following which they were transferred into pots (25 cm high × 20 cm diameter; two plants per pot) containing one of thirteen different nutrient solutions in hydroponic system. The complete nutrient solution contained 234.6 mg K; 210.1 mg N; 200.4 mg Ca; 64.2 mg S; 48.6 mg Mg; 31 mg P; 648 μg Cl, 502 μg Mn, 500 μg B; 50 μg Zn; 20 μg Cu; 11 μg Mo and 5.022 μg Fe and per liter and was adjusted to pH 6.0 (Hoagland and Arnon, 1950).

The sources (analytical standard) used in the complete solution and solutions with omission of nutrients are presented in the supplemental data. The compositions of the twelve remaining nutrient solutions were identical except that, for each solution, one of the elements (excluding Cl) was omitted while maintaining the levels of all of the other elements. Plants were grown on in a greenhouse under natural light and 70% relative humidity for 55 days, and nutrient solutions were changed every week. The experiment was of completely randomized design, and each of the thirteen treatments was replicated four times with each replicate comprising two plants.

Visual symptoms of deficiency of an element were assessed daily as described in the literature. The symptoms observed were: the presence of chlorosis (marginal, internerval, or total leaves) reddish or purplish, blasting, shortening of internodes, foliar anomalies, necrosis, and the general appearance of the plant (Malavolta, 2006). Plants were harvested at the end of the growth period, and the variables stem diameter (mm), root size (cm) and number of leaves determined. Total dry matter (g per plant), shoot dry matter (SDM; g per plant), root dry matter (RDM; g per plant), and shoot-to-root dry matter ratio (SDM/RDM) were evaluated with samples of plant material that had been dried in a heating chamber at 40 °C for 72 h.

The analyzes of the elements presentin the leaves followed the routine analysis of determining elements of Foliar Analysis Laboratory of the Department of Chemistry at UFLA, according to the recommendations of Malavolta et al. (1997). The dried material was ground and the samples submitted to nitro-perchloric and sulfuric extraction. Phosphorus concentrations were determined by the colorimetric method metavanadate, the K, by flame photometry; the Ca, Mg, Cu, Fe, Mn and Zn by atomic absorption spectrometry, the S turbidimetrically the $BaSO_4$. The N by the Kjeldal method, the B by colorometric azomethine-H. The Mo content could not be quantified because it is not a routine laboratory analysis.

Analysis of photosynthetic pigments and extraction of essential oils were performed using fully expanded leaves that had been freshly harvested, wrapped in aluminum foil and transported immediately to the laboratory in polystyrene boxes containing ice. Extraction of leaf tissue and evaluation of the photosynthetic pigments chlorophylls *a* and *b*, chlorophyll *a*/chlorophyll *b* ratio, total chlorophyll and carotenoids were carried out using the methods described by Lichtenthaler and Buschmann (2001).

Essential oils were extracted separately from duplicate 20 g shoot samples for each treatment by hydrodistillation with 500 mL of water for 90 min in a Clevenger-type apparatus. The distillates were partitioned against dichloromethane, and the organic phases were separated, dried over excess anhydrous magnesium sulfate and filtered. The resulting solutions were transferred to dark vials that were left partially open and stored at room temperature in order to allow excess solvent to evaporate. The oil content of each sample was determined as the weight of oil (g) per 100 g of dry leaf biomass, and total dry matter and oil content were expressed as percentages of the respective values for control plants grown in complete nutrient solution.

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