An Acad Bras Cienc (2020) 92(suppl.1): e20180321 DOI 10.1590/0001-3765202020180321
Anais da Academia Brasileira de Ciências | *Annals of the Brazilian Academy of Sciences*
Printed ISSN 0001-3765 I Online ISSN 1678-2690
www.scielo.br/aabc | www.fb.com/aabcjournal

AGRARIAN SCIENCES

Productive, metabolic and anatomical parameters of menthol mint are influenced by light intensity

MARCO ANDRE A. DE SOUZA, RENAN P. BRAGA, ANDRÉ M. DOS SANTOS, JOECILDO F. ROCHA, ROSANE N. CASTRO, MANLIO S. FERNANDES & SONIA R. DE SOUZA*

Abstract: The cultivation of aromatic species to obtain essential oils has great economic importance, presenting an increasing demand from different industrial sectors, especially to menthol mint (*Mentha arvensis* L.) essential oil, rich in menthol (70-80%). Consortium cultivation has been an important practice in agricultural systems whose land use is necessary, consequently promoting strong competition for light in reduced space. Thus, this study aimed verifying if different light intensities might promote chemical, metabolical and anatomical alterations in menthol mint. Plants were grown in greenhouse at different average of light intensities (137, 254, 406 and 543 µmol photons m^{-2} s^{-1}). Samples were collected 43 days after germination and submitted to following analyses: Gravimetric test, photosynthetic pigments, soluble fractions, enzymatic activity, N-total, trichome density and histochemistry and chemometric test based on essential oil chemical profile. Fresh mass gain, trichome density, essential oil content and soluble sugars were positively influenced by light intensity increase. On the other hand, total-N, NO_3^--N and pigments content have decreased influenced by light intensity increase. In the secretion from the trichomes, phenolic substances were reported, as well as lipophilic ones in the peltate ones. The increase of oxygenated monoterpenes was favored by light intensity decrease.

Key words: *Mentha arvensis*, glandular trichomes, histochemistry, essential oil, nitrate reductase.

INTRODUCTION

Among various essential oils types, the ones from *Mentha* genus have been the most requested, mainly from *M. arvensis* L. (menthol mint, japanese mint, vique) with menthol as its principal component (Croteau et al. 2005, Shasany et al. 2010, Kamatou et al. 2013). Menthol is an oxygenated monoterpene with a crystalline appearance, with aromatic and medicinal properties, which has a soothing and refreshing effect on the skin and mucous membranes and is, therefore, an ingredient widely exploited by the pharmaceutical and cosmetic industry among others (Mishra et al. 2018, Zhao et al. 2018). In the reason of its high demand, menthol mint essential oil production has been the second greatest worldwide, and second one only to citrus fruits considered as juice industry sub-products (Baser & Buchbauer 2010, Bizzo et al. 2009, Kothari 2005). According to Jain (2017) total global production of mint essential oil was 48,000 tons between 2016-2017, of which India contributed 80%, followed by China and Brazil with 9% and 7% respectively.

Menthol mint volatiles biosynthesis exclusively occurs in the secretory cells from the capitate and peltate secretory trichomes, as well as genes involved with menthol route synthesis are expressed in these cells, making this a single site where all biochemical reactions in regarding to volatiles synthesis have occurred (McConkey et al. 2000, Sharma et al. 2003, Croteau et al. 2005, Tiwari 2016). Some works have pointed to a positive correlation between the glandular trichomes density and the essential oil yield and content (Gupta et al. 2017, Mishra et al. 2018, Souza et al. 2016). Thus, the extensive research on the cultivation and elucidation of metabolic pathway responsible for essential oil biosynthesis has made substantial contribution in research focusing on trichomes and production of essential oil (Tiwari 2016).

Availability of natural light to crop suffers little or no management in agricultural systems, however, inclusion of intensive soil practices promoted an intense dispute over this resource in intercropping systems. Light restriction compromises plant development, as well as may directly affect metabolism and relevant quantitative aspects such as volatiles production (Fernandes et al. 2013, Shafiee-Hajiabad et al. 2016, Fadil et al. 2016). In addition, there are works showing the effect of light intensity on the biomass and essential oil yield, several metabolites and glandular trichomes density, as well as the relation between them (Fernandes et al. 2013, Gupta et al. 2017, Souza et al. 2016).

In this context, this study aimed verifying if light intensities variations might promote alterations in biomass production, anatomical characteristics, nutritional aspects, influencing volatiles production and quality in menthol mint.

MATERIALS AND METHODS

General aspects and seedlings preparation

Menthol mint (*M. arvensis* L.) seedlings from IAC 701 variety provided by Linax® (Votuporanga, SP) in a greenhouse at Department of Chemistry from Universidade Federal Rural do Rio de Janeiro (UFRRJ) were cultivated. Plants were produced from shoots middle-third cuttings treated with 2% sodium hypochlorite (10 min), washed with distilled water and fixed on polystyrene support. Part from cuttings was immersed in a Hoagland and Arnon nutrient solution modified by 15 mM NO_3^--N at one-fourth strength under constant aeration. After 2 weeks, seedlings by leaves number and root size were standardized. Furthermore, a sample from plants used in this experiment to herborization was subjected and in the herbarium (RBR, Botany Department, UFRRJ) under RBR32886 code was registered.

Hydroponic system and nutrient solution

Seedlings were placed in 2 cm thick Styrofoam plates with the help of synthetic foam, and then allocated on 1.8 L pots connected by flexible caliber tubes to an electromagnetic air compressor at 65 L min^{-1} flow rate, adjusted at 45 min activity per hour. Hydroponic system was composed by twenty-four pots and in each one two plants were placed. During the experiment a Hoagland and Arnon nutrient modified solution with 2 mM NO_3^--N, 2 mM NH_4^+-N, 5 mM Ca_2^+ and 9.5 mM S at one-fourth strength were used (Hoagland & Arnon 1950).

Experiment description and treatment

The experiment was carried out in a greenhouse at Chemistry Institute (UFRRJ). The mean temperature ranged from 18°C to 31°C and the mean radiation was 543 µmol photons m^{-2} s^{-1} inside, as well as 797 µmol m^{-2} s^{-1} outside greenhouse. Three types of share cloths aiming

25, 50 and 75% radiation reduction were used, and the mean radiation was 137, 254 and 406 µmol photons m^{-2} s^{-1}, respectively. Every day nutrient solution volume and pH value were adjusted to 1.6 L and 5.8, respectively, and radiation (from 400 to 700 nm) was measured by a Basic Quantum meter QMSW from Apogee Instruments® Inc. (Logan, USA) at 9, 12 and 15 hours (local time). Nutrient solution was weekly replaced.

Fresh mass and photosynthetic pigments analyses

After 43 days, harvest was performed and roots, stems and leaves were separated and used for fresh mass analysis. Leaf samples discs with 9 mm diameter (4th node) were immersed in dimethyl sulfoxide and placed in a steam bath at 64°C for 3 hours pigment extraction (Hiscox & Israelstam 1979). The extract was used for *a* and *b* chlorophyll, and carotenoids analyses (Wellburn 1994).

Soluble fraction analysis and dry matter content

One gram from roots, stems and 4th node from leaves in 80% ethanol was separately homogenized. After chloroform partitioning, soluble fraction for determining $NH4^+$-N, NO_3^--N, free amino-N and free sugar levels (3rd and 4th nodes) were weighed and dried in a forced-air oven at 65°C for 72h (Felker 1977, Cataldo et al. 1975, Yemm & Cocking 1955, Yemm & Willis 1954, respectively), then grounded and weighed for evaluating total-N and phosphorus according to Kjeldahl method (Tedesco et al. 1995).

Nitrate reductase activity (NRA)

Nitrate reductase (NR, EC1.7.1.1) activity (NRA) in 0.2 g fresh plant tissue samples (roots, stems, 3rd and 4th nodes from leaves) from each plant according to methodology cited by literature was determined (Jaworski 1971). Samples were placed in 5 mL phosphate buffer solution (100 mM KH_2PO_4 pH 7.5, 3% *n*-propanol and 200 mM KNO_3) and kept in bath at 30°C for 60 min. Samples aliquots (0.4 mL) were combined with 0.3 mL sulfanilamide 1% in 3 M HCL and 0.3 mL *n*-naphthyl-ethylenediamine 0.02%. After 20 min, 4 mL water was added, and absorbance (540 nm) against $NaNO_2$ standard by spectrophotometry equipment was measured.

Essential oil extraction and analysis

Menthol mint essential oil was extracted by hydrodistillation in a Clevenger apparatus from 100 g fresh plant material for 30 min. Essential oil content (% w/w) was evaluated and composition by gas chromatography (GC) was analyzed. A Hewlett Packard 5890 Series II (Palo Alto, USA) with flame ionization detection and a splity/splitless injection in a 1:20 split ratio was used for separating and detecting essential oil constituents. Substances were separated by a CP-Sil-8CB (30 m x 0,25mm x 0,25µm) fused silica capillary column. Equipment operating conditions were the same ones previously published (Souza et al. 2016). Gas chromatography coupled to mass spectrometry (GC-MS) for essential oil analysis by a Varian Saturn 2000 (Palo Alto Ca) was used. Helium gas carrier flow capillary column and temperature conditions for the GC-MS analysis were the same ones described for the GC, and previously published (Souza et al. 2016). Essential oils constituent identification was based comparing their GC linear retention indexes (LRI) and their mass spectra with those ones from authentic standards [(-)- pulegone, (-)- menthone, (+)- neomenthol and (-)- menthol from Sigma – Aldrich (USA)], NIST – 2008 database and literature retention indexes (Adams 2007). LRI was obtained from a C_8-C_{40} (Fluka, Louis, USA) alkane standard solution co-injection and

calculated according to literature (van Den Dool & Kratz 1963).

Density and glandular trichome histochemistry

For anatomical study, fresh material and 70% ethyl alcohol stored samples (Johansen 1940) were selected by the Ranvier microtome in the middle-third region of the leaf blade. Section (10-12 µm) were clarified by 20% sodium hypochlorite, neutralized in 1% acetic water, washed in distilled water, stained by blue astra-safranin (Bukatsch 1972) and confectioned between lamina and laminula with 50% glycerin (Strasburger 1924). Photomicrographies were confectioned by Olympus BX-51 microscopy with Q color 5 camera capture system and Image-Pro Express software. When necessary images were edited in Corel Photo-Paint® 15 and boards in Corel DRAW® 15.

To verify different chemical compounds in trichomes and nature of fresh material from all walls, these ones were treated with Sudan IV (Johansen 1940) and Sudan Black B (Pearse 1980) for lipid groups identification; 10% Potassium Dichromate for phenolic compounds detection (Gabe 1968); 0.02% Ruthenium Red for pectic compounds (Jensen 1962); Xylidine Ponceau for protein detection (Amaral et al. 2001, Cortelazzo & Vidal 1991); Periodic Acid/Schiff Reagent (PAS) for neutral polysaccharides (Amaral et al. 2001, Cortelazzo 1992, Taboga & Vilamaior 2001). Control treatment was applied to histochemical tests according to authors mentioned above.

Experimental design and statistical

Each treatment was composed by three replicates (six plants) arranged in a completely randomized experimental design and in each pot, two plants (one replicate) were placed. Average, standard deviation (SD), standard error mean (SEM) and graphics were calculated and created in GraphPad Prism, version 6.01 (Graph Pad Software® Inc., San Diego, USA). Principal Component Analysis (PCA) and its graphics were made by PAST Program, version 3.13 (Hammer et al. 2001).

RESULTS AND DISCUSSIONS

Biomass and photosynthetic pigments

Positive linear correlation between light intensity increase and fresh mass gain of stems, leaves and root/shoot ratio was observed (Figure 1a-c), highlighting the increase in roots mass was proportionally higher than increases in aerial part mass (Figure 1c). On the other hand, negative linear correlation in regarding to light intensity and pigments concentration was observed (Figure 1d-f).

Results previously obtained have shown that fresh mass production in genus Mentha is modulated by environmental and nutritional factors (Souza et al. 2007, 2014, 2016, Fernandes et al. 2014), mainly luminosity and day and night temperatures with strong influence (Burbott & Loomis 1967, Clark & Menary 1980a). Availability of light has also influenced photosynthetic pigments concentration, since light intensity increase has usually decreased chlorophyll content, and light reduction has induced to an increase of these pigments concentration in order to enlarge light capture area (Enríquez & Sand-Jensen 2003, Zervoudakis et al. 2012).

Carotenoids are photoprotective pigments cooperating with light harvest complexes (Lichtenthaler & Buschmann 2001). However, carotenoids concentration increase was not observed with light intensity increase, in contrast in higher light intensity treatment drastic decrease in pigments concentration (Figure 1f), below values usually found in plants with C3 type metabolism was not reported.

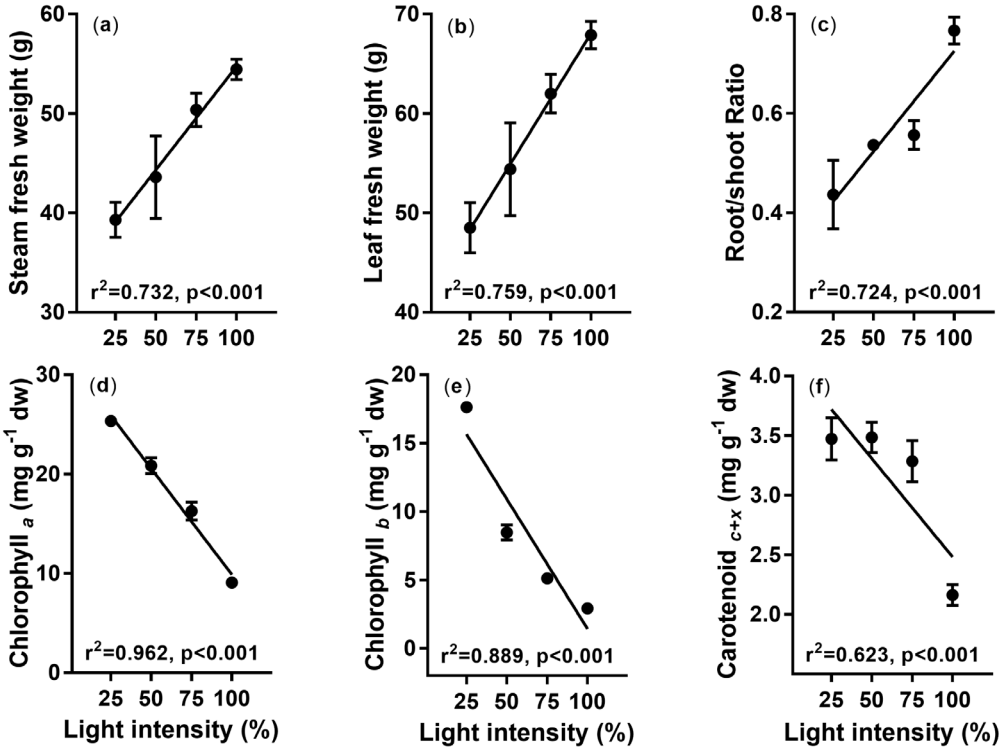

Figure 1. Stems (a) and fresh mass (b), roots/shoot ratio (c), chlorophyll a (d), chlorophyll b (e) and carotenoids (c+x) (f) contents in menthol mint for 43 days and submitted to different light intensities (25, 50, 75 and 100%). Lines correspond to linear correlations and bars to average standard deviation (n = 3).

Soluble fractions, total N and nitrate reductase activity (NRA)

Increased soluble sugars values were observed, mainly in stems due to light intensity increase (Figure 2a). On the other hand, light intensity increase resulted in NO_3^--N contents decrease in menthol mint plant parts (Figure 2b). Marked decrease in N-amino contents in stems due to light intensity was observed. Roots presented the lowest N-amino content (Figure 2c).

As shown in Figure 2, stems presented the highest soluble fractions contents. The nitrate accumulation in the stems has been observed as a reserve N strategy, likewise stem high soluble sugars content indicated that stem might be a storage place or a photoassimilates intense traffic between source and drain tissues (Hirel et al. 2007, Fageria et al. 2008).

Nitrate reductase activity (NRA) in roots was positively influenced in regarding to light intensity increase, however, NRA decreased in leaves (Figure 2e). NRA in the leaf was higher than in the roots, except at 100% light treatment, presenting higher NRA in the roots. Reduction in light availability also reduces photoassimilates one in menthol mint (Figure 2a). Thus, menthol mint more intensively reduced leaf nitrate as a result of photoassimilates supply, however, when photoassimilates levels are higher, nitrate reduction may also occur in the roots.

Most of the nitrogen in plants is assimilated into amino acids, manly composing proteins structure. However, until proteins can be synthetized, much energy is necessary for the maintenance of process involving absorption, assimilation and their own synthesis. For this reason, sugars flow to anabolic processes is essential, and it has been expected that light intensity increasing, higher total-N contents will be found. However, this result was not confirmed in this study in the reason that higher leaves total-N contents followed by roots and stems have decreased due to light intensity increase, mainly in leaves (Figure 2d).

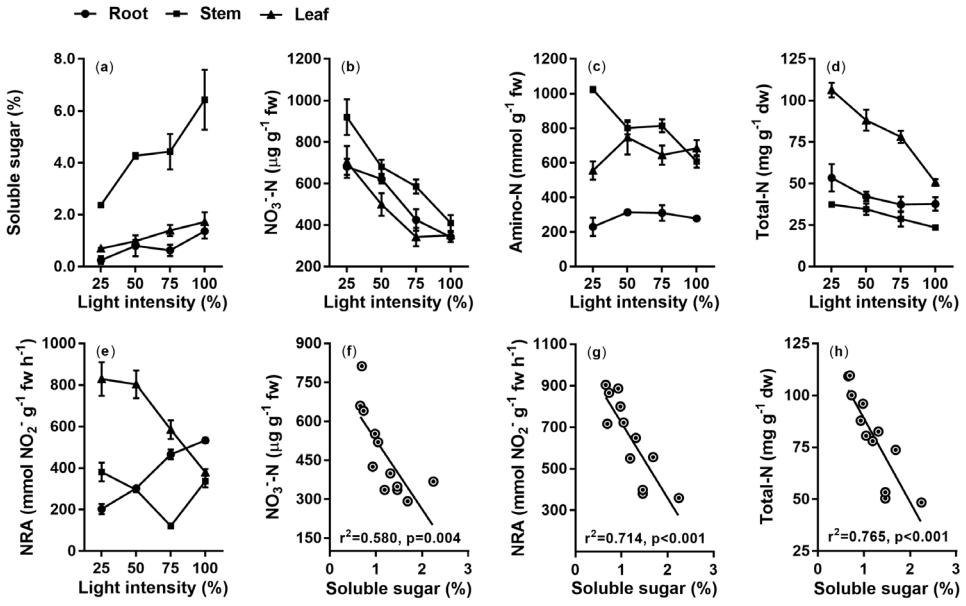

Figure 2. Soluble sugar (a), NO_3^--N (b), amino-N (c), total-N (d) contents and nitrate reductase activity (e). Correlation among NO_3^--N (f), NRA (g) and total-N (h) with soluble sugar variable. Menthol mint cultivated in hydroponic system for 43 days and submitted to different light intensities (25, 50, 75 and 100%). Lines correspond to linear correlations and bars to average standard deviation (n = 3).

Plants with low light intensity have probably invested most energy resources in proteins synthesis related to leaves light energy capture and processing (light harvest complex and photosystems), as well as absorption and roots and stems nutrients transport to the detriment of the vegetative growth (biomass) which is stimulated in higher light intensity conditions (Enríquez & Sand-Jensen 2003, Zervoudakis et al. 2012). Linear negative correlation among soluble sugars contents, NO_3^--N, NRA and total-N in menthol mint leaves was observed (Figure 2f-h). These results corroborated the thesis in regarding tissues where endergonic processes have been intense, the soluble sugar contents are lower (Fernandes 1990).

Density and glandular trichome histochemistry

Secretory trichomes found on both sides of leaves lamina were classified as two types: peltate and capitate, formed by 10 and 3 cells, respectively (Figure 3). Peltate secretory trichomes are composed by a basal cell, an unicellular peduncle, and an eight secretory cells head, covered by thin cuticle. Presence of conspicuous subcutilar space filled with secretion was observed. Thicker sidewalls from the peduncle cells and impregnated by lipid substances were reported (Figure 3a, b).

Capitate secretory trichomes consist of a basal cell, an unicellular peduncle and a globular or oval secretory head, covered by thin cuticle. Sidewalls from peduncle cells are impregnated by lipids substances and also thicker. Subcuticular space is undeveloped and filled with secretion (Figure 3c, d).

The anatomical description of the secretory trichomes from IAC 701 cultivar is in line with results presented by other authors (Shanker et al. 1999, Sharma et al. 2003, Deschamps et al. 2006, Mishra et al. 2017).

Histochemical tests showed lipid substances, proteins, phenolic substances, acid and neutral polysaccharides. Reactions were equally positive in the peltate and capitate trichomes cells protoplast (Table I). Great amount of phenolic and lipophilic substances deposition in the endoplasmatic reticulum and in peppermint vesicles justifying positive tests for these substances in cytoplasm were reported (Turner et al. 2000).

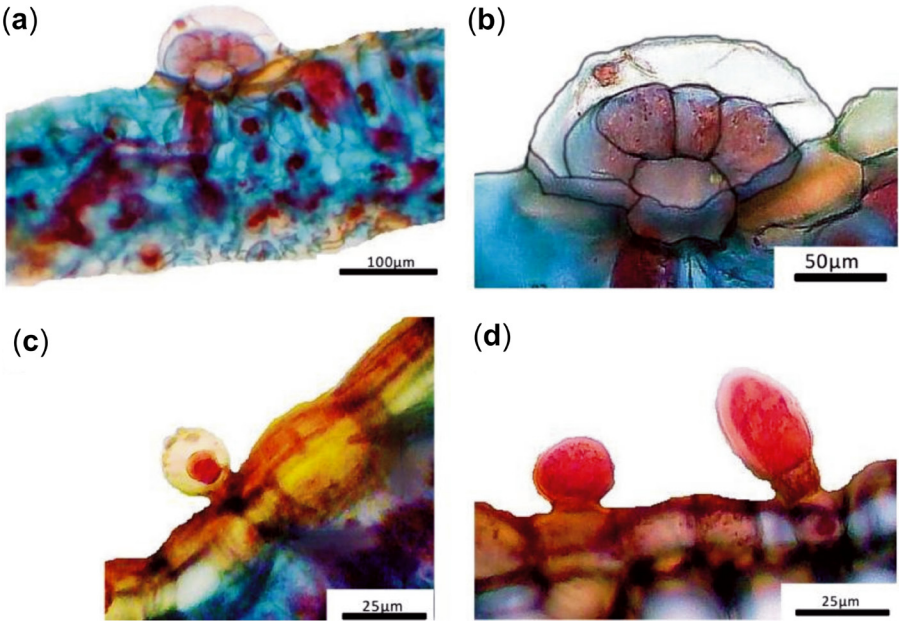

Figure 3. Peltate secretory trichome (a, b) and capitate secretory trichomes (c, d) in menthol mint leaves, IAC 701 cultivar.

In subcuticular space from both trichomes it might be observed lipophilic material drops composing the secretion. Reaction for phenolic substances is more intense in protoplasm of capitate trichomes cells, on the other hand, no proteins, pectic substances and neutral polysaccharides in peltate and capitate trichomes secretion were detected (Table I).

Results presented in Table I were also observed in *Mentha* genus (Martins 2002), as well as were divergent in regarding to pectin presence in peltate and capitate trichomes of *M. pulegium* (Rodrigues et al. 2013). However, according to some authors capitate trichomes from peppermint plants did not present any monoterpenes secretion (Brun et al. 1991), and its secretion is mainly composed by proteins, lipids and polysaccharides mixture (Turner et al. 2000).

In regarding to secretory trichomes density, 4th and 5th nodes leaves were evaluated and was verified that from 25-50% to 75-100% light intensity increase promoted significant increase in trichomes density, mainly the capitate ones, being decreased density from proximal to distal region in both foliar faces (Figure 4). No studies in the literature were observed in relation to light intensity effect over secretory trichomes density or distribution in menthol mint leaves, showing this result reported in this study as pioneer. However, Fernandes et al. (2013) verified that light intensity variations did not affect peltate and capitate secretory trichomes density in *Ocimum gratissimum* leaves.

It was also observed that secretory trichomes (peltate + capitate) density average is twice as large in the abaxial face, and capitate secretory trichome density average is three times higher than secretory peltate one in foliar surface (Figure 4). In menthol mint plant, relation of four peltate trichomes for each capitate one was described in literature (Shanker et al. 1999, Sharma et al. 2003).

For menthol mint, spearmint and water mint, higher concentration of peltate and capitate trichomes in abaxial face were reported by previously published results, however, peppermint demonstrated higher trichomes density in adaxial face (Turner et al. 2000, Martins 2002, Deschamps et al. 2006).

Presence of phenolic compounds has been cited as a group of substances of great

importance in regarding to protection against herbivores, microorganisms, ultraviolet radiation excess, and also protecting cellular protoplast maintaining its integrity when submitted to water stress (Carmelo et al. 1995, Paiva & Machado 2008, Swain 1979, Taiz & Zeiger 2006). Pharmacologically, phenolic substances present astringent, healing, antiseptic, antioxidant, vasoconstricting, hemostatic and anti-inflamatory properties (Cunha & Batista 1999, Kuklinski 2000, Rocha et al. 2002, Osadebe & Okoye 2003, Raphael & Kuttan 2003). According to Rocha et al. (2002) other functions related to phenolic substances may be present although some doubts in relation to the totality of their roles were reported. In addition to secretory structures, production and secretion sites of secondary metabolites as phenolic compounds in non-specialized cells, as the parenchyma one, were also reported. According to Barros & Teixeira (2008) secondary metabolites production in Indigofera (Leguminosae) species non-specialized cells were observed.

Mucilage, described as a mixed nature secretion, mainly consisting by acid and/or neutral heteropolysaccharides, proteins and phenolic substances present wide distribution in plants constituting colloidal solutions becoming viscous in contact with water (Gregory & Baas 1989, Priolo de Lufrano & Caffini 1981, Roshchina & Roshchina 1993). These substances may play different roles in plants, including protection of developing structures or organs water retention, carbohydrates reserves, perspiration reduction, radiation protection by dispersing or reflecting incident light, protection against herbivores, roots apex lubricant, insects capture in insectivorous plants as an adhesive in seed dispersion and in seed germination regulation (Gregory & Baas 1989, Clifford 2002, Pimentel et al. 2011, Fahn 1979, Martini et al. 2003, Roshchina & Roshchina 1993).

Mucilage in secretory trichomes from the species studied in this survey is composed by acid and neutral polysaccharides and phenolic substances. Gregory & Baas (1989) suggested that in *Althaea officinalis* different mucilage fractions presented different functions. Water reserve is made by the acid fraction reaching its production peaks in the summer, as well as carbohydrates reserve is made by the neutral one showing maximum production in the winter. Presence of phenolic compounds in mucilage, mainly tannins, has antimicrobial importance and protection against herbivores, constituting a relevant chemical protection barrier, as well (Carmelo et al. 1995, Swain 1979). Pimentel et al. (2011) identified phenolic substances presence in different morphological types of secretory structures and reported these substances might perform functions attributed by authors above cited.

Mucilage produced by menthol mint trichomes probably presented as main function desiccation as reported by Meyberg (1988) for *Nymphoides peltata* (S. G. Gmel) Kuntze secretory trichomes. In different morphological types from menthol mint secretory trichomes, diversity of chemical substances classes was reported. These substances protect in plant surface against desiccation and may perform as water and carbohydrates reserves inside the plant, in water balance, drought resistance and due to phenolic compounds presence protecting against herbivores and pathogens, as well. It was considered that reports of the use from other genus species with medicinal purposes and different classes of chemical substances evidenced in menthol mint secretory trichomes have been sufficiently strong indications for further varieties investigations, from pharmacological point of view.

Table I. Histochemistry of peltate and capitate trichomes in menthol mint leaves.

Reagents	Substances	Reactivity			
		Peltate		Capitate	
		Cytoplasm	Secretion	Cytoplasm	Secretion
Sudan IV	Lipophilic	+	+	+	+
Sudan Black B	Lipophilic	+	+	+	+
Potassium Dichromate	Phenolic	+	–	++	–
Ruthenium Red	Pectic	+	–	+	–
Periodic Acid + Schiff Reagent	Neutral Polysaccharides.	+	–	+	–
Xylidine Ponceau	Proteins	+	–	+	–

Secretion - content in the subcuticular space; negative reaction (–); positive reaction (+) and more intense reaction (++).

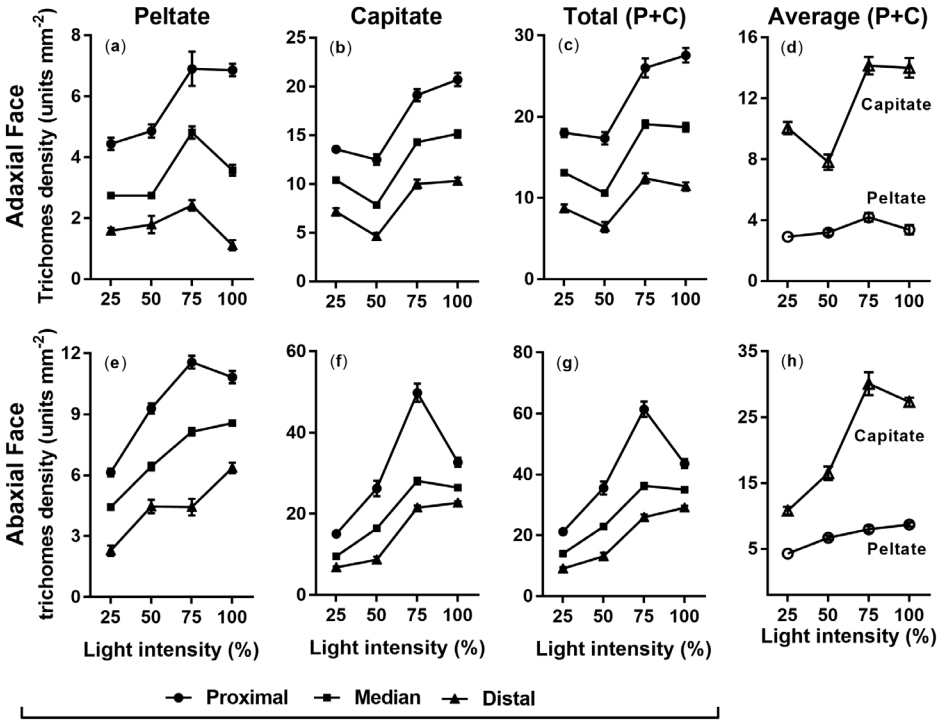

Figure 4. Secretory trichome density according to type (peltate and capitate), face (adaxial an abaxial) and region (proximal, medial and distal) in menthol mint leaves cultivated in hydroponic system for 43 days and submitted to different light intensities (25, 50, 75 and 100%). Bars represent averages standard deviation (n = 3). Density of secreted trichomes pelted on the adaxial face (a), capitate (b), total (P+C = peltate + capitate) (c) and average (P+C = peltate + capitate) (d) in menthol mint leaves. Density of secreted trichomes pelted on the abaxial face (e), capitate (f), total (P+C = peltate + capitate) (g) and average (P+C = peltate + capitate) (h) in menthol mint leaves.

Essential oil

Essential oils may present insect repellent function or be toxic to animals, operating against hervivores (Rosenthal & Janzen 1979), lipophilic secretions, mainly terpenes have generally been reported as plants chemical defensors, protecting against herbivores and pathogens (Werker & Fahn 1981, Ascensão et al. 1995, Corsi & Bottega 1999), as well as might also perform in attracting or repelling animals (Rodriguez et al. 1984).

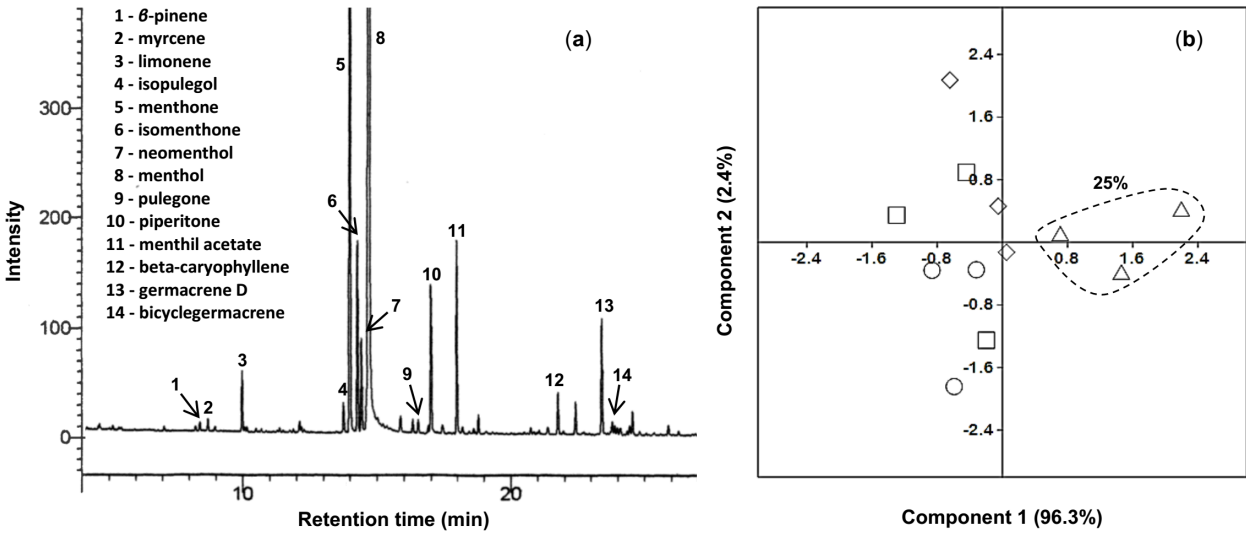

Figure 5. Total ions chromatogram, GC-MS (a) and principal component analysis, scores (b), based on the menthol mint essential oil composition cultivated at different light intensities (triangle diamond, square and circle: 25, 50, 75 and 100, respectively). Components 1 and 2 represents 96.3 and 2.4% variance, respectively.

Essential oils main substances is presented in Figure 5a. Profile related in all treatments is according to a menthol mint essential oil presenting around 70% menthol, and other *p*-mentans such as: mentone, iso-mentone, neomenthol. Significant difference among treatments was observed from menthol (68.9% minimum and 72.5% maximum) and alcohols/esters monoterpenes number (71.5% minimum and 76.1% maximum) (Figure 5a).

Main components analysis based on essential oil chemical profile allowed to only differentiate plants group submitted to the lowest light intensity, monoterpenes, alcohols and esters positively contributed for group separation (25%), as well as mentone and Ketone monoterpenes negatively contributed (Figure 5b).

Previously published results pointed to shading effect in the anticipation of essential oil maturation in peppermint plants, characterized by Ketone monoterpenes contents decrease and monoterpenes alcohols and esters contents increase (Burbott & Loomis 1967, Costa & Chagas 2014, Clark & Menary 1980b).

Essential oil content reported in this study presented moderate correlation with soluble sugars and leaves production, on the other hand, strong one with secretory trichomes density. Secretory trichomes density presented strong correlation with leaves fresh mass and soluble sugar levels (Table II).

Previously published studies pointed to the existence of a positive correlation between quantitative aspects, such as fresh leaves mass and essential oil content (Enríquez & Sand-Jensen 2003). Correlation analysis among descriptions from different menthol mint accesses showed no correlation between secretory trichomes density and essential oil, however, moderate negative correlation between secretory trichomes density and menthol contents was observed (Mishra et al. 2017). Studies relating secretory trichome density versus fresh leaves mass and soluble sugars were not found in literature.

Previously published results related secretory trichomes density increase with foliar maturation and with essential oil production menthol mint plants, as well (Shanker et al. 1999). On the other hand, Deschamps et al.

Table II. Pearson correlation coefficient among essential oil contents values and secretory trichomes total density and other associated variables to menthol mint leaves.

Associated variables	Essential oil (%)	Secretory trichomes density
Essential oil (%)	-	0.928
Secretory trichomes density	0.928	-
Fresh leaves mass	0.592	0.920
Soluble sugars	0.722	0.977

(2006) reported lower essential oil production with higher secretory trichomes density in menthol mint leaves.

Results corroborated strong influence of light availability in menthol mint plants cultivation, since increase in light intensity positively influenced fresh mass gain, which was strongly correlated to essential oil content and secretory trichomes density. Therefore, it might be suggested further studies to establish the best conditions for menthol mint cultivation, indicating crop spacing and/or ideal intercropping condition with other crops, as well.

Acknowledgments

The authors are grateful to Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ) for the scholarship and grants that supported this work. This work is part of the D.Sc. thesis of the first author in Programa de Pós-Graduação em Química (PPGQ) at Universidade Federal Rural do Rio de Janeiro (UFRRJ).

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How to cite

SOUZA MAA, BRAGA RP, SANTOS AM, ROCHA JF, CASTRO RN, FERNANDES MS & SOUZA SR. 2020. Productive, metabolic and anatomical parameters of menthol mint are influenced by light intensity. An Acad Bras Cienc 92: e20180321. DOI 10.1590/0001-3765202020180321.

Manuscript received on April 9, 2018;
accepted for publication on June 20, 2019

MARCO ANDRE A. DE SOUZA[1]
https://orcid.org/0000-0003-2173-3513

RENAN P. BRAGA[2]
https://orcid.org/0000-0003-0218-204X

ANDRÉ M. DOS SANTOS[1]
https://orcid.org/0000-0002-2397-3775

JOECILDO F. ROCHA[3]
https://orcid.org/0000-0002-0070-1291

ROSANE N. CASTRO[1]
https://orcid.org/0000-0001-8983-3786

MANLIO S. FERNANDES[2]
https://orcid.org/0000-0001-5329-6122

SONIA R. DE SOUZA[1*]
https://orcid.org/0000-0001-5206-1030

[1]Universidade Federal Rural do Rio de Janeiro/ UFRRJ, Instituto de Química, Departamento de Bioquímica, Laboratório de Bioquímica de Plantas, BR 465, Km 7, 23897-000 Seropédica, RJ, Brazil

[2]Universidade Federal Rural do Rio de Janeiro/ UFRRJ, Instituto de Química, Departamento de Solos, Laboratório de Nutrição Mineral de Plantas, BR 465, Km 7, 23897-000 Seropédica, RJ, Brazil

[3]Universidade Federal Rural do Rio de Janeiro/UFRRJ, Instituto de Ciências Biológicas e da Saúde, Departamento de Botânica, BR 465, Km 7, 23897-000 Seropédica, RJ, Brazil

Correspondence to: **André Marques dos Santos**
E-mail: amarques@ufrrj.br / amarques.ufrrj@gmail.com
**in memoriam*

Author contributions

Marco Andre A. de Souza contributed in running the experiments and laboratory work, analysis of the data and drafted the paper. Renan P. Braga contributed in running the experiments/ laboratory work, analysis of the data. André M. dos Santos contributed analysis data, discussion about plant metabolism, and preparation and critical reading of the manuscript. Joecildo Francisco Rocha contributed in histochemical analysis and discussion of the results associated with these analyzes. Rosane N. Castro contributed in extraction and essential oil analysis and discussion about chemical profile of essential oil. Manlio S. Fernandes contributed to critical reading of the manuscript and plant metabolism discussion. Sonia R. de Souza designed the study, supervised the laboratory work and contributed to critical reading of the manuscript. All the authors have read the final manuscript and approved the submission.