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ARTICLE

Plants of *Achillea millefolium* L. grown under colored shading nets have altered secondary metabolism

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ABSTRACT: (Plants of *Achillea millefolium* L. grown under colored shading nets have altered secondary metabolism). Here we evaluated the effect of quality and quantity of light on the growth and secondary metabolism of *Achillea millefolium* L. plants. Plants were cultivated under either full light or colored shading nets (blue, red or black). Analyses were performed after eight weeks of shading and at two and four weeks after the removal of the nets. Plants grown under nets presented lower dry weight of leaves and flowers. In addition, the synthesis of phenols and flavonoids decreased in the leaves and flowers of shaded plants. On the other hand, the blue net increased the content of essential oil in the leaves. The concentrations of the majority of the compounds analyzed in the essential oil increased when using the black net. Farnesol and chamazulene were the most plentiful compounds in the oil of leaves and flowers under all conditions. Overall, these results indicate that in *A. millefolium* plants different compounds respond differently to specific light wavelengths. Thus, photoselective treatments should be directed toward the production of the target metabolite.
Keywords: Photoselective nets, secondary metabolism, essential oil, shading.

RESUMO: (Plantas de *Achillea millefolium* L. crescidas sob sombreamento com malhas coloridas possuem alterações no metabolismo secundário). O efeito da qualidade e quantidade de luz no crescimento e metabolismo secundário foram avaliados em plantas de *Achillea millefolium* L.. As plantas foram cultivadas sob luz plena ou sob redes coloridas (azul, vermelho e preto) e avaliadas após oito semanas de sombreamento e após duas e quatro semanas da retirada das redes. Plantas sombreadas apresentaram menores valores de matéria seca de folhas e flores. Além disso, a síntese de fenóis e flavonóides diminuiu em folhas e flores de plantas sombreadas. Por outro lado, a rede azul aumentou o teor de óleo essencial nas folhas. A concentração da maioria dos compostos analisados no óleo essencial das plantas aumentou quando se utilizou a rede preta. Farnesol e chamazuleno foram os compostos mais abundantes no óleo essencial de folhas e flores em todas as condições. De forma geral, esses resultados demonstram como os compostos secundários respondem diferentemente à qualidade da luz em plantas de *A. millefolium*. Assim, os tratamentos fotosseletivos devem ser direcionados para a produção do metabólito de interesse.
Palavras-chave: redes fotoseletivas, metabolismo secundário, óleo essencial, sombreamento.

INTRODUCTION

Medicinal plants are important sources of natural antioxidants and secondary metabolites, useful for the production of drugs and other bioactive compounds (Al-Snafi 2015). However, predicting the metabolites that in fact will be in the plant extract is a challenge for bringing medicinal plants into successful commercial cultivation (Canter *et al*. 2005). In order to minimize this problem, specific cultivation techniques can be used to maximize the production of particular chemical substances (such as phenols, flavonoids and terpenes). An interesting strategy is to utilize light at specific wavelengths in the cultivation of medicinal plants. It is well known that light quality affects plant development and physiology, such as the production of metabolites via the selective activation of different photoreceptors (Rai *et al*. 2017), and that these effects vary between different plant species and varieties (Silva *et al*. 2016, Yu *et al*. 2017, Ribeiro *et al*. 2018).

Photoreceptors are able to perceive the day length as well as the quality and quantity of light (Batista *et al*. 2018). Phytochromes are an important group of photoreceptors responding to red (650–680 nm) and far-red (FR) (710–740 nm) light by reversibly interconverting between the active and inactive forms (Vierstra & Zhang 2011). In addition, cryptochromes, containing flavin adenine dinucleotide as a chromophore, are photoreceptors responsible for perceiving UVA and blue light (Batista *et al*. 2018).

Artificial light (*e.g.* fluorescent and light-emitting diode lamps) is commonly used to provide light at specific wavelengths, but this technique becomes expensive and unfeasible in large-scale cultures (Batista *et al*. 2018). Thus, the use of colored shading nets is indicated to transmit sunlight wavelengths in a specific manner and to protect against hail, wind and excessive solar radiation and to hinder insect pests entering the production environment (Kotilainen *et al*. 2018). The use of colored shading nets has been further shown to increase plant growth (Strydom *et al*. 2018) and the production of antioxidants (Zhang *et al*. 2015), to develop more nutritious fruits (Ilic *et al*.

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2018) and to accumulate higher contents of metabolites of pharmaceutical interest (Ribeiro *et al*. 2018).

A study has monitored polytunnels equipped with red, blue or black nets (all ChromatiNet®, Polysack Plastic Industries, D.N. Negev, Israel) for light quality, temperature, relative humidity and wind resistance over a period of one year (Arthurs *et al*. 2013). The authors reported that black, red and blue nets reduced the transmittance of photosynthetically active radiation by approximately 55%, 41% and 51%, respectively, depending on the season. It was also found that blue nets transmitted peaks in the blue (450–495 nm) as well as in FR/near infrared (beyond 750 nm), while red nets showed a minor peak at 400 nm and major transmittance beyond 600 nm. In addition, Arthurs *et al*. (2013) found that black and red nets gave an R:FR ratio (defined as 600–700 nm/700–800 nm) similar to natural conditions (R:FR ratio *ca.* 1.0), while blue nets lowered the R:FR ratio by a small amount (*ca.* 0.8).

Achillea millefolium L. (Asteraceae) is a widespread plant with important medicinal properties such as antibacterial, anti-inflammatory, antitumoral, antifungal and antioxidant (Ahmadi-Dastgerdi *et al*. 2017) activities. Studies of *A. millefolium* have demonstrated the importance of this plant in alternative and traditional medicine (Teixeira *et al*. 2003, Benedek & Kopp 2007, Santoro *et al*. 2007) and in the pharmaceutical area (Alvarenga *et al*. 2015); however, only one study has assessed the potential of *A. millefolium* plants with photoselective treatments (Alvarenga *et al*. 2015). Despite the valuable contributions of this study regarding the production of secondary metabolites *in vitro*, information on growth and production of secondary metabolites in *A. millefolium* cultivated *ex vitro* remain unexplored. In addition, no information on the effect of the light quality on *A. millefolium* flowers can be found. The aim of this study was to evaluate growth and to monitor yield and composition of essential oils in *A. millefolium* plants grown under different colored shading nets.

MATERIAL AND METHODS

Plant material and experimental conditions

The plant material was obtained from a single clump to ensure uniformity of data. Seedlings of *A. millefolium* were grown in a greenhouse for four months and then transferred to the field (see environmental conditions in Table 1). In the field, the soil, characterized as Planosol, was corrected for phosphorus and potassium using triple superphosphate and potassium chloride. Plants were irrigated through a drip system throughout the entire experiment. Shading with colored nets (ChromatiNet®-Polysack Industries, Nir Yitzhak, Israel) was used to modify the transmitted light. The red, blue and black nets attenuated the transmitted light by *ca.* 58%, 66% and 83%, respectively. In addition to decreasing incident light, the colored nets also changed the quality of light. The red net transmitted greater light at wavelengths above 590 nm, while the blue net transmitted greater light above 470 nm. As a control, a group of plants was grown in full light. After eight weeks in the field, the first harvest was performed (8WS) and the nets were removed. Two and four weeks after the removal of the nets (2WAS and 4WAS, respectively), additional samplings were made. Herbarium specimens were prepared and deposited at the PEL Herbarium (24.600).

Plant growth

Leaves and flowers were maintained in a forced-air oven at 35 ºC until reaching constant weight, and the dry weight measured using an analytical scale.

Total phenolic content

Dried leaves or flowers (1.0 g) were crushed into a fine powder using liquid nitrogen. Then, the homogenates were suspended in 10 mL of methanol/ chloroform/water 12:5:5 (v/v/v) followed by overnight extraction and centrifugation at 4000 *g* for 10 min. To the supernatant was added chloroform and water, followed by centrifugation at 4000 *g* for 10 min. The aqueous extract was collected and concentrated by evaporation at 37 ºC for 24 h in order to eliminate excess methanol and chloroform.

The total phenolic content was assayed using the Folin–Ciocâlteu reagent, as described by Bieleski & Turner (1966). Briefly, 0.5 mL of the aqueous extract was mixed with 0.5 mL of 1 N Folin–Ciocâlteu reagent and allowed to react for 15 min. Then 5 mL of saturated sodium carbonate solution was added to the samples and the mixture incubated at 30 ºC for 60 min. The absorbance was measured at 760 nm and the phenolic acid was used as the standard (Jennings 1981).

Determination of total flavonoid content

The total flavonoids were extracted by grinding 0.25 g of dried leaves or flowers with 70% methanol. The homogenates were incubated for 24 h in the dark and the samples filtered using filter paper (Mendes *et al*. 2005). Next, the plant extracts were added to 5% aluminum chloride solution in 70% methanol following by incubation at 30 ºC for 10 min. Readings were obtained at 420 nm and the standard curve was prepared using quercitin (Challice & Markham 1984).

Extraction and composition of essential oil

Shade-dried powdered materials (70 g) of leaves or flowers were hydro-distilled in a Clevenger-type apparatus for 4 h. The extracted volatile oils were dried over anhydrous sodium sulfate and the yield of the oils calculated based on dry weight of plant materials (Santos *et al*. 2004).

GC-MS analysis was carried out on a Shimadzu (GC-FID) gas chromatograph fitted with a fused silica DB-5 capillary column (30 m × 0.25 mm; film thickness 0.25 μm). The oven temperature was programmed from 40–280 ºC at 5 ºC min^{-1}. Nitrogen was used as carrier gas at a flow rate of 1.2 mL min^{-1} at 80 kPa. Identification of components of essential oils were carried out based on

Table 1. Temperature (ºC), relative humidity (%) and solar energy (cal cm^{-2} dia^{-1}) in the field during the experiment. In January the plants were transplanted to the field, in May, the plants were shaded, in June the first harvest and the removal of the nets were performed; and in July the second harvest was performed.

Months	Climatological data*								
	Temperature (ºC)			Relative Humidity (%)			Solar energy(cal cm^{-2} dia^{-1})		
	Avarege	Max	Min	Avarege	Max	Min	Avarege	Max	Min
January	23.7	29.5	19.2	80.4	89.8	63.7	521.5	754.6	732.0
February	24.4	30.0	20.2	86.6	96.1	71.1	439.7	696.0	112.5
March	22.6	28.2	18.5	82.0	93.0	65.5	433.6	636.3	114.6
April	19.3	25.3	14.8	80.1	94.1	65.8	312.9	455.4	64.0
May	16.7	20.9	13.7	87.9	96.9	56.0	202.1	377.2	37.7
June	14.0	18.7	10.2	84.3	97.5	63.0	197.3	304.5	22.8
July	13.2	18.7	8.7	84.4	97.8	61.5	213.5	342.9	18.2

*Source: Weather report – EMBRAPA Clima Temperado (http://agromet.cpact.embrapa.br/)

retention indices and fragmentation patterns of the mass spectra (Neto *et al*. 2003).

Experimental design and statistical analysis

Experiments were arranged in a completely randomized block design, in a 4 × 3 factorial (nets and harvest periods; for content of total phenolics) and 4 × 2 (nets and harvest periods; for analysis of dry weight, content of flavonoids and for yield and composition of essential oil). All analyses were carried out using four repetitions ($n = 4$), with each biological sample consisting of five plants. Data were first submitted to Shapiro–Wilk test to check the normality and to Levene's test to verify homoscedasticity, followed by ANOVA analysis. The post hoc comparisons were tested using the Tukey test at the significance level of $P < 0.05$ with the statistical program SAS (SAS SYSTEM, 2002). The graphs were produced using SigmaPlot 11.0.

RESULTS

Analyses of total phenols, total flavonoids and essential oil yield were affected by the significant interaction between the factors shading by color nets and harvest periods. However, there was no interaction between these factors for biomass accumulation. The use of nets resulted in decreases in the dry weight of leaves and flowers. Leaves shaded with red net decreased the biomass weight 1.3-fold compared to non-shaded plants, while the dry weight of plant leaves shaded with blue or black nets decreased 1.8-fold compared to control plants. In addition, plants shaded with red, blue or black nets decreased flower biomass 2-fold when compared to plants maintained in full light (Table 2).

The content of total phenols and flavonoids in leaves decreased eight weeks after shading with the different nets (Fig. 1A–B). Total flavonoids in flowers were also reduced by the use of shading nets at eight weeks (Fig. 1C). Overall, the levels of phenols and flavonoids in leaves increased after the shading nets were removed (Fig. 1A–B), but not in flowers (Fig. 1C).

The essential oil of leaves and flowers of *A. millefolium* can be characterized as viscous with intense blue coloration (data not shown). In addition, the yield varied according to the part of the plant from which it was extracted, showing that leaves produced 0.41 mL g^{-1} DW and the flowers 1.67 mL g^{-1} DW (data not shown). The essential oil yield was higher in all leaves and flowers harvested after 8WS compared to 4WAS (Table 3). The essential oil yield in the leaves increased 1.5- and 1.6-fold when using the blue net in the harvest period 8WS or red in the harvest period 4WAS, respectively, in comparison to control plants (Table 3). In flowers harvested after 8WS there was no difference in essential oil yield in shaded and non-shaded plants. However, flowers harvested 4WAS presented lower (13%) essential oil content when shaded with blue net compared to unshaded plants (Table 3).

The 11 compounds identified in the essential oil of leaves were terpenic, in which the monoterpenes (10 C)

Table 2. Dry weight of leaves and flowers of *Achillea millefolium* L. grown under full light (no net) or under colored shading nets (black, blue and red) and at different harvest periods (eight weeks of shading – 8WS and four weeks after shading – 4WAS). Values are given in g $plant^{-1}$.

Net	Harvest Period			
	Leaves		Flowers	
	8WS	4WAS	8WS	4WAS
No net	67.18 ± 16.31 Aa*	80.29 ± 8.21 Aa	11.01 ± 3.45 Aa	26.37 ± 5.65 Aa
Red	54.20 ± 4.81 Ba	62.13 ± 13.21 Ba	8.96 ± 2.21 Ba	12.24 ± 4.20 Ba
Blue	38.35 ± 6.88 Ca	44.22 ± 7.28 Ca	7.32 ± 2.53 Ba	6.64 ± 1.64 Ba
Black	36.71 ± 4.10 Ca	42.58 ± 7.34 Ca	7.07 ± 2.07 Ba	12.16 ± 1.18 Ba

* Distinct uppercase letters indicate significant differences in the column while distinct lowercase letters indicate significant differences in the line (Tukey; $P < 0.05$).

were 1–8 cineol, γ-terpinene, borneol, menthol, terpineol and bornil acetate. Meanwhile, the sesquiterpenes (15 C) were trans-caryophyllene, α-humulene, caryophyllene oxide, farnesol and camazulene (Table 4). The most plentiful compounds extracted from the essential oil from leaves grown under full light in the first harvest (8WS) were farnesol (11.28%), trans-caryophyllene (5.41%), caryophyllene oxide (3.94%), borneol (2.44%), and camazulene (2.37%). These compounds were also the most abundant in black-shaded plants in the harvest period 8WS, especially farnesol (21.30%) followed by trans-caryophyllene (9.47%), camazulene (5.75%), caryophyllene oxide (4.66%) and borneol (3.87%). The compound γ-terpinene, although not very abundant, was increased in this shading condition (Table 4). In plants shaded with blue net in 8WS, a significant increase can be observed in the proportion of camazulene (9.23%) and farnesol (14.17%) in leaves, compared to plants grown under full light. In addition, trans-caryophyllene (2.94%), caryophyllene oxide (2.26%) and borneol (1.23%) were also found, but in lesser amounts than in non-shaded plants (Table 4). The main compounds in essential oil extracted from leaves submitted to treatment with red net for eight weeks (8WS) were farnesol (12.09%), camazulene (4.29%), caryophyllene oxide (3.15%), trans-caryophyllene (2.80%) and borneol (1.15%). The proportion of camazulene was higher in this treatment when compared to plants grown under full light (Table 4). Four weeks after shading (4WAS), the essential oil composition of leaves was similar to that obtained in plants grown under full light, especially farnesol, caryophyllene oxide, trans-caryophyllene and borneol. The compound caryophyllene oxide increased significantly in the previously shaded plants, especially those from the black net treatment. Plants shaded with blue net also increased trans-caryophyllene, borneol, bornil acetate and α-humulene (Table 4).

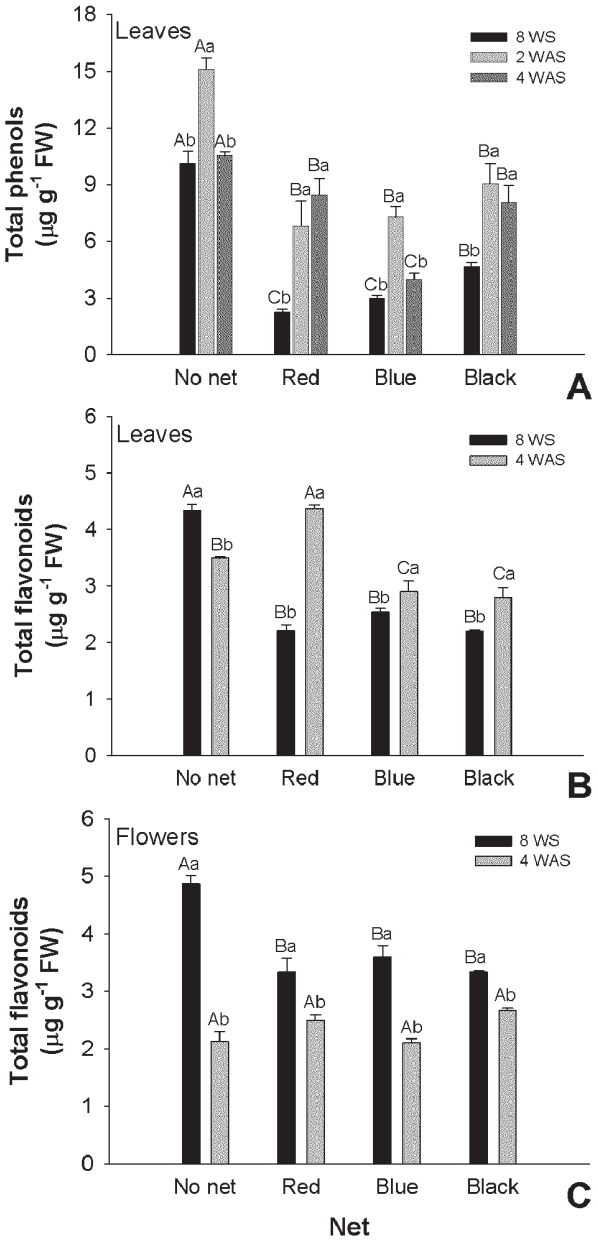

Figure 1. Total phenol (A, leaves) and total flavonoid content (B, leaves; C, flowers) in *A. millefolium* L. plants grown under full light (no net) or under colored shading nets (black, blue and red) and at different harvest periods (eight weeks of shading – 8WS, two weeks after shading – 2WAS and four weeks after shading – 4WAS). Values are the mean ± standard deviation of four biological replicates. Distinct uppercase letters compare the same harvest period within the different shading nets, while distinct lowercase letters compare the different harvest periods within the same shading condition (Tukey test; $P < 0.05$).

In flowers, of the 16 compounds identified in the essential oil, 15 of them were terpenic, in which the monoterpenes found were α-pinene, β-pinene, 1–8 cineol, γ-terpinene, α-terpinene, camphor, borneol, menthol, terpineol and bornil acetate. The sesquiterpenes were trans-caryophyllene, α-humulene, caryophyllene oxide, farnesol and camazulene. The only non-terpene compound identified in this oil was eugenol, a phenolic compound (Table 5). The most abundant compounds in the flower essential oil of plants grown under full light for eight weeks were farnesol (44.61%), camazulene (22.29%), caryophyllene oxide (3.79%), trans-caryophyllene (3.60%) and borneol (2.43%). At the end of the experiment (4WAS), the most plentiful compounds were camazulene (30.84%), trans-caryophyllene (15.54%), borneol (11.34%), farnesol (9.91%) and caryophyllene oxide (9.02%) (Table 5).

Regarding the effect of the black net treatment for eight weeks on the composition of the essential oil of flowers, the most abundant compounds were farnesol (35.44%), camazulene (7.18%), trans-caryophyllene (4.30%), caryophyllene oxide (2.33%) and borneol (1.65%). In the oil obtained from flowers of plants shaded with blue net for eight weeks were mainly camazulene (22.07%), followed by trans-caryophyllene (10.09%), farnesol (6.45%), camphor (3.58%) and caryophyllene oxide (3.19%). In plants shaded with red net the composition was changed to a higher proportion of farnesol (38.50%), followed by camazulene (19.34%), trans-caryophyllene

Table 3. Yield (%) of the essential oil of leaves and flowers of *Achillea millefolium* L. plants grown under full light (no net) or under colored shading nets (black, blue and red) and at different harvest periods (eight weeks of shading – 8WS and four weeks after shading – 4WAS).

Net	Harvest Period			
	Leaves		Flowers	
	8WS	4WAS	8WS	4WAS
No net	0.107 ± 0.018 Ba*	0.046 ± 0.001 Bb	0.726 ± 0.027 Aa	0.646 ± 0.019 Bb
Red	0.105 ± 0.014 Ba	0.074 ± 0.008 Ab	0.740 ± 0.023Aa	0.687 ± 0.018 Ab
Blue	0.159 ± 0.010 Aa	0.044 ± 0.005 Bb	0.744 ± 0.025Aa	0.562 ± 0.011 Cb
Black	0.106 ± 0.011 Ba	0.042 ± 0.002 Bb	0.779 ± 0.031 Aa	0.653 ± 0.017 Bb

***** Distinct uppercase letters indicate significant differences in the column while distinct lowercase letters indicate significant differences in the line (Tukey; $P < 0.05$).

(5.95%), caryophyllene oxide (2.83%) and borneol (2.38%) (Table 5).The most abundant compounds in the oil extracted from the flowers shaded with black net four weeks after return to full light (4WAS), were trans-caryophyllene (17.90%), camazulene (15.61%), borneol (9, 53%), farnesol (6.97%) and caryophyllene oxide (6.71%). In the plants previously shaded with blue net, the composition of the main components of the oil modified especially to the proportion of camazulene (23.44%), followed by similar proportions of trans-caryophyllene (20.94%), borneol (10.57%), caryophyllene oxide (8.58%) and farnesol (5.04%). On the other hand, the compounds extracted from flowers shaded with red net 4WAS were camazulene (31.29%), followed by trans-caryophyllene (14.27%), farnesol (11.15%), borneol (6.94%) and caryophyllene oxide (6.48%) (Table 5).

DISCUSSION

The colored nets attenuated the transmitted light by *ca.* 58 (red), 66 (blue) and 83% (black), which would explain the decrease in biomass accumulation in shaded plants compared to plants grown under full light (Table 2). In optimum temperature conditions, *A. millefolium* presents photosynthesis saturation above 1200 µmol m^{-2} s^{-1} (Loveys *et al*. 2003). Thus, the decrease in irradiance may have affected the photosynthetic potential of the studied plants, resulting in a lower biomass. An increase in the growth rate of shaded plants generally occurs with plants that exhibit low photosynthetic light saturation (Medina *et al*. 2002). In addition, the higher dry mass of leaves exhibited by plants cultivated under red shading nets compared to the other nets is likely to be related to the greater red:far-red light ratio that the red net offers. This ratio affects directly the phytochromes that control the transport of growth regulators, such as auxin. In plants grown under red light these compounds are plentiful, while in plants cultivated under blue light they are found in very low concentrations. Since the red wavelengths fits accurately with the absorption peak of chlorophylls, shoot growth and apical dominance is common in plants grown under red light (Darko *et al*. 2014). However, blue light acts to inhibit the growth of the plant (Silva *et al*. 2016). As expected, four weeks after the removal of nets (4WAS), the inhibitory effects of shading on dry matter increase were reversed and the plant growth was restored.

Phenols, besides being excellent antioxidants, also work as photoprotectors (Ebrahimzadeh *et al*. 2014).

Table 4. Chemical composition (%) of essential oil of leaves of *Achillea millefolium* L. grown under full light or under colored shading nets (black, blue and red) and harvest periods (eight weeks of shading – 8WS and four weeks after shading – 4WAS).

Compounds*	Net								
	No net			Red		Blue		Black	
	RI	Harvest I	Harvest III	8WS	4WAS	8WS	4WAS	8WS	4WAS
1-8 cineol	5.88	0.47	n.d.	0.15	n.d.	0.16	0.09	0.72	0.09
γ-terpinene	6.54	0.16	n.d.	0.12	n.d.	0.13	n.d.	0.40	n.d.
borneol	9.77	2.44	1.99	1.15	1.98	1.23	3.06	3.87	3.64
menthol	10.02	n.d.	n.d.	n.d.	n.d.	0.06	0.01	0.006	n.d.
terpineol	10.61	0.41	0.35	0.17	0.32	0.16	0.36	0.60	0.42
bornyl acetate	13.70	0.21	0.13	0.09	0.13	0.08	0.27	0.33	0.46
trans-caryophyllene	17.62	5.41	3.37	2.80	3.38	2.94	5.23	9.47	9.36
α-humulene	18.55	0.77	0.63	0.51	0.61	0.41	0.99	1.24	1.66
caryophyllene oxide	21.90	3.94	4.15	3.15	4.26	2.26	6.76	4.66	9.74
farnesol	24.67	11.28	4.30	12.09	3.45	14.17	5.26	21.30	3.83
chamazulene	30.56	2.37	0.95	4.29	1.04	9.23	0.62	5.75	1.16
Total		**27.47**	**15.86**	**24.53**	**15.18**	**30.83**	**22.65**	**48.34**	**30.38**

***** Listed in order of elution; RI, retention index (min); n.d., not detected.

Table 5. Chemical composition (%) of essential oil of flowers of *Achillea millefolium* L. grown under full light or under colored shading nets (black, blue and red) and harvest periods (eight weeks of shading – 8WS and four weeks after shading – 4WAS).

Compounds*	Nets								
	No net			Red		Blue		Black	
	RI	Harvest I	Harvest III	8WS	4WAS	8WS	4WAS	8WS	4WAS
α-pinene	4.11	n.d.	n.d.	n.d.	0.39	n.d.	0.30	n.d.	0.40
β-pinene	4.81	n.d.	n.d.	n.d.	0.26	n.d.	0.18	n.d.	0.29
α-terpinene	5.57	0.21	0.22	n.d.	0.53	n.d.	n.d.	n.d.	n.d.
1-8 cineol	5.88	1.06	1.39	0.82	3.14	1.21	2.79	0.38	3.19
γ-terpinene	6.54	0.52	0.72	0.45	1.09	0.43	1.09	0.28	1.22
camphor	9.02	n.d.	n.d.	n.d.	n.d.	3.58	0.21	n.d.	0.19
borneol	9.77	2.43	11.34	2.38	6.94	1.00	10.57	1.65	9.53
menthol	10.02	0.04	n.d.	0.05	n.d.	0.12	n.d.	0.01	n.d.
terpineol	10.61	0.73	2.91	0.62	1.49	0.74	2.35	0.42	2.29
bornyl acetate	13.70	n.d.	0.45	n.d.	0.39	n.d.	0.61	n.d.	0.51
eugenol	15.82	0.43	0.68	0.46	0.33	n.d.	0.43	0.30	0.55
trans- caryophyllene	17.62	3.60	15.54	5.95	14.27	10.09	20.94	4.30	17.90
α-humulene	18.55	0.89	2.70	1.02	2.34	1.57	2.92	0.91	2.65
caryophyllene oxide	21.90	3.79	9.02	2.83	6.48	3.19	8.58	2.33	6.71
farnesol	24.67	44.61	9.91	38.50	11.15	6.45	5.04	35.44	6.97
chamazulene	30.56	22.29	30.84	19.34	31.29	22.07	23.44	7.18	15.61
Total		**80.59**	**85.72**	**72.43**	**80.12**	**50.46**	**79.47**	**53.21**	**68.04**

***** Listed in order of elution; RI, retention index (min); n.d., not detected.

Thus, the higher concentration of phenols in *A. millefolium* leaves grown under full light may be associated with the mechanism of protection against excess radiation. With shading, the light stress is probably diminished, so diminishing the phenolic compounds (Fig. 1). Similarly, the content of phenolic compounds in leaves of *Psidium cattleyanum* Sabine decreased in shaded plants (Junior *et al*. 1999). In general, the flavonoid content also decreased in leaves and flowers with shading. However four weeks after plants were returned to full light conditions, the total flavonoid content increased in the leaves of plants previously shaded with red net, reaching higher concentrations than plants kept under full light (Fig. 1), suggesting that the differential light spectrum to which the plants were subjected influences the response when re-exposed to full light. Some studies have shown that red light influences positively the accumulation of flavonoids (Fu *et al*. 2016, Pedroso *et al*. 2017). In addition, the red light activates related enzymes and transcription factor genes in the flavonoid pathway (Miao *et al*. 2016).

The higher yield of the essential oil in leaves shaded with blue net (Table 3) at 8WS indicates that the blue light can promote the activation of secondary metabolism routes in *A. millefolium*. Indeed, the activity of enzymes related to secondary metabolism and the production of compounds present in the essential oil increases when the seedlings of *Pisum sativum* L. are submitted to blue light (Loschke *et al*. 2001). In tea leaves, blue light increases the content of volatiles, especially volatile phenylpropanoids/benzenoids and several amino acids including l-phenylalanine, suggesting the activation of a plastid-located shikimate pathway (Darko *et al*. 2014). Meanwhile, four weeks after removing the nets (4WAS), the yield of the essential oil of *A. millefolium* decreased in all treatments. However, this effect was less intense in leaves previously shaded with red net, which maintained a high yield of essential oil (Table 3). This is an excellent strategy in crops directed toward the production of essential oil by plants, maintaining higher levels of oil throughout the plant development. In a study with trichomes, it was observed that some plants (*Ocimum selloi* Benth.) shaded with blue and red nets presented greater numbers of glandular trichomes responsible for the production of essential oils (Costa *et al*. 2010). Regarding the studied organs, it was apparent that the yield of the essential oil was 8.6-fold higher in reproductive, relative to photosynthetic structures, indicating that flowers are the main site of production and storage of active principals. In addition, the chemical compounds α-pinene, β-pinene, α-terpinene, camphor and eugenol were detected only in the flowers of *A. millefolium*, showing that these are the plant structures containing the greater variety of compounds (Table 5).

Plants shaded with blue or black nets presented higher contents of secondary compounds in their essential oil when compared to plants grown in full light. However, the composition of the essential oil varied greatly with the color of the nets and in the plant organs (Table 5). In leaves, farnesol was the major compound in all treatments, but was more abundant in plants shaded with black net. In flowers, farnesol production increased in plants shaded with red net and decreased in plants

cultivated under blue or black nets, indicating that the quality of light influences differentially the biosynthesis of this compound in the different organs of the plant. The production of the compounds trans-caryophyllene and caryophyllene in leaves also benefited by the use of the blue nets, but the same did not occur in flowers. In flowers the most produced compound in plants shaded with blue or black nets was cineol. As a matter of fact, blue and black nets have been proved to be efficient in the activation of routes for the production of secondary compounds in the essential oil of *Plectranthus amboinicus* (Lour.) Spreng. and *Pogostemon cablin* Benth. (Noguchia & Amaki 2016, Ribeiro *et al*. 2018).

The biosynthesis of essential oil compounds may also vary according to the type of crop. In a study with *A. millefolium* grown *in vitro* under different light spectra, the production of secondary compounds differed from that found in our study (Alvarenga *et al*. 2015). This can be explained by the fact that plants grown *in vitro* respond differently to plants grown *ex vitro*, since the growth conditions are completely different (Hazarika 2006). Indeed, the growing conditions of *A. millefolium* affect the synthesis of camazulene. In some environments this compound is the major component in the essential oil extracted from flowers, whereas in others this compound is not found at all in the oil of this plant (Ahmadi-Dastgerdi *et al*. 2017). In addition, the age of the plant and the method of light supply may also have influenced the responses found (Figueiredo *et al*. 2008, Ahmadi-Dastgerdi *et al*. 2017).

Regarding the harvest period (*i.e.* 8WS, 2WAS and 4WAS), there was fluctuation in the parameters studied even when the plants were not subjected to shading. Abiotic factors such as air humidity, precipitation and temperature can have great influence on the content of antioxidants, photoprotectors and secondary metabolites in plants (Ahmadi-Dastgerdi *et al*. 2017). We cannot rule out these variations also occurring in the different stages of plant development, since some compounds may increase and others decrease according to the age of the plant. These variations have already been reported in *A. millefolium* and demonstrate how important it is to choose a growing season and a suitable age for harvesting plant material (Ahmadi-Dastgerdi *et al*. 2017).

The establishment of specific light conditions is fundamental in maximizing the production of active substances. The quality of the light should be chosen according to the purpose of cultivation (*e.g.* production of antioxidants, increase in oil yield, production of essential oil compounds), because each component responds very specifically to different light spectra. Furthermore, in addition to the net colors, other factors should also be observed, such as plant age at harvest and the environmental conditions. Overall, our results indicate that unshaded plants grow better and produce higher contents of phenols and flavonoids. Under these conditions, flowers are more abundant in essential oils and have a richer chemical composition. However, if the goal is to increase the content of chemical compounds in leaves, it is better to shade plants for eight weeks with black net.

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