

Organic systems in the growth and essential-oil production of the yarrow¹

Sistemas orgânicos no crescimento e produção de óleo essencial em mil-folhas

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ABSTRACT - Fertilization of the soil with organic fertilizers has been gaining importance within the concept of sustainable crop production. This study aimed evaluate the effects of dosages of cattle and poultry manure on *Achillea millefolium* L. as regards the vegetative growth and the content and chemical composition of its essential oil. For the cattle manure fertilization the dosages evaluated were: 1) soil with no fertilizer; 2) soil + 3.0 kg m⁻²; 3) soil + 6.0 kg m⁻²; 4) soil + 9.0 kg m⁻² and 5) soil + 12.0 kg m⁻². For fertilization with poultry manure: 1) soil without fertilizer; 2) soil + 1.5 kg m⁻²; 3) soil + 3.0 kg m⁻²; 4) soil + 4.5 kg m⁻² and 5) soil + 6.0 kg m⁻². The experimental design was completely randomized, with four replications per treatment and four plants per experimental plot. Harvesting took place at 110 days and the following variables were measured: shoot and root dry biomass; root to shoot ratio and the content, yield and chemical composition of the essential oil. Data were submitted to variance and regression analyses. *A. millefolium* has more intense response in fertilization with poultry manure than to that of cattle, where the dosage of 6 kg m⁻² presented the greatest shoot dry weight and highest yield of essential oil. Without regard to fertilization, the essential oil of *A. millefolium* consists mainly of chamazulene, with the applied treatments not significantly interfering in the oil chemical composition and content.

Key words: *Achillea millefolium* L.. Essential oil. Organic fertilizer. Soil fertility. Biomass.

RESUMO - A fertilização dos solos com adubos orgânicos tem adquirido importância do ponto de vista da concepção de produção vegetal sustentável. Foi objetivo deste trabalho avaliar os efeitos de dosagens de esterco bovino e avícola em *Achillea millefolium* L. no crescimento vegetativo, teor e composição química do óleo essencial. Na fertilização com o esterco bovino avaliaram-se as doses: 1) Solo sem adubação; 2) solo + 3,0 kg m⁻²; 3) solo + 6,0 kg m⁻²; 4) solo + 9,0 kg m⁻²; 5) solo + 12,0 kg m⁻². Na adubação com esterco avícola: 1) solo sem adubação; 2) solo + 1,5 kg m⁻²; 3) solo + 3,0 kg m⁻²; 4) solo + 4,5 kg m⁻² e 5) solo + 6,0 kg m⁻². O delineamento experimental utilizado foi o inteiramente casualizado com quatro repetições por tratamento e quatro plantas por parcela experimental. A colheita ocorreu aos 110 dias e avaliaram-se as biomassas secas da parte aérea e raiz, razão raiz/parte aérea, teor, rendimento e composição química do óleo essencial. Os dados foram submetidos à análise de variância e de regressão. *A. millefolium* responde com maior intensidade a adubação com esterco avícola que com a bovina, onde a dose de 6 kg m⁻² apresentou o maior acúmulo de biomassa seca da parte aérea e maior rendimento de óleo essencial. Independente da adubação das plantas, o óleo essencial da *A. millefolium* é constituído majoritariamente por camazuleno e os tratamentos aplicados não interferiram expressivamente na composição e teor dos constituintes químicos do óleo.

Palavras-chave: *Achillea millefolium* L.. Óleos essenciais. Fertilizante orgânicos. Fertilidade do solo. Biomassa vegetal.

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INTRODUCTION

Organic production systems have seen an exponential growth in popularity in recent years. However protocols for horticultural organic-production systems are still in their infancy (SURRAGE *et al.*, 2010). In the production of medicinal and aromatic plants, these are becoming almost mandatory, since nowadays most large companies producing herbal pharmaceuticals, as well as those marketing herbs, give preference to plant materials which come from certified biodynamic or organic crops (SCHIPPMANN *et al.*, 2002).

Achillea millefolium L. (Asteraceae), popularly known as yarrow, is one of the species listed on the National Register of Medicinal Plants of Interest to the Brazilian Health Service (RENISUS), being species which are widely used by the Brazilian population, and for which there is already evidence as to their appropriate application, however further studies are needed to determine growing conditions and agricultural production, safety, efficacy and definitions of the most suitable pharmaceutical form (BRAZIL, 2009). *A. millefolium* has been used in folk medicine against various disorders, including skin inflammations, spasmodic, gastrointestinal and hepatobiliary disorders (BENEDEK; KOPP, 2007). Phytochemical studies of *Achillea millefolium* have identified several components, including essential oils, sesquiterpenes and phenolic compounds such as flavonoids and fatty phenyl-carbonics (BENEDEK; KOPP; MELZIG, 2007). The species is native to temperate regions and has adapted well to the climatic conditions in Brazil, however studies aimed at plant productivity and the levels and chemical composition of the essential oil are just beginning (LORENZI; MATOS, 2002).

Several aromatic species have been studied in order to assess the effects of organic fertilization on plant productivity and the synthesis of their volatile constituents, such as *Plectranthus neochilus* Schltr. (ROSAL *et al.*, 2011), *Aloysia triphylla* Britton. (BRANT *et al.*, 2010), *Origanum vulgare* L. (CORRÉA *et al.*, 2010), *Ocimum gratissimum* L. (BIASI *et al.*, 2009), *Salvia fruticosa* Mill. (KAPLAN *et al.*, 2009), *Melissa officinallis* L. (SANTOS *et al.*, 2009), *Chamomila recutita* L. (AMARAL *et al.*, 2008), *Cymbopogon citratus* D.C. (COSTA *et al.*, 2008), *Baccharis trimera* (Less.) D.C. (SILVA *et al.*, 2007), *Ocimum basilicum* L. (BLANK *et al.*, 2005). However the different responses to organic fertilizer described in those studies, both in the production of biomass and the levels and chemical composition of essential oils, demonstrate the need for individual evaluation of each plant species.

Within this context, aimed evaluate the effect of different dosages of cattle and poultry manure on biomass production and the content, yield and chemical composition of the essential oil of *A. millefolium*.

MATERIAL AND METHODS

Trials were carried out from February to June of 2009 in a greenhouse of the Laboratory of Tissue Culture and Medicinal, Aromatic and Spice Herbs in the Department of Agriculture (DAG) of the Federal University of Lavras (UFLA). Plants of *A. millefolium* L. were obtained by propagating rhizomes from mother plants of the Medicinal Garden at UFLA. After 60 days, the plants were transplanted to plastic pots, each with a capacity of 10 L, containing soil (dark-red latosol) mixed with organic fertilizers, in two distinct trials: Trial I (cattle manure - CM) : 1) soil with no fertilizer; 2) soil + 3.0 kg m⁻² CM; 3) soil + 6.0 kg m⁻² CM; 4) soil + 9.0 kg m⁻² CM; 5) soil + 12.0 kg m⁻² CM; and Trial II (poultry manure - PM): 1) soil with no fertilizer; 2) soil + 1.5 kg m⁻² PM; 3) soil + 3.0 kg m⁻² PM; 4) soil + 4.5 kg m⁻² PM; 5) soil + 6.0 kg m⁻² PM. The experimental design was completely randomised with four replications and four plants per lot.

The soil and fertilizer used in the tests were analysed by the Laboratory for Soil Analysis of UFLA. The chemical characteristics of the soil were: pH in water = 5.6; P and K (mg dm⁻³) = 0.6 and 14; Ca²⁺, Mg²⁺, Al³⁺, H+Al (cmol_c dm⁻³) = 0.5, 0.1, 0.0, 2.1; base saturation V (%) = 23.4; organic matter (dag kg⁻¹) = 1.4; The organic fertilizers resulted in the following values for the cattle manure N, P, K, Na, Ca, Mg, S (g kg⁻¹) = 18.0; 5.1; 13.0; 1.5; 4.1; 3.2; 2.6; B, Cu, Fe, Mn, Zn (mg g⁻¹) = 5.6; 39.0; 12,848.0; 461.0; 150.0. For the poultry manure the values were: N, P, K, Na, Ca, Mg, S (g kg⁻¹) = 21.0; 20.0; 7.3; 2.2; 46.0; 2.8; 3.1; B Cu, Fe, Mn, Zn (mg kg⁻¹) = 17.0; 74.0; 4,601.0; 315.0; 314.0.

At 110 days after planting, the plants were harvested, separated into roots and shoots, and oven dried with forced air at 38 ± 1 °C until reaching constant weight, and weighed with an analytical balance. Plant growth was determined by the accumulation of leaf dry weight (LDW, g plant⁻¹), root dry weight (RDW, g plant⁻¹) and the root to shoot ratio (R/S).

The essential oil of *A. millefolium* was extracted by hydrodistillation with a modified Clevenger apparatus, using 40 g of leaf dry biomass (LDW) in 500 mL of distilled water for 90 minutes. A completely randomised experimental design was used with four replications per treatment. The essential oil was purified by liquid-liquid partition with dichloromethane. The organic phase was collected and treated with anhydrous magnesium sulphate, filtered, and the solvent then evaporated at room temperature in a gas exhaustion chamber. The content (g 100 g⁻¹ LDW) and yield (g plant⁻¹) of the essential oil from the dry base of the leaves were determined.

Analysis of the chemical composition of the essential oil from the leaves of *A. millefolium* was carried

out at the Laboratory for Phytochemistry, at the Department of Medicinal Plants of DAG/UFLA. A composite sample was used in these analyses, made up by combining the aliquot parts contained in equivalent weights of the essential oil from the replications of each treatment.

Quantitative analyses of the oil were conducted employing gas chromatography coupled with a hydrogen-flame ionization detector (GC-FID) using an Agilent® 7890A system equipped with a fused-silica capillary column HP-5ms (30 m length × 0.25 mm inner diameter × 0.25 µm film thickness) (California, USA). Helium was used as the carrier gas at a flow rate of 1.0 mL min⁻¹; the temperature of the injector and detector were kept at 220 to 240 °C respectively. The initial temperature of the oven was 60 °C with a temperature ramp of 3 °C min⁻¹ to 150 °C followed by an isotherm of 10 minutes and then a ramp of 10 °C min⁻¹ to 270 °C. The oil was dissolved in ethyl acetate (1%, v/v) and automatically injected into the chromatograph using an injection volume of 1.0 µL, in split mode, at an injection ratio of 1:50. The concentration of the constituents was calculated using their complete respective peak areas relative to the total area of all the constituents of the sample.

Qualitative analyses of the oil were carried out by gas chromatography-mass spectrometry (GC-MS) using an Agilent® 5975C system operated with electron impact ionization at 70 e V, in scanning mode, at a speed of 1.0 scan sec⁻¹, with a mass acquisition range of 40-400 m/z. The chromatographic conditions were the same as those employed in the quantitative analyses. The components were identified by comparing their Kovats retention indices with mass-spectra and retention-index data taken from the literature (ADAMS, 2007; KOTAN *et al.*, 2010) and by comparison of the mass spectra with the NIST/EPA/NIH library database (NATIONAL INSTITUTE OF STANDARDS AND TECHNOLOGY, 2008).

Data of the shoot dry weight (SDW), root dry weight (RDW), root to shoot ratio (R/S), and content and yield of the essential oil were subjected to regression analysis ($p < 0.05$) using the SISVAR® statistical software (FERREIRA, 2007).

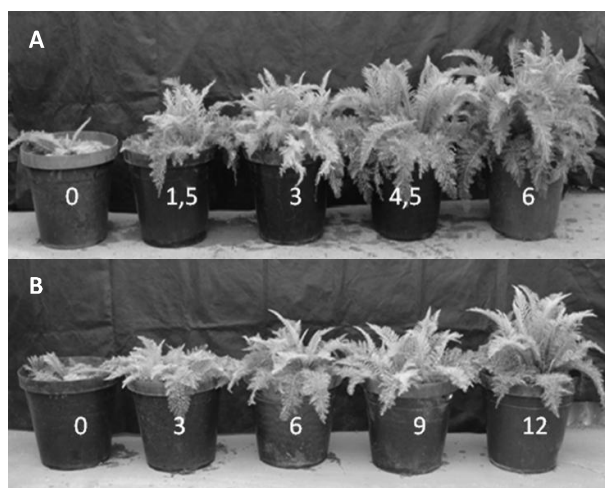
RESULTS AND DISCUSSION

Achillea millefolium plants responded positively in vegetative growth relative to the dosages and sources of manure, unlike the plants which received no fertilizer and which presented reduced size (Figure 1).

However, *A. millefolium* responded better to fertilization with poultry manure, showing increased SDW production at a value of 36 g plant⁻¹ and a dosage of 6 kg m⁻² compared to 25 g plant⁻¹ at a dosage of 12 kg m⁻² of cattle

manure (Figures 2 A and B). The superiority of the poultry manure was 30.5% when applying half the dosage of cattle manure needed to obtain maximum SDW accumulation. Comparing the chemical analyses of the fertilizers, it can be seen that on average the poultry manure had higher nutrient levels, probably resulting in better gains in SDW. Studies by Scheffer *et al.* (1993) showed a positive response of *A. millefolium* to organic fertilization, confirming the results observed in the present study. Other studies, which have evaluated the increase in biomass production in medicinal and aromatic plants by the application of organic fertilizers, showed similar results (BRANT *et al.*, 2010; CORRÊA *et al.*, 2010; COSTA *et al.*, 2008; ROSAL *et al.*, 2011).

Figure 1 - Plants of *Achillea millefolium* L. grown in pots under different dosages of organic fertilization. A - poultry manure (0.0, 1.5, 3.0, 4.5 and 6 kg m⁻²). B - cattle manure (0.0, 3.0, 6.0, 9.0 and 12.0 kg m⁻²)



Analysing the RDW, a quadratic response was seen, where the point of maximum accumulation was 35 g plant⁻¹ at a dosage of 6 kg m⁻² for poultry manure and 28 g plant⁻¹ at a dosage of 12 kg m⁻² for cattle manure (Figures 2 C and D). A quadratic tendency was seen for R/S for poultry manure, with values ranging from 0.71 to 1.12 (Figures 2 E and F). The poultry manure at a dosage of 6 kg m⁻² showed a close relationship to a value of one (R/S = 0.96) (Figure 2 D). Cattle manure on the other hand, exhibited linear behaviour with values of between 0.71 and 1.30. At a dosage of 12 kg m⁻², plants fertilized with cattle manure had an R/S of 1.12, indicating higher drainage to the root (Figure 2 F).

The essential oil extracted from the leaves of *A. millefolium* was characterised as a high viscosity liquid oil with intense blue colouration. There was no statistical

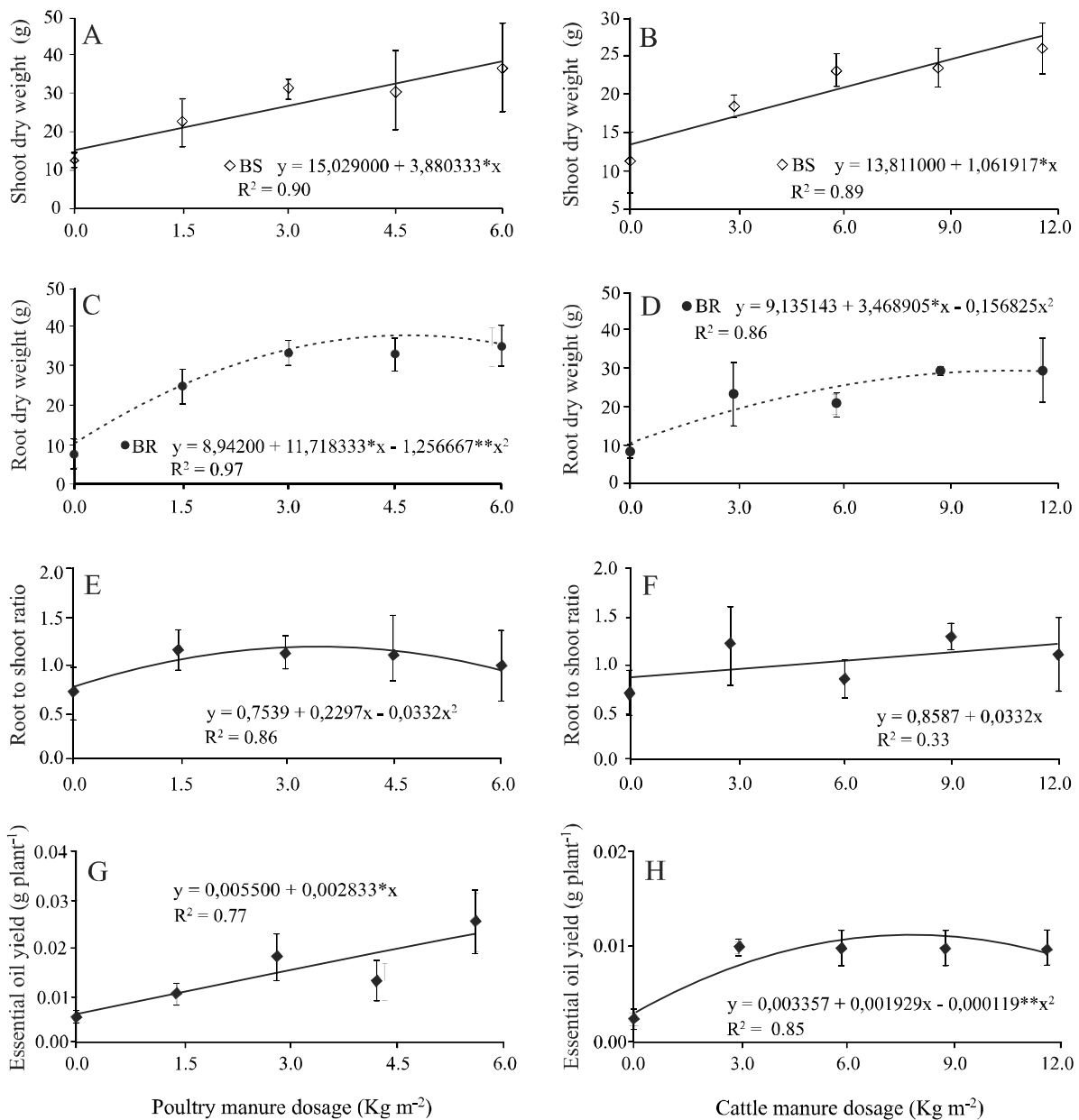
difference between the dosages and sources of organic fertilizers for the levels of the essential oil of *A. millefolium*, which were in the range of 0.05 to 0.07%.

The yield of essential oil showed a significant difference between the dosages of both organic fertilizers (Figures 2 G and H). The poultry manure showed an increasing linear curve adjustment where the maximum yield was 0.025 g plant⁻¹ at a dosage of

6 kg m⁻² (Figure 2 G). The cattle manure also had a significant response to the applied dosages compared to the control, displaying a quadratic curve adjustment, and presenting the highest yield of oil (0.01 g plant⁻¹) at a dosage of 9 kg m⁻² (Figure 2 H).

Confirming this study, Amaral *et al.* (2008) noted that there was an increase in the yield of essential oil of *Chamomila recutita*, due to the

Figure 2 - Production of shoot dry weight (A and B), root dry weight (C and D), root to shoot ratio (E and F) and essential oil yield (G and H) of *Achillea millefolium* L. Plants, fertilized with different dosages of poultry manure (left column) and cattle manure (right column)



*Significant by the F-test at 5%

increase in biomass from fertilization with nitrogen. In *Ocimum selloi*, Costa *et al.* (2008) found that plants fertilized with 4.0 kg m⁻² of poultry manure had a higher yield of essential oil (0.031 g plant⁻¹) compared to plants fertilized with 8.1 kg m⁻² of cattle manure (0.023 g plant⁻¹), concluding that the increase in oil may have been influenced by the increase in the levels of available nutrients in the soil.

Organic fertilization with poultry and cattle manure resulted in no marked qualitative or quantitative differences to the essential oils. Chromatographic analysis indicated a very complex chemical composition of the essential oil of *A. millefolium*, containing around 48 chemical components (Tables 1 and 2). The main constituents were borneol, spathulenol, *E*-nerolidol

and chamazulene, which made up nearly 59% of the relative area of the chromatographic peaks.

Achillea millefolium, regardless of the amount of fertilization, presented high levels of chamazulene: in plants fertilized with poultry manure its concentrations ranged from 44.25 to 46.14%, in those fertilized with cattle manure, from 40.49 to 52.01%. For levels of the constituents, borneol (3.13 to 5.45%), spathulenol (4.48 to 6.29%), *e*-nerolidol (3.94 to 5.23%) and β -cubebene (0.83 to 4.03%), the differences were less significant. Phytochemical studies carried out in various regions of the world have also shown the high complexity and diversity of the volatile chemical composition of *A. millefolium* (GUDAITYTE; VENSKUTONIS, 2007; JUDZENTIENE; MOCKUTE, 2010).

Table 1 - Chemical composition of the essential oil of *Achillea millefolium* cultivated with different dosages of poultry manure

Constituents	IR*	Poultry manure (kg m ⁻²)				
		0	1.5	3	4.5	6
γ -terpinene	1059	0.12	0.09	nd	0.07	0.11
terpinolene	1087	0.31	0.24	0.31	0.22	0.32
borneol	1152	4.13	3.31	5.16	3.13	4.46
cis-dihydro- α -terpineol	1164	0.48	0.36	0.64	0.39	0.71
terpinen-4-ol	1178	1.2	1.19	1.81	1.12	1.54
bornyl acetate	1274	0.15	0.17	0.2	0.14	0.2
thymol acetate	1347	nd	0.23	0.26	0.22	0.24
<i>Z</i> -caryophyllene	1409	1.41	2.46	2.4	1.94	2.28
humulene	1442	0.27	0.48	0.45	0.4	0.44
β -cubebene	1470	1.34	0.83	1.56	1.97	4.03
β -ionone	1476	0.25	0.2	0.28	0.26	0.31
germacrene D	1485	nd	nd	nd	0.23	0.31
α -farnesene	1498	0.18	0.2	0.39	0.51	0.81
cubebol	1513	nd	0.17	nd	0.13	0.18
δ -cadinene	1524	nd	0.21	0.4	0.39	0.36
cis-nerolidol	1540	0.79	0.56	0.84	0.91	0.9
α -calacorene	1543	nd	nd	0.29	0.32	0.27
aromadendrene oxide	1550	2.32	3.02	3.61	3.44	3.19
spathulenol	1561	5.47	4.82	5.5	5.47	4.95
<i>E</i> -nerolidol	1566	4.37	5.23	5.05	4.73	4.25
caryophyllene alcohol	1571	0.48	0.39	0.35	0.37	0.35
sesquisabinene hydrate	1574	0.47	0.28	0.24	0.16	0.22
caryophyllene oxide	1576	1.4	1.65	1.62	1.7	1.55
globulol	1588	0.29	0.2	nd	0.16	0.25
β -copaen-4-ol	1590	0.85	1.23	1.07	1.17	0.92

Continuação Tabela 1

viridiflorol	1594	1.4	0.85	0.77	0.8	1.46
ledol	1604	0.17	0.38	0.31	0.37	0.25
humulene epoxide	1606	nd	0.27	nd	0.21	0.22
β -himachalene oxide	1618	nd	0.26	nd	0.13	nd
10-epi- γ -eudesmol	1625	1.08	0.89	0.82	0.9	0.76
1-epi-cubenol	1630	0.87	0.58	0.57	0.73	0.7
α/β -caryophylla-4(14),8(15)-dien-5-ol	1637	2.87	1.76	1.72	2.05	2.19
t-muurolol	1639	0.36	0.51	0.4	0.52	0.39
α -bisabolol oxide B	1648	0.42	0.43	0.31	0.39	0.4
β -eudesmol	1652	nd	0.29	0.23	0.41	0.26
selin-11-en-4- α -ol	1657	0.44	0.46	nd	0.4	nd
14-hydroxy-9-epi- β -caryophyllene	1668	nd	0.78	0.68	0.89	nd
α -cadinol	1676	2.46	1.48	1.25	1.49	1.69
α -bisabolol	1683	1.09	0.99	1.02	1.07	0.97
2Z,6Z-farnesol	1701	1.07	1.27	1	1.26	0.94
chamazulene	1734	45.4	44.25	46.14	42.67	43.38
2E,6E-farnesol	1741	0.35	0.28	nd	0.24	nd
α -bisabolol oxide A	1744	nd	0.26	nd	0.28	nd
14-hydroxy- α -muurolene	1779	2.03	1.48	1.74	2.16	1.73
β -eudesmol acetate	1791	2.68	2.77	2.95	3.46	2.65
epi- α -bisabolol acetate	1800	2.18	1.67	1.74	1.88	1.59
isolongifolol oxide	1816	0.73	0.54	0.61	0.78	0.61
2E,6E-farnesyl acetate	1849	0.65	0.46	0.48	0.65	0.51
Total monoterpenes	7.8	8.05	10.78	7.23	8.75	
Total sesquiterpenes	84.7	82.38	84.39	86.06	84.3	
Total	92.5	90.43	95.17	93.29	93.04	

*Retention index relative to the *n*-alkanes (C8-C20) series in a HP-5MS column. nd: not detected**Table 2** - Chemical composition of the essential oil of *Achillea millefolium* cultivated with different dosages of cattle manure

Constituents	IR*	Cattle manure (kg m ⁻²)				
		0	3	6	9	12
γ -terpinene	1059	0.12	0.20	0.11	0.18	0.22
terpinolene	1087	0.31	0.43	0.29	0.38	0.37
borneol	1152	4.13	5.45	3.88	4.56	4.32
cis-dihydro- α -terpineol	1164	0.48	0.77	0.40	0.57	0.58
terpinen-4-ol	1178	1.20	1.66	1.18	1.40	1.36
bornyl acetate	1274	0.15	0.23	0.15	0.17	0.19
thymol acetate	1347	nd	0.24	0.17	0.16	0.21
Z-caryophyllene	1409	1.41	1.99	1.70	1.83	2.18
humulene	1442	0.27	0.37	0.33	0.35	0.41

Continuação Tabela 2

β -cubebene	1470	1.34	2.26	1.80	2.33	2.18
β -ionone	1476	0.25	0.27	0.24	0.29	0.25
germacrene D	1485	nd	0.17	nd	nd	nd
α -farnesene	1498	0.18	0.32	0.30	0.41	0.49
cubebol	1513	nd	0.20	0.30	nd	nd
δ -cadinene	1524	nd	0.20	0.29	nd	0.22
cis-nerolidol	1540	0.79	0.89	1.05	0.68	0.49
α -calacorene	1543	nd	0.12	0.18	nd	nd
aromadendrene oxide	1550	2.32	3.05	3.37	2.62	2.65
spathulenol	1561	5.47	5.38	6.29	5.13	4.48
<i>E</i> -nerolidol	1566	4.37	4.83	4.87	4.21	3.94
caryophyllene alcohol	1571	0.48	0.44	0.47	0.33	0.28
sesquisabinene hydrate	1574	0.47	0.42	0.41	0.30	0.20
caryophyllene oxide	1576	1.40	1.58	1.60	1.45	1.34
globulol	1588	0.29	0.34	0.38	0.20	nd
β -copaen-4-ol	1590	0.85	0.96	1.00	0.86	0.83
viridiflorol	1594	1.40	1.62	1.67	1.17	0.79
ledol	1604	0.17	0.32	0.31	0.25	0.21
humulene epoxide	1606	nd	0.27	nd	0.21	0.22
β -himachalene oxide	1618	nd	0.19	0.16	nd	nd
10-epi- γ -eudesmol	1625	1.08	1.01	1.11	0.91	0.71
1-epi-cubenol	1630	0.87	0.81	0.92	0.74	0.52
α/β -caryophyll-4(14),8(15)-dien-5-ol	1637	2.87	2.37	2.74	2.18	1.66
t-muurolol	1639	0.36	0.38	0.33	0.34	0.33
α -bisabolol B oxide	1648	0.42	0.44	0.40	0.39	0.32
β -eudesmol	1652	nd	0.25	0.20	nd	nd
selim-11-en-4- α -ol	1657	0.44	0.41	nd	nd	nd
14-hydroxy-9-epi- β -caryophyllene	1668	2.16	1.20	0.70	2.07	0.98
α -cadinol	1676	2.46	1.87	2.16	1.96	1.47
α -bisabolol	1683	1.09	0.92	1.00	1.06	1.03
2 <i>Z</i> ,6 <i>Z</i> -farnesol	1701	1.07	0.93	1.19	1.03	1.12
chamazulene	1734	45.35	40.49	42.48	47.27	52.01
2 <i>E</i> ,6 <i>E</i> -farnesol	1741	0.35	0.23	0.30	nd	0.30
14-hydroxy- α -muurolene	1779	2.03	1.46	2.14	1.87	1.61
β -eudesmol acetate	1791	2.68	2.13	2.14	2.90	2.60
epi- α -bisabolol acetate	1800	2.18	1.39	1.79	1.84	1.76
isolongifolol oxide	1816	0.73	0.49	0.71	0.66	0.58
2 <i>E</i> ,6 <i>E</i> -farnesyl acetate	1849	0.65	0.43	0.62	0.60	0.47
Total monoterpenes		6.38	8.96	6.19	7.43	7.25
Total sesquiterpenes		88.24	83.42	87.66	88.39	88.65
Total		94.62	92.38	93.84	95.82	95.90

*Retention index relative to the *n*-alkanes (C8-C20) series in a HP-5MS column. nd: not detected

CONCLUSIONS

1. For the accumulation of dry biomass, and thus a higher yield of essential oil, *Achillea millefolium* is a medicinal plant which needs organic manure. However, fertilization with cattle manure (0 to 12.0 kg-m²) or poultry manure (0 to 6.0 kg m²) does not significantly affect either the composition or concentration of the chemical constituents of the oil;
2. Fertilization with poultry manure at a dosage of 6 kg m² produced a greater accumulation of shoot biomass and greater yield of essential oil. However the chamazulene content is higher in plants fertilized with 12 kg m² of cattle manure.

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