



Growth and production of volatile compounds of yarrow (*Achillea millefolium* L.) under different irrigation depths

IVAN C.A. ALVARENGA¹, FERNANDA V. PACHECO¹, AMAURI A. ALVARENGA²,
SUZAN K.V. BERTOLUCCI¹ and JOSÉ EDUARDO B.P. PINTO¹

¹Laboratório de Cultura de Tecidos e Plantas Mediciniais, Departamento de Agricultura,
Universidade Federal de Lavras, Caixa Postal 3037, 37200-000 Lavras, MG, Brazil

²Laboratório de Crescimento e Desenvolvimento de Plantas, Departamento de Fisiologia Vegetal, Setor de
Biologia, Universidade Federal de Lavras, Caixa Postal 3037, 37200-000 Lavras, MG, Brazil

Manuscript received on January 29, 2018; accepted for publication on July 11, 2018

ABSTRACT

Yarrow (*Achillea millefolium* L., Asteraceae) is an important medicinal plant used worldwide for its medicinal properties such as the analgesic, antioxidant and anti-inflammatory ones. The aim of this study was to evaluate the growth and production of photosynthetic pigments and of volatile constituents of *Achillea millefolium* L. under different irrigation depths. The treatments were the application of 55, 110, 220, 440 and 880 mm of water for a period of 110 days. Data were submitted to polynomial regression analysis at 5% probability, while the volatile constituents were analyzed by standard deviation. Different irrigation depths provided quadratic growth responses being the highest dry matter production at the depth of 440 mm. The contents of chlorophyll a, b, total and carotenoids were higher at the lower depth tested (55 mm). The major volatile compounds identified were sabinene, 1,8-cineol, borneol and β -caryophyllene. Increased water availability reduced the complexity of the volatile fraction of essential oil. Thus, it is recommended that the species be cultivated at 440 mm irrigation depth to have a higher production of dry matter and lower variation in the volatile profile of the essential oil.

Key words: water stress, 1,8 cineol, chlorophyll, terpenes.

INTRODUCTION

Yarrow (*Achillea millefolium* L., Asteraceae) is a perennial herb, native to Europe and growing in different countries. The species produces essential oil in its leaves and inflorescences, and is used as an analgesic, antioxidant, anti-inflammatory and is gastroprotective (Judzentiene and Mokute 2010). In addition, its essential oil is used commercially

in the cosmetics industry, skin care products and aromatherapy (Mohammadhosseini et al. 2017). This essential oil presents great diversity of chemical compounds, and caryophyllene, sabinene, γ -terpinene, borneol and chamazulene are mentioned as majoritarian (Kindlovits and Németh 2012). However, the chemical composition of its essential oil varies due to different factors. For example, Pinto et al. (2014a) observed significant changes in the essential oil compounds of yarrow when cultivated under different light conditions;

Correspondence to: Ivan Caldeira Almeida Alvarenga
E-mail: pytchoagro@yahoo.com.br

the same response was observed when there was lack of macro and microelements in a hydroponic system (Alvarenga et al. 2015).

Irrigated cultivation allows medicinal plants to be grown year-round, thus providing the continuous supply of raw material. However, the plant productivity response as a function of water availability is specific, since it is related to the genetic characteristics of the plant, nutrient availability, and the ability of the plant to allocate photoassimilates (Marschner et al. 1996). In most cases, pigments such as chlorophylls and carotenoids are rapidly degraded under water limiting conditions; however, they can also be accumulated depending on the species (Anjum et al. 2011, Manivannan et al. 2007).

The species *Lippia sidoides* Cham., cultivated under water limitation, for example, showed a reduction in the production of dry matter and essential oil (Alvarenga et al. 2012). However, the moderate water limitation in a cultivation of lemongrass (*Cymbopogon citratus* (DC.) Stapf) increased the production of essential oil (Pinto et al. 2014b). For basil (*Ocimum basilicum* L.) and tea tree (*Melaleuca alternifolia* Chen.), intermediate water depths have proved ideal for the production of dry matter (Silva et al. 2012, Pravuschi et al. 2010).

Therefore, water availability for the production of medicinal plants alters their growth and the synthesis of secondary compounds. However, it is necessary to understand how the availability of water changes the plant metabolism, because alterations in the levels of active constituents can compromise the parameters of quality, efficacy and safety of the plant material (Baghalian et al. 2008). Misra and Srivastava (2000) observed significant increases in menthol and menthone levels for *Mentha arvensis* L. under water limitation (field moisture capacity of 10%). On the other hand, for *Mentha piperita* L., menthol content decreased by 36% and menthone content by 50%, under the

condition of less water availability (Khorasaninejad et al. 2011). Thus, it can be observed that water availability influences both the essential oil content and its quality, yielding significant changes in the terpenic compounds. In addition, the proper irrigation management combines the production of vegetable matter with the contents of secondary metabolites (Alvarenga et al. 2012).

Due to the influence of water availability on plant production, and lack of knowledge of the response of yarrow to irrigation, the objective of this study was to evaluate the growth, production of photosynthetic pigments and of volatile constituents of *Achillea millefolium* L. under different irrigation depths.

MATERIALS AND METHODS

The experiment was conducted in a greenhouse, in the sector of Plant Physiology at the Department of Biology of the Universidade Federal de Lavras (UFLA). A species specialist identified the plants and deposited the exsiccatae in the Bioscience Institute of the Universidade Federal do Rio Grande do Sul, Brazil, with register number ICN 187014.

Drainage microlysimeters with capacity of 15L and mean diameter of 25 cm were used. They were filled with substrate composed of soil and sand in the ratio of 2:1, and fertilized with NPK 4-14-8 in the ratio of 2 kg per m³ of substrate. The physicochemical characteristics of the soil were analyzed at the Laboratory of Analysis of Soil of UFLA, and were: pH: 5.4; P: 4.13 mg dm⁻³; K: 73.32 mg dm⁻³; Ca: 2.30 cmolc dm⁻³; Mg: 0.30 cmolc dm⁻³; Al: 0.10 cmolc dm⁻³; H + Al: 2.90 cmolc dm⁻³; V: 49.00%; organic matter: 2.10 dag kg⁻¹; Clay: 70.00 dag kg⁻¹; Silt: 16.00 dag kg⁻¹ and Sand: 14.00 dag kg⁻¹.

To start the experiment, the microlysimeters were saturated with water, and then covered with plastic to prevent evaporation to allow the substrate to have 100% of field capacity. By the time water

percolation ceased, the planting of seedlings was carried out.

The seedlings (derived from cuttings) were produced in expanded polypropylene trays filled with commercial substrate, and kept in intermittent mist chamber. After sixty days, with 10 cm of height and four expanded leaves, the seedlings were transplanted to the microlysimeters.

The treatments consisted of application of five irrigation depths: 55, 110, 220, 440 and 880 mm, distributed in daily applications during the growing period. For each treatment, five replicates were used with a plant per repetition, in a total of 25 plants, distributed in a completely randomized design. Plants from which samples were taken for chlorophyll analysis were not accounted for assessment of the production of dry matter of the aerial part and total dry matter, being used for analysis of the other variables.

The average temperature during the experiment was of 22.08 °C, with average maximum temperature of 30.91 °C, and average minimum temperature of 13.26 °C and average relative humidity of 78.50%. The temperature and relative humidity in the greenhouse were recorded with the aid of a portable thermo-hygrometer.

After 110 days of cultivation (Netto and Raffaelli 2004), plants were collected for evaluation of growth. The growth was evaluated through measurement of root length, stem diameter, and by counting the number of leaves and shoots. The dry matter production of the aerial part, stem and roots as well as total dry matter production were evaluated. In order to obtain the dry materials, plants were dried in a forced-air oven at 40 °C until the weight stabilized.

The photosynthetic pigments analyzed were chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids. The extraction was performed from fully expanded leaf collected from five plants per treatment on the 110th day of cultivation. For the extraction, 0.2 g of fresh leaves were weighed, and

homogenized with 30 mL of 80% acetone (v/v). The contents of chlorophyll a, chlorophyll b and carotenoids were calculated with the equations proposed by Lichtenthaler and Buschmann (2001) from the absorbance readings at 663.2 nm, 646.8 nm and 470 nm, respectively. All procedures were performed in the dark and the readings were performed in triplicates.

The constituents of the volatiles were analyzed from 40 mg of dry matter of leaves, and four composite samples per treatment were utilized. Dried leaves were placed in a 20 mL headspace vial, sealed with septum silicone / PTFE. The analyses were performed with the "headspace" technique in a Gas Chromatograph coupled to a Mass Spectrophotometer (GC/MS: Agilent® 7890 A / Agilent® MSD 5975C, HP-5MS column). After optimization, the following operating conditions were established: Sample incubation temperature of 100 °C for 30 min, syringe temperature of 110 °C. The volume of 500 µL of the vapor phase was injected into the chromatographic column, in split mode at a ratio of 1:50. The volatile fraction was analyzed in an Agilent® 7890A Gas Chromatograph coupled to an Agilent® 5975C Mass Selective Detector (MSD) (Agilent Technologies, CA), operated by electron impact ionization using 70 eV, scanning mode at a scan rate of 1.0 / s, acquisition mass range of 40-400 m / z. We used an HP-5MS fused-silica capillary column (30 m length x 0.25 mm internal diameter x 0.25 mm film thickness) (California, USA). Helium was used as carrier gas with a flow rate of 1.0 ml / min; the injector temperature and the transfer line to the MS were maintained at 220 °C and 240 °C, respectively. The initial oven temperature was of 60 °C held isothermally for 1.5 min, followed by a temperature ramp of 3 °C / min until the temperature of 240 °C was reached, followed by a ramp of 10 °C / min until the temperature of 270 °C was reached.

The identification of the volatile constituents was performed by comparing retention indices

relative to a standard solution of n alkanes (C_8 - C_{18}) and with the mass spectra found in specialized literature (Adams 2007, NIST 2008). The retention indices were calculated using the equation proposed by Van den Dool and Kratz (1963).

For the growth and production of photosynthetic pigments, the data were analyzed through the F test ($p < 0.05$) and, when significant, polynomial regression was performed. The volatile constituents were analyzed by standard deviation ($n=4$) and represented in a table showing the identified compounds with their respective retention indices and relative areas (%).

RESULTS AND DISCUSSION

The plants presented significant ($p < 0.05$) responses to the different irrigation depths, showing mostly quadratic curves. The increase in stem diameter and the number of leaves were favored with increasing water supply, and the highest values of these variables were observed for the greatest depth applied (880 mm). Meanwhile, root length was greater for the depth of 440 mm and the number of shoots was larger for the depth of 55 mm (Figure 1).

The increase in stem diameter is related to the increase in the number of leaves, since the plant grows in a rosette form. The larger number of leaves, observed for the greatest depth, is a way the plant has to increase evaporation surface and improve the flow of nutrients and water (Peuke 2010). The results corroborate those observed by Meira et al. (2013), where there was an increase in these variables for the treatment with the greatest depth applied to *Melissa officinalis* L.

The yarrow plant ceased its root growth at the lowest water availability (55mm), unlike many species under water limiting conditions, which expend their energy on root growth and, consequently, reach deeper and wet layers of soil (Das and Kar 2018).

The increase in the number of shoots with the lowest water availability may be associated with hormonal imbalance between auxins and cytokinins and with a stimulating effect on shoot initiation (Fu and Harberd 2003).

For leaves, roots, stem and total dry matter production, a quadratic behavior of the curve was observed with increasing responses up to the depth of 440 mm (Figure 2). The reduction of dry matter production in all parts of the plant at 880 mm may be attributed to a possible excess of water in the root zone. This excess water might have led to a condition of hypoxia, which compromises the production of dry matter. Under hypoxia, the roots favor anaerobic respiration due to reduced oxygen levels and ATP generation decreases. This promotes a reduction in energy production and, consequently, in reduced overall plant growth (Hasibeder et al. 2014). The higher soil water availability also promoted reductions in dry matter production of *Ocimum basilicum* L. (Khalid 2006).

The results for dry matter production indicate that the species grows better under intermediate water conditions, with higher growth under a depth of 440 mm. Similar results, where intermediate depths were also ideal for the production of dry matter, were observed for *Ocimum basilicum* L. and *Melaleuca alternifolia* Cheel. (Silva et al. 2012, Pravuschi et al. 2010). Nonetheless, it is important to notice that the species is able to survive under low water availability.

The contents of chlorophyll a, b, total chlorophyll and carotenoids were higher at the smallest depth tested (55 mm), which provided an accumulation of almost double the contents of the treatment of 440 mm (Figure 3). Pigments such as chlorophylls and carotenoids may be used as indicators of both nutritional and water stress (Reddy et al. 2004). Usually, under water limitation conditions, chlorophyll is rapidly degraded, which affects photosynthesis and results in production losses (Havaux and Tardy 1999). However, in some

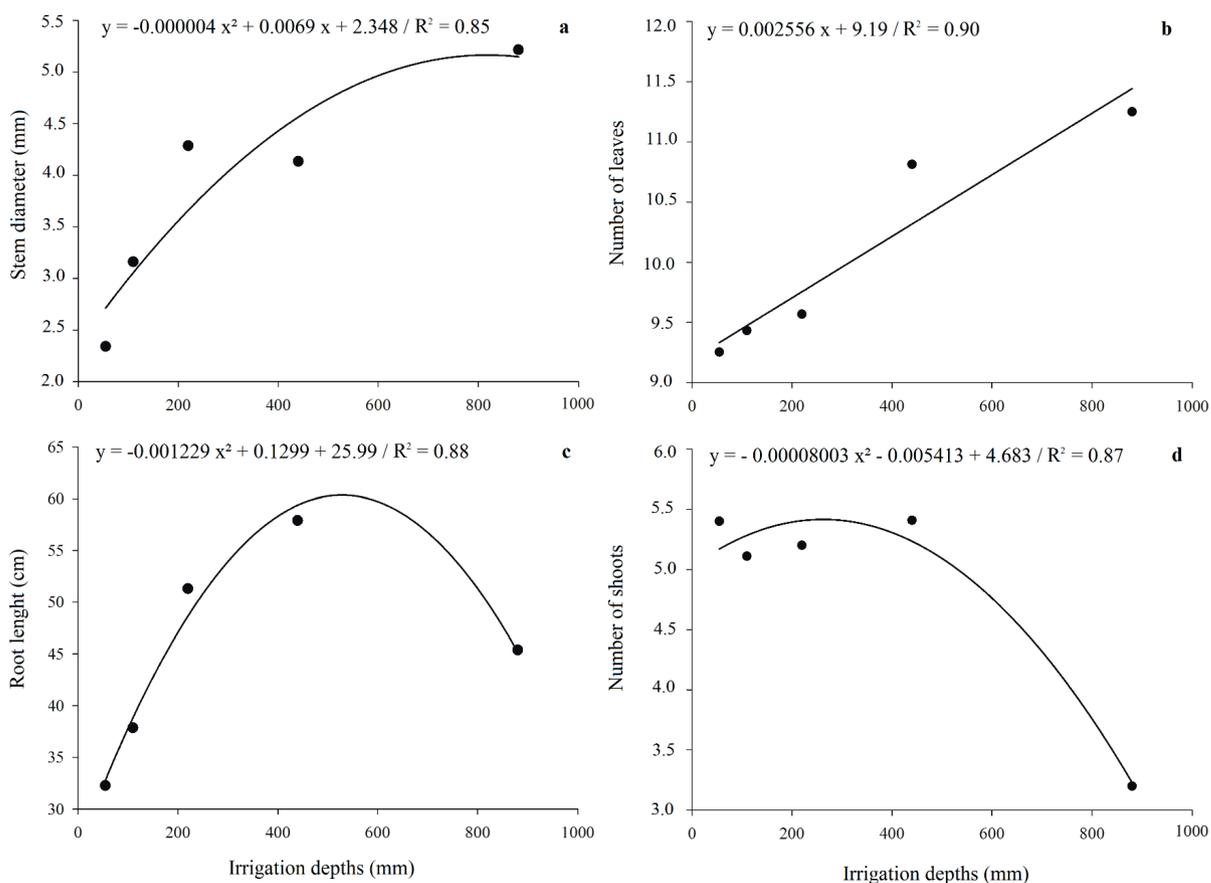


Figure 1 - Stem diameter (a), number of leaves (b), root length (c) and number of shoots (d) of *Achillea millefolium* L. grown with application of 55, 110, 220, 440 and 880 mm irrigation depths.

cases, there may be an accumulation of pigments as a way to mitigate stress, which reduces the action of reactive species of oxygen and, at the same time, maintains a photosynthetic rate (Dinakar et al. 2012).

Therefore, photosynthetic pigments may work as protection against oxidative damage in yarrow. Another point to be noticed was the lowest pigment contents observed for the depth of 440 mm, which corresponded to the highest dry matter production, which may indicate an adequate adjustment of the photosynthetic apparatus. This response is corroborated by the work of Khalil et al. (2011) who observed that yarrow did not significantly alter its stomatal conductance under conditions of water limitation.

Table I shows the presence of 27 constituents in the composition of the volatile fraction of the essential oil of yarrow. The largest number of constituents (27) was observed for the treatment with the smallest depth (55 mm), and the smallest number of constituents (17) for the treatment with the greatest depth (880 mm). The identified volatile constituents belong to the classes of monoterpenes (contents between 47.85 and 63.64%), oxygenated monoterpenes (18.31-30.49%), sesquiterpenes (12.03-22.98%) and oxygenated sesquiterpenes (0.29-0.63%), among others (0.98-3.4%). The major constituents (Figure 4) were sabinene (32.21-50.97%), 1,8-cineol (6.05-12.85%), borneol (6.69%-13.00%) and β -caryophyllene (10.30-20.07%), as observed in studies on chemical

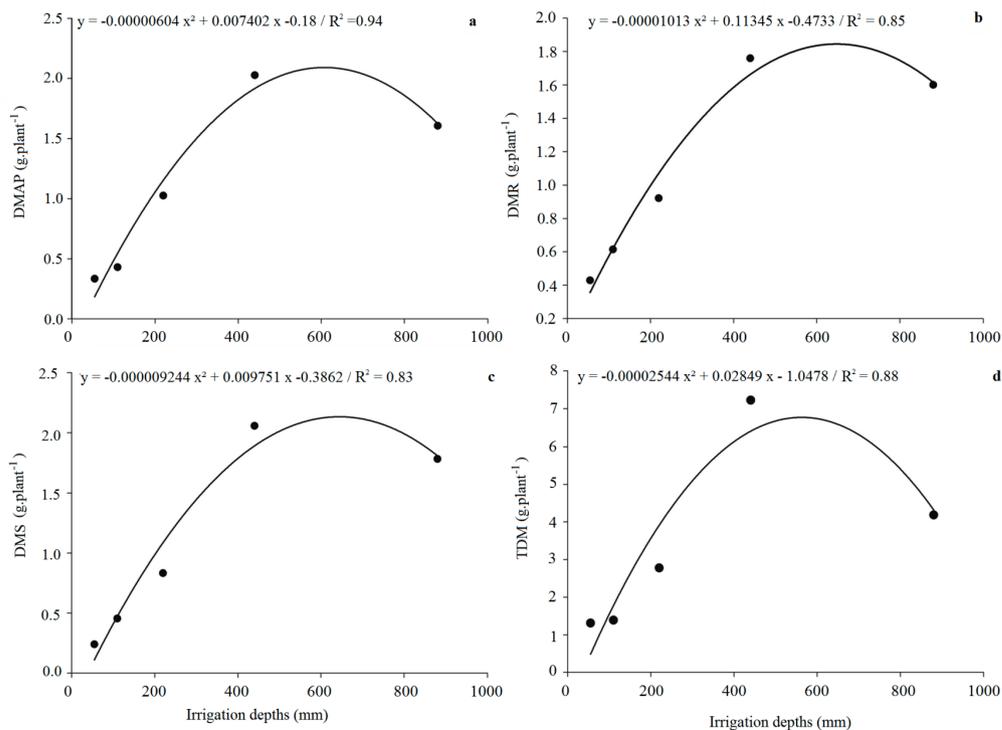


Figure 2 - Production of *Achillea millefolium* L. grown with application of 55, 110, 220, 440 and 880 mm irrigation depths. (a) Dry matter of aerial part (DMAP); (b) Dry matter of roots (DMR); (c) Dry matter of stem (DMS); (d) Total dry matter (TDM).

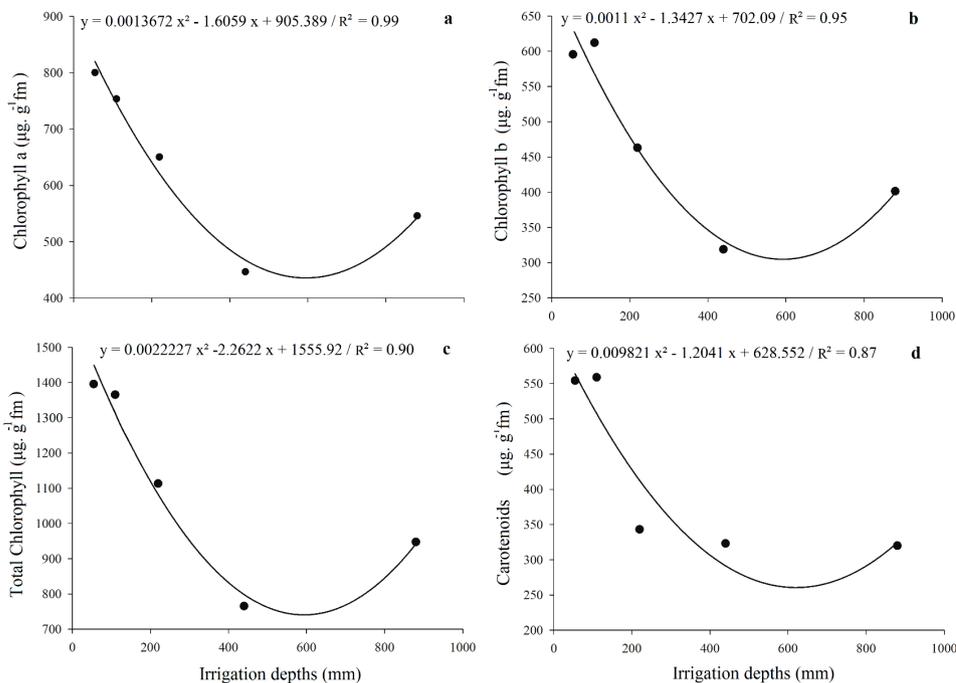


Figure 3 - Photosynthetic pigments of *Achillea millefolium* L. grown with application of 55, 110, 220, 440 and 880 mm irrigation depths. (a) Chlorophyll a; (b) chlorophyll b; (c) total chlorophyll; (d) carotenoids.

TABLE I
Volatile compounds of *Achillea millefolium* L. cultivated with different irrigations depths (Relative area \pm SD).

Compounds	RI ¹	Irrigation Depths				
		55 mm	110 mm	220 mm	440 mm	880 mm
Monoterpenes		54.84	47.85	56.85	50.37	63.64
α -thugene	925	1.10 \pm 0.012	1.16 \pm 0.01	1.65 \pm 0.02	1.43 \pm 0.04	1.77 \pm 0.05
α -pinene	932	1.33 \pm 0.17	1.40 \pm 0.07	2.27 \pm 0.03	1.36 \pm 0.02	2.00 \pm 0.13
camphene	947	1.05 \pm 0.08	0.96 \pm 0.15	1.56 \pm 0.08	0.91 \pm 0.05	1.04 \pm 0.06
sabinene	973	36.04 \pm 1.74	32.21 \pm 1.32	41.54 \pm 1.14	38.85 \pm 0.74	50.97 \pm 0.9
β -pinene	976	3.00 \pm 0.15	3.03 \pm 0.03	2.32 \pm 0.1	2.24 \pm 0.12	2.450.05 \pm
myrcene	990	2.74 \pm 0.23	2.32 \pm 0.44	1.67 \pm 0.07	1.38 \pm 0.01	1.67 \pm 0.02
α -terpinene	1016	0.36 \pm 0.01	0.23 \pm 0.07	0.23 \pm 0.01	—	—
<i>para</i> -cymene	1023	1.97 \pm 0.04	1.51 \pm 0.56	1.17 \pm 0.01	1.38 \pm 0.06	0.79 \pm 0.04
d-limonene	1027	3.68 \pm 0.19	2.68 \pm 0.56	1.89 \pm 0.04	1.41 \pm 0.04	1.630.01 \pm
<i>E</i> - β -ocimene	1046	0.11 \pm 0.02	—	—	—	—
γ -terpinene	1057	3.10 \pm 0.05	2.07 \pm 0.23	1.22 \pm 0.03	1.41 \pm 0.02	1.33 \pm 0.01
terpinolene	1088	0.37 \pm 0.01	0.29 \pm 0.05	1.33 \pm 0.05	—	—
Oxygenated Monoterpenes		29.25	30.49	26.47	22.94	18.31
1,8-cineol	1030	12.85 \pm 0.12	10.76 \pm 0.74	9.79 \pm 0.64	6.05 \pm 0.04	8.39 \pm 0.54
3-hidroxi-1-nonene	1040	0.44 \pm 0.01	0.34 \pm 0.02	—	0.46 \pm 0.07	—
<i>trans</i> -sabinene hydrate	1065	3.03 \pm 0.05	3.33 \pm 0.24	2.06 \pm 0.02	1.52 \pm 0.12	1.16 \pm 0.03
camphor	1143	0.14 \pm 0.01	0.14 \pm 0.012	—	—	—
borneol	1164	10.56 \pm 0.64	13.00 \pm 0.75	12.14 \pm 0.98	12.41 \pm 0.33	6.96 \pm 0.18
terpinen-4-ol	1176	0.48 \pm 0.05	0.48 \pm 0.01	0.33 \pm 0.02	0.57 \pm 0.09	—
α -terpineol	1190	1.74 \pm 0.17	2.44 \pm 0.66	2.15 \pm 0.12	1.92 \pm 0.10	1.81 \pm 0.03
Sesquiterpenes		12.03	17.51	15.14	22.98	17.07
β -caryophyllene	1417	10.30 \pm 0.65	15.41 \pm 1.28	12.98 \pm 1.82	20.07 \pm 0.69	13.54 \pm 1.01
α -humulene	1451	1.08 \pm 0.06	1.51 \pm 0.21	1.09 \pm 0.17	1.96 \pm 0.07	1.12 \pm 0.11
β -cubebene	1479	0.66 \pm 0.22	0.59 \pm 0.12	1.07 \pm 0.11	0.95 \pm 0.16	2.41 \pm 0.31
Oxygenated Sesquiterpenes		0.29	—	—	0.63	—
caryophyllene oxide	1580	0.29 \pm 0.02	—	—	0.63 \pm 0.02	—
Others		2.55	3.04	2.46	2.07	0.98
3-metil-pentanol	849	0.13 \pm 0.01	0.14 \pm 0.02	0.31 \pm 0.02	0.51 \pm 0.05	—
6-metil-5-hepten-2-ona	986	0.14 \pm 0.04	0.11 \pm 0.02	—	—	—
thujanol	1098	1.54 \pm 0.12	1.85 \pm 0.03	1.33 \pm 0.04	1.56 \pm 0.09	0.98 \pm 0.01
bornyl acetate	1285	0.74 \pm 0.01	0.94 \pm 0.04	0.82 \pm 0.11	—	—
Total identification (%)		98.96	98.89	100	98.99	100
Number of compounds		27	25	22	21	17

¹RI: retention indices relative to C₈–C₁₈ *n*-alkanes on the HP-5MS column in order of elution.

— Not identified. SD: standard deviation (*n*=4).

characterization of the species (Kindlovits and Németh 2012, Mohammadhosseini et al. 2017).

Increased water availability reduces the complexity of essential oil by reducing the

number of constituents. Moreover, the conditions of lower water availability promoted an increase in the content of oxygenated monoterpenes and a decrease in the contents of sesquiterpenes. The

increase in the contents of monoterpenes may be associated with a way the plant has to neutralize reactive species of oxygen. The accumulation of monoterpenes may mitigate the oxidative damage to chloroplast caused by free radicals, besides replacing photorespiration in order to protect against photooxidation under stress conditions (Peñuelas and Llusià 2002). The results demonstrated that the species has adaptability to conditions of low water availability. Contrary results were observed for *Rosmarinus officinalis* L. and *Mentha spicata* L., where the contents of monoterpene constituents increased with increased water availability (Delfine et al. 2005).

The highest sabinene content was observed for the greatest depth applied (880 mm), with an increase of approximately 70% when compared to the smallest depth, while the contents of 1,8 cineol and borneol decreased with higher water availability (880 mm). Decrease in the contents of these compounds (1,8 cineol and borneol) with higher water availability was also observed for *Salvia officinalis* L. and *Alpinia zerumbet* (Pers.) B. L. Burt & R. M. Sm (Bettaieb et al. 2009, Rezende et al. 2011).

The β -caryophyllene content presented a varying response in relation to the different depths tested, being higher for the treatment with 440 mm (Figure 4). The variation in contents and the increased number of constituents under low water availability may be attributed to the activity of enzymes, from the terpene synthases family, which have the ability to transform a substrate in different products, and many of which are more active under stress conditions (Degenhardt et al. 2009).

CONCLUSIONS

The data observed in this study can make it possible to infer that yarrow presents some adaptation to low water availability conditions, such as growth stabilization and increased number of shoots, the

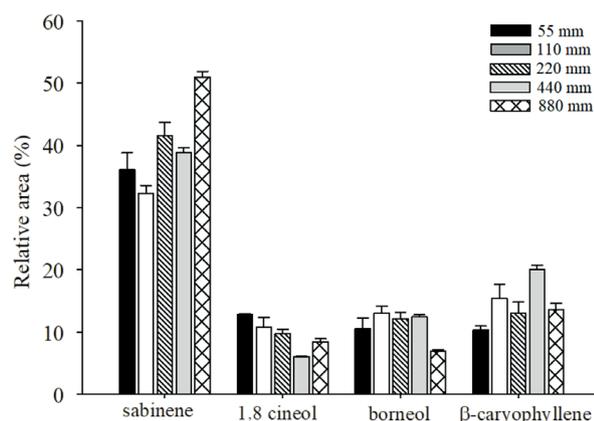


Figure 4 - Major volatile compounds of *Achillea millefollium* L. cultivated with application of 55, 110, 220, 440 and 880 mm irrigation depths; bars show standard deviations ($n = 4$).

accumulation of pigments and increased number of volatile components of the leaves, which on further studies can be used as an indicator of stress. In addition, the depth of 440 mm of water was the one that provided the greatest growth with higher aerial, root and total dry matter production of yarrow. Regarding the volatile components, it was observed that water availability affects the metabolism of terpenes; however, the major components: sabinene, borneol, 1,8 cineol and β -caryophyllene, were identified in all-treatments.

ACKNOWLEDGMENTS

The authors wish to thank Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), and Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG) for granting scholarships and for their financial support.

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