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# Influence of mineral nutrition on plant development and chemical composition of volatile oils *of Porophyllum ruderale* (Jacq.) Cass subspecies

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**The objective of this study was to find out the influence of soil nutrition on the composition of volatile oils of *Porophyllum ruderale* subsp. *macrocephalum* and *Porophyllum ruderale* subsp. *ruderale.* The seedlings were transplanted into pots containing sand as substrate. Every seven days, different Hoagland solutions were applied: Complete solution and solution lacking, respectively nitrogen, phosphorus, potassium, calcium, magnesium, sulfur and a control. The experiment was done in a randomized block with eight treatments and five replicates. For biometrics, height, leaf area and dry mass of shoots were analyzed. The average was compared by Tukey test (5%) probability. Aerial parts were collected and oil was extracted by hydrodistillation in Clevenger apparatus for 4 h. The major components of *P. ruderale* subsp. *ruderale* were trans-β-ocimene monoterpene, limonene and hydrocarbon undecene. As for the *P. ruderale* subsp. *macrocephalum,* the major component was monoterpene limonene and undecene hydrocarbon. The results of biometric analyses in this work showed that the two species have different growth. Treatments without nitrogen, phosphorus and the control had the lowest average and increased undecene content in the two subspecies. The chemical composition of volatile oils nutrition did not interfere significantly in their composition. Monoterpene limonene was the highest in *macrocephalum* subspecies.**

**Key words:** Brazilian medicinal plants, fertilizers.

## INTRODUCTION

Brazil is a country with a rich genetic diversity in both flora and fauna. In its flora, there have been cataloged more than 55,000 species of an estimated total of 350,000 to 550,000 species. The increased genetic diversity and chemical diversity allow the use of plants as invaluable source of bioactive compounds for the development of new drugs (Gottlieb et al., 1996). Plants produce a wide variety of organic compounds that are

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economically important, such as alkaloids, resins, tannins, volatile oils, among others (Croteau et al., 2000). The growing demand for medicinal plants constitutes a concern for conservation. Because extraction threatens more native species (Ioris et al., 1999), a more detailed study is needed on native vegetation for the recovery of species of medicinal value (Dias, 2000).

According to Di Stasi et al. (2002), among the plant species of therapeutic interest, the Asteraceae family can be considered as one of the most important. This is because the large number of plants belonging to it, such as *Baccharis trimera* (gorse) and *Achillea millefolium* (“novalgina”) in addition to arnicas, guacos and bidens, are popularly used as drugs. Most of these species are native to Brazil, while several others have acclimatized and can be found throughout Brazilian territory, which have been incorporated in traditional medicine.

*Porophyllum ruderale* (Jacq.) Cass., popularly known as arnica is a ruderal herb used in folk medicine for healing, as haemostatic (Goleniowski et al., 2006), antitinflamatória (Lima et al., 2010) and for combating leishmaniasis (Takahashi et al., 2011). There are two subspecies of *Porophyllum* used for the same purpose: *P. ruderale* subsp. *ruderale* and *P. ruderale* subsp. *macrocephalum*. The two subspecies have the same common name and share some morphological characters, such as herbaceous and similar inflorescences, attributted to the misuse of species. The chemical composition of the volatile oil of the two subspecies is different. The major compounds in *P. ruderale* subsp. *ruderale* are monoterpenes trans-β-ocimene, α-pinene and limonene, while in *P. ruderale* subsp. *macrocephalum*, there are monoterpenes limonene and β-pinene (Raggi et al., 2010). There are few works done on the subspecies, *ruderale* and *macrocephalum*. Some authors have reported the presence of β-phellandrene (Fonseca et al., 2006), sabinene (Loayza et al., 1999), limonene and 7-tetradecene (Guillet et al., 1998) as major compounds in the chemical composition of the volatile oil of *P. ruderale*.

According to Martins et al. (1995), of all the factors that can interfere with the active principles of plants, nutrition is one of those that require more attention, as excess or deficient nutrients can be directly correlated to changes in the production of active substances. Evaluating the influence of mineral nutrition on yield and volatile oil composition of *Ocimum basilicum* (basil), *Coriandrum sativum* (coriander), *Anethum graveolens* L. (dill), and *Mentha piperita* L. (Mint) and Hornok (1983) reported the occurrence of variations based on the four levels of NPK used. With increased phosphorus level, there was an increase in the volatile oil content of mint and basil and low volatile oil content of biomass and dill. High nitrogen levels increased the essential oil of mint and basil, but reduced the percentage of menthol and linalool. Also in relation to nitrogen levels, the authors observed an increase in the production of green biomass of dill and cilantro, but not seeds.

The species of the genus Porophyllum are widely used in folk medicine of South and Central America. However, there are phytotechnical works related to soil nutrition and the chemical composition of the volatile oils of species *P. ruderale* subsp*. macrocephalum* and *P. ruderale* subsp. *ruderale.* The objective of this project was, consequently, to study the effect nutrition management on plant development and production of volatile oils of arnica’s subspecies.

This work aimed to evaluate the management of mineral nutrition for plant growth, yield and chemical composition of the volatile oils of *P. ruderale* subsp. *ruderale* (Jacq.) Cass. and *P. ruderale* (Jacq.) Cass. subsp. *macrocephalum* (DC.) R. R. Johnson.

**MATERIALS AND METHODS**

Seedlings of *P. ruderale* subsp. *ruderale* and *P. ruderale* subsp. *macrocephalum* were obtained from seeds and grown in a greenhouse at the Experimental Field of Ornamental Plants Research Center, São Paulo Botanic Institute.

The seeds used were obtained from mother plants of PEFI (State Park Ipiranga Font) and sown in polystyrene trays with 128 cells, using Tropstrato HT Hortaliças® as substrate. After the appearance of four pairs of permanent leaves, the seedlings were transplanted into plastic pots of 10.5L, using sand as substrate and placed in a greenhouse equipped with microsprinkler irrigation system, in the Experimental Field Ornamental Plants. The pots were fertilized once a week with 50 ml of nutrient solutions of Hoagland and Arnon (1950), under different managements: complete solution, solution without nitrogen (-$N^{3-}$), solution without phosphorus (-$P^{5+}$), solution without potassium (-$K^{+}$), solution without magnesium (-$Mg^{2+}$), solution without calcium (-$Ca^{2+}$), solution without sulfur (-$S^{2-}$), and a control with 50 mL distilled water. The experiment was done in a randomized block design with eight treatments for each species, five replications and four vessels.

The height of both subspecies was taken with a measuring tape in centimetre from the soil surface to the highest part of the plant. The first measurement was done 30 days after transplanting, while the other was done every 20 days until the end of the experiment (130 days) after treatments application. To obtain dry mass, three individuals from each treatment were weighed on an analytical balance, placed in paper bags and oven-dried at 45°C for 15 days. The leaf area was determined in a meter Model LI-3100C expressed in square decimetres. Leaf area is defined as the average of the areas of all the leaf blades of the three subjects per treatment (Benincasa, 2003).

All data collected were submitted to Tukey test at 5% significance using the SISVAR program (Ferreira, 2002).

For extraction and identification of volatile oil, plant materials were collected at 130 days after treatment application, in the morning. The plant materials were stored in transparent bags and kept in a freezer at -22°C for later extraction in the Center for Research in Physiology and Biochemistry, São Paulo Botanic Institute. The shoots were subjected to extraction to obtain volatile oil in a continuous process by hydrodistillation using Clevenger apparatus. They were adapted to a round bottom flask of 6000 mL, for 4 h in three replicates for each treatment. After this period, the volatile oil was removed from the apparatus and stored in an

Erlenmeyer. For the complete removal of the volatile oil, solvent pentane was added. Anhydrous sodium sulfate was used to remove any water present in the volatile oil. The volatile oil containing pentane was transferred to a vial with cap. With the aid of rotatory evaporator at room temperature, pentane was completely removed leaving only the pure volatile oil. The oil mass was determined with the aid of an analytical balance. The oils were stored in cardboard box in a freezer at -22°C for further identification of its constituents.
The yield of each oil was calculated from the fresh mass (Santos et al., 2004; Pino et al., 2006; Kelen and Tepe, 2008).

To identify the constituents, the extracted volatile oils were dissolved in acetone 1:10 (v/v) and 2 uL of each diluted sample was analyzed by gas chromatography on Agilent apparatus (6890 series) HP, coupled to spectrophotometer mass with quadrupole system (Agilent 5973 Network mass Selective Detector), and 70 eV ionization energy. The capillary column used was HP-5 MS (30 m × 0.25 mm internal diameter, 0.25 thickness) under the following conditions: gun (with flow-split split/splitless) to 250°C (split ratio 1: 20), the column of heating temperature of 40 to 240°C to 3°C $min^{-1}$, 240°C for 10 min using helium as carrier gas at a pressure of 80 kPa and a linear speed of 1 ml $min^{-1}$. The total analysis time was 78 min. Nitrogen, synthetic air and hydrogen are used as auxiliary gases in the ratio of 1:1:10, respectively. The retention index (RI) was calculated on HP-5 MS column using a homologous series of n-alkanes (C5 to C30) under the same conditions for chromatographic analysis. The identification of the compounds was made by comparing mass spectra to those registered in the library database 275.

## RESULTS AND DISCUSSION

### Growth of *P. ruderale* subspecies under differents nutrient solutions of Hoagland

It was observed that the growth of plants *P. ruderale* subsp. *ruderale* and *P. ruderale* subsp. *macrocephalum* was differently influenced by the different treatments.

The mean height of the subspecies *ruderale* treated with complete nutrient solution at 130 days after treatment application (DAT) was 144.8 cm; while *macrocephalum* was 126.1 cm in the same period.

The highest mean of plant height was obtained with the complete solution treatment and treatments without $Ca^{2+}$, $Mg^{2+}$ and $S^{2-}$, respectively. Subspecies *ruderale* that had the lowest plant height mean was treated with the nutrient solution minus nitrogen ($–N^{3-}$) and the control. The means obtained with these treatments ($–N^3$ and control) signifcantly differed from the others height means.

The descending order height for *macrocephalum* subspecies was: $-Ca^{2+}$, $-Mg^{2+}$, - $S^{2-}$, complete solution, -$K^+$, $-P^{5+}$, $-N^{3-}$ and control; for subspecies *ruderale*: $-Mg^{2+}$, $-Ca^{2+}$, $-S^{2-}$, $-K^+$, complete solution, $-P^{5+}$, $-N^{3-}$ and control. Importantly for both subspecies, in $-N^{3-}$ treatment and control, the plants are not developed satisfactorily.

In this study, the two subspecies responded similarly to the treatments without nitrogen ($N^{3-}$) and the control; they had significantly lower growth compared to other treatments (Tables 1 and 2). According to Kerbauy (2008), nitrogen is essential for plant growth and its deficiency significantly influences the growth of the plant. Nitrogen is the mineral element required in larger quantities by plants and is the constituent of many components of the plant cell (Castro, 2007). It has a structural function in the plant and parts of many cell components such as proteins, nitrogenous bases, nucleic acids, enzymes, co-enzymes, vitamins and pigments. It, also, participates in processes such as ion absorption, photosynthesis, respiration, cell multiplication and differentiation (Malavolta, 2006). Its deficiency rapidly inhibits plant growth (Taiz and Zeiger, 2004).

At 130 days, the height mean of plants treated with the nutrient solutions $-Ca^{2+}$ and $-Mg^{2+}$ were not statistically different from each other and were similar to the one of the plants which received full Hoagland nutrient solution. This showed that the absence of this nutrient did not negatively affect the development of *macrocephalum* subspecies (Tables 2 and 3).

Although many studies conducted showed that the absence of calcium and magnesium dramatically affects the development of plant, their absence effect differed in the subspecies *Porophyllum* since in both species the absence of these nutrients did not affect their growth.

The application of nutrient solution minus P did not negatively affect the height, dry mass and leaf area of *P. ruderale* subsp. *ruderale* comparatively to the ones of the plants that received full nutrient solution of Hoagland. Phosphorus is involved in the storage and transfer of energy and nitrogen fixation; nitrogen is connected to the rapid development of roots, accelerated fruit ripening, increased fruiting as carbohydrate content, oils, fats and proteins (Malavolta, 2006).

According to Malavolta (2006), nitrogen is required for flowering. Without this nutrient, the emission of flowers will be retarted. This is because the limitation of nutrient reduces the assimilation of $CO_2$ through photosynthesis, and after a variable period it ends up decreasing biomass production, photosynthesis and stomatal conductance (Fujita et al., 2003).

Phosphorus has fundamental function in the life of plants. As part of compounds, it is rich in energy like adenosine triphosphate (ATP) (Malavolta, 1985), and through this energy, seeds germinates, plant performs photosynthesis, absorbs actively soil nutrients and synthesizes various organic compounds (Naiff, 2007). Phosphorus is also involved in essential functions of cellular metabolism, which acts in the synthesis of metabolites and complex molecules such as DNA, RNA and phospholipids, in the electron transport chain, redox reactions promoting the regulation of the rate of several enzymatic reactions and metabolic processes, such as respiration and photosynthesis (Alves et al., 1996).

As also noted with *ruderale* subspecies, the indicative biometric variables of *macrocephalum* subspecies growth were also significantly affected by nitrogen omission.

Symptoms of deficiency or excess of a mineral element

**Table 1.** Mean values of dry matter of shoot and leaf area of *Porophyllum ruderale* subsp. *ruderale* at 130 days after treatment application.

| Parameter | Nutrient solutions | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Full | $-N^{3-}$ | $-P^{5+}$ | $-K^{+}$ | $-Ca^{2+}$ | $-Mg^{2+}$ | $-S^{2-}$ | Control |
| Height (cm) | $158.4^{a}$ | $99.0^{b}$ | $147.8^{a}$ | $165.6^{a}$ | $168.2^{a}$ | $173.4^{a}$ | $168.4^{a}$ | $78.6^{b}$ |
| Fresh mass (g) | 217.5 | 68.6 | 158.7 | 229.0 | 220.7 | 251.4 | 198.5 | 45.6 |
| Dry mass (g) | $14.4^{a}$ | $3.6^{b}$ | $10.9^{a}$ | $14.8^{a}$ | $12.8^{a}$ | $13.7^{a}$ | $12.9^{a}$ | $2.8^{b}$ |
| Leaf area ($dm^2$/plant) | $46.25^{a}$ | $8.43^{c}$ | $33.74^{ab}$ | $44.31^{ab}$ | $29.17^{b}$ | $36.52^{ab}$ | $31.66^{ab}$ | $3.57^{c}$ |

Means followed by the same letter in the same lines do not differ significantly by Tukey test at 5% probability.

**Table 2.** Mean values of dry matter of shoot and leaf area of *Porophyllum ruderale* subsp. *macrocephalum* at 130 days after treatment application.

| Parameter | Treatments | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Full | $-N^{3-}$ | $-P^{5+}$ | $-K^{+}$ | $-Ca^{2+}$ | $-Mg^{2+}$ | $-S^{2-}$ | Control |
| Height (cm) | $151.8^{ab}$ | $58.4^{c}$ | $134.0^{b}$ | $139.4^{ab}$ | $164.4^{a}$ | $160.8^{a}$ | $157.2^{ab}$ | $44.2^{c}$ |
| Fresh mass (g) | 168.3 | 58.1 | 221.1 | 179.6 | 209.6 | 239.8 | 191.5 | 27.7 |
| Dry mass (g) | $6.8^{a}$ | 0.7c | $3.9^{b}$ | $7.3^{a}$ | $6.3^{ab}$ | $7.4^{a}$ | $7.6^{a}$ | $0.1^{c}$ |
| Leaf area ($dm^2$/plant) | $12.23^{a}$ | $2.23^{bc}$ | $8.53^{ab}$ | $10.68^{a}$ | $11.40^{a}$ | $14.50^{a}$ | $9.37^{ab}$ | $0.98^{c}$ |

Means followed by the same letter on the lines do not differ significantly by Tukey test at 5% probability.

are similar in all plant species (Leal and Prado, 2008). According to Pozza et al. (2001), nutrients perform specific functions in plant metabolism, not only in influencing its growth, but also its production. Thus, a nutrient in abnormal levels can damage production causing a nutritional stress (Deon, 2007). The dry matter production data of shoot and leaf area of the subspecies *Porophyllum* are shown in Table 2.

Analyzing the variables, for the shoot dry matter production was differently affected by the treatments. The plants treated with the nutrient solution minus nitrogen and the control had significantly lower shoot dry matter of 3.6 and 2.8 g, respectively. As well, the leaf area was differently influenced by the treatments: the control had the lowest mean of 3.57 $dm^2$ per plant and the highest mean of 46.25 $dm^2$ per plant was obtained with the complete nutrient solution.

Table 3 shows the height mean values of shoot dry matter and leaf area of the subspecies *macrocephalum*. Significant differences were observed between the treatments: the treatments without potassium ($-K^{+}$), calcium ($-Ca^{2+}$) and magnesium ($-Mg^{2+}$) and the complete nutrient solution had the highest mean leaf area.

## Chemical composition of the essential oils of the aerial parts of *P. ruderale* subsp. *ruderale* as influenced by different nutrient solution of Hoagland

The chemical composition of volatile oils of *P. ruderale* subsp. *ruderale* is presented in Table 3. Twenty-two compounds were identified out of thirty compounds. The yield of volatile oil was 0.07% for treatments with complete Hoagland solution and without sulfur ($-S^{2-}$); 0.06% for treatments without phosphorus ($-P^{5+}$), potassium ($-K^{+}$), magnesium ($-Mg^{2+}$) and calcium ($-Ca^{2+}$); 0.03% for the control treatment and 0.02% for treatment without nitrogen ($-N^{3-}$). Monoterpenes were the predominant constituents, ranging from 48.5% (treatment $-N^{3-}$) to 95.7% ($-Ca^{2+}$ treatment). The other major compounds were trans-β-ocimene, limonene and hydrocarbon. The different nutrient solution handlings also influenced the chemical composition of the volatile oils except limonene; even the treatments with a greater influence negative ($-N^{3-}$ and control) remained constant at about 20% of the total chemical composition. The major compound was trans-β-ocimene, accounting for about 62%, on average, of the total chemical composition of the volatile oil of subspecies *ruderale* for almost all treatments except N in which it was 22.2%. The content of the hydrocarbon undecene was greater about eight times in the N treatment (18.9%) and six times in the control treatment (13.7%), relative to the treatment with addition of the complete Hoagland solution.

The presence of sesquiterpenes was also higher in the treatment without nitrogen. The hydrocarbon of undecene was also observed by Raggi et al. (2012) who compared the chemical composition of volatile oils of green material collected at 270 days and aging plant collected at 300 days. Undecene increased by about 10 times in plants of

**Table 3.** Chemical composition of the essential oils of the aerial parts of *P. ruderale* subsp. *ruderale* grown under different managements of nutrient solution.

| Compound | Retention time | Kovats index | Peak area (%) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Full | $-N^{3-}$ | $-P^{5+}$ | $-K^{+}$ | $-Mg^{2+}$ | $-Ca^{2+}$ | $-S^{2-}$ | Control |
| Pentanone | 6.39 | 822 | 0.8 | 1.8 | 1.0 | 0.7 | 0.8 | 0.8 | 0.9 | 0.8 |
| α-Pineno | 9.87 | 931 | 0.6 | | - | 0.4 | 0.2 | 0.2 | 0.2 | |
| Sabinene | 11.61 | 971 | 0.9 | - | 0.6 | 0.8 | 0.8 | 0.8 | 0.8 | - |
| β-Pineno | 11.79 | 975 | 7.9 | 3.2 | 6.5 | 7.3 | 7.6 | 7.4 | 6.8 | 3.9 |
| Myrcene | 12.42 | 988 | 0.1 | - | - | - | - | - | - | - |
| Limonene | 14.28 | 1029 | 20.5 | 21.3 | 24.0 | 20.8 | 21.0 | 20.4 | 20.7 | 17.4 |
| Cis-β-Ocimene | 14.62 | 1036 | 1.0 | - | 0.9 | 1.0 | 1.0 | 0.9 | 0.9 | 0.8 |
| Trans-β-Ocimene | 15.13 | 1046 | 62.9 | 22.2 | 59.0 | 62.5 | 64.3 | 65.1 | 62.9 | 58.6 |
| Undecene | 17.28 | 1087 | 2.2 | 18.9 | 3.7 | 2.5 | 2.4 | 2.5 | 3.0 | 13.7 |
| Decanol | 22.71 | 1199 | - | 1.0 | - | - | - | - | - | - |
| NI | 22.80 | 1201 | 0.5 | 2.8 | 1.1 | 0.8 | 0.5 | 0.4 | 0.8 | - |
| NI | 25.57 | 1264 | - | 2.2 | 1.0 | 0.2 | - | - | 0.2 | - |
| Silfineno | 28.88 | 1338 | - | 0.6 | - | - | - | - | - | - |
| NI | 30.47 | 1374 | - | 1.7 | 0.5 | - | - | - | - | - |
| α-Isocomeno | 30.72 | 1379 | - | 0.9 | - | - | - | - | - | - |
| β-Isocomeno | 31.66 | 1399 | - | 0.8 | - | - | - | - | - | - |
| β-Cariofileno | 32.04 | 1408 | 0.5 | 2.0 | - | 0.4 | - | - | 0.4 | 1.2 |
| NI | 33.94 | 1449 | - | 1.4 | - | - | - | - | - | - |
| NI | 34.15 | 1453 | - | 0.6 | - | - | - | - | - | - |
| NI | 34.25 | 1455 | - | 0.3 | - | - | - | - | - | - |
| Germacrene-D | 34.60 | 1462 | 0.5 | - | - | 0.6 | 0.2 | - | 0.4 | - |
| Biciclogermacrene | 35.19 | 1474 | 0.4 | - | - | 0.6 | 0.5 | 0.5 | 0.5 | 1.1 |
| NI | 36.88 | 1512 | - | 1.6 | 0.3 | - | - | - | - | - |
| NI | 37.64 | 1536 | - | 1.9 | - | - | - | - | - | 0.7 |
| NI | 37.91 | 1544 | | 1.2 | - | - | - | - | - | - |
| NI | 38.16 | 1551 | - | 0.3 | - | - | - | - | - | - |
| Spathulenol | 38.39 | 1558 | 1.2 | 5.1 | 1.5 | 1.3 | 1.0 | 0.9 | 1.4 | 0.6 |
| Caryophyllene oxide | 38.58 | 1564 | - | 1.3 | - | - | - | - | - | - |
| NI | 38.70 | 1568 | - | 0.3 | - | - | - | - | - | - |
| Phthalate * | 48.64 | 1774 | - | 0.8 | - | - | - | - | - | - |
| Hexadecanoic acid | 52.19 | 1957 | - | 3.6 | - | - | - | - | - | 1.3 |
| Linoleic acid | 57.34 | 2106 | - | 1.0 | - | - | - | - | - | - |
| Linoleate ethyl | 57.50 | 2108 | - | 1.2 | - | - | - | - | - | - |
| Total | - | - | 99.5 | 85.8 | 97.1 | 99.0 | 99.6 | 99.6 | 98.9 | 99.3 |
| Monoterpenes | - | - | 94.6 | 48.5 | 92.0 | 93.5 | 95.6 | 95.7 | 93.2 | 81.5 |

**Table 3.** Contd.

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Sesquiterpenes | - | - | 2.6 | 10.7 | 1.5 | 3.0 | 1.6 | 1.4 | 2.7 | 2.8 |
| Outhers | - | - | 2.2 | 26.6 | 3.7 | 2.5 | 2.4 | 2.5 | 3.0 | 15.0 |
| Yield (%) | - | - | 0.07±0.01 | 0.02 | 0.06±0.01 | 0.06±0.01 | 0.06±0.00 | 0.06±0.00 | 0.07±0.02 | 0.03 |

NI: Not identified; *Poisoning; Full: complete solution; -$N^{3}$-: solution with no nitrogen; -$P^{5+}$: solution with no phosphorus; -$K^{+}$: solution with no potassium; -$Mg^{2+}$: magnesium absence solution; -$Ca^{2+}$: solution absence of calcium; -$S^{2-}$: solution with no sulfur; Control: treatment with no nutrient solution. Amounts related to the average of three extractions except –$N^{3-}$ and control. Income calculated based on the fresh weight + standard deviation.

300 days old relative to the green material. Though, there are no studies on the hydrocarbon of undecene in different plant growth stages.

For the biometric analysis, treatments that led to nutritional deficiency in the chemical composition of volatile oils were -$N^{3-}$ and control.

## Chemical composition of the essential oils of the aerial parts of *P. ruderale* subsp. *macrocephalum* as influenced by different nutrient solution of Hoagland

The yield of volatile oil of *macrocephalum* subspecies was lower compared to that of *ruderal*e. For -$K^{+}$ and -$Ca^{2+}$ treatments, the yield was 0.04%; for complete and -$S^{2-}$, -$Mg^{2+}$ treatments, the yield was 0.03% and for phosphorus, the yield was 0.02%. In -$N^{3-}$ and control treatments, there was no much oil yield due to the small amount of oil obtained. As noted in the biometric analysis, -$N^{3-}$, -$P^{5+}$ and control treatments did not produce sufficient biomass to conduct the volatile oil extraction in three repetitions, as the other treatments.

The chemical composition of volatile oils of *macrocephalum* subspecies is described in Table 4. Of the twenty compounds, thirteen were identified. Monoterpene limonene was the major constituent in almost all treatments except control, whose main component was undecene hydrocarbon (29%). The other treatments showed, on average, 15% of hydrocarbon. -$Mg^{2+}$ treatment found to have the highest percentage of limonene (70.5 %) and the lowest content of this constituent was found in the control treatment (27.4%). Treatments without potassium and sulfur had the highest percentages of myrcene (18.2 and 18.4%, respectively), compared to the same constituent in the other treatments on average, 8%, except the control treatment containing only 2.6%. The sesquiterpenes content was higher in the control (12.4%) compared to other treatments, 2.7% on average.

The chemical composition of volatile oil of *Porophylum* is well reported in the literature. However, little is said about the studied subspecies. The levels of the major constituents were very different. Rondón et al. (2008), working with *P. ruderale* collected in Venezuela, identified a mixture of limonene and β-phellandrene as the main components of the chemical composition of volatile oils. For plants collected in Bolivia, the main constituent was sabinene (Loayaza et al., 1999). Fonseca et al. (2006) identified β-phellandrene monoterpene in plants collected in Minas Gerais. In Ceará, Neto et al. (1994) said the major compound was limonene (74%).

Many articles report that geographical and climatic variations are responsible for the variation in the chemical composition of volatile oils. However, besides these, there is also correct identification of the material to be studied and the quality of tillage. Raggi et al. (2014), working with both species, concluded that the major components in subspecies *macrocephalum* and *ruderale* were limonene and E-β-ocimene, respectively. They noted that the difference in the two species is not caused by climate, but by genetic issues.

Monoterpene limonene present mainly in subspecies *macrocephalum* has different biological activities such as antimicrobial and anti-inflammatory. These activities are related to its medicinal use (Souza et al., 2003; Lorenzi and Matos, 2008).

## Conclusion

Both species did not have proper growth development with nitrogen deficiency, phosphorus and control. The chemical composition of volatile oils nutrition did not interefere significantly in their composition. Monoterpene limonene was the highest in *macrocephalum* subspecies,

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interest.

**Table 4.** Chemical composition of the essential oils of the aerial parts of P. ruderale subs. macrocephalum grown under different managements.

| Compound | Retention time | Kovats index | Peak area (%) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Full | $-N^{3-}$ | $-P^{5+}$ | $-K^{+}$ | $-Mg^{2+}$ | $-Ca^{2+}$ | $-S^{2-}$ | Control |
| Pentanone | 6.39 | 822 | 0.7 | 1.1 | 0.8 | 0.9 | 0.7 | 0.8 | 0.6 | 1.1 |
| Sabinene | 11.61 | 971 | 0.5 | 0.8 | 0.9 | 0.3 | 1.1 | 0.8 | 0.8 | - |
| Beta Pinene | 11.80 | 975 | 0.6 | | 0.8 | 1.3 | 0.7 | 0.6 | 1.5 | - |
| Myrcene | 12.42 | 988 | 8.8 | 8.9 | 6.9 | 18.2 | 7.7 | 8.2 | 18.4 | 2.6 |
| Limonene | 14.27 | 1028 | 58.0 | 68.3 | 64.8 | 54.8 | 70.5 | 66.5 | 54.1 | 27.4 |
| Trans Beta Ocineme | 15.09 | 1046 | 3.7 | 4.4 | 3.2 | 3.7 | 4.0 | 3.8 | 3.9 | 2.4 |
| Undecene | 17.28 | 1087 | 17.2 | 14.0 | 18.6 | 15.2 | 12.1 | 15.0 | 16.3 | 29.0 |
| Decano | 22.72 | 1199 | 0.2 | - | - | 0.2 | - | - | 0.2 | 1.2 |
| NI | 30.43 | 1373 | 02 | - | - | - | - | - | - | 1.2 |
| Alfa Isocomeno | 30.71 | 1379 | 2.0 | 1.1 | 1.3 | 1.4 | 1.0 | 1.2 | 1.4 | 5.7 |
| Beta Isocomeno | 31.66 | 1399 | 1.1 | - | 0.7 | 0.6 | 0.5 | 0.7 | 0.7 | 3.1 |
| Beta Caryophyllene | 32.05 | 1408 | 0.3 | - | - | - | - | - | - | 1.3 |
| Germacrene-D | 34.60 | 1462 | 1.3 | - | 0.7 | 0.7 | 0.6 | 0.7 | 0.7 | 2.3 |
| NI | 36.11 | 1492 | 0.5 | - | - | 1.1 | - | 1.2 | - | 2.3 |
| NI | 48.64 | 1774 | - | - | - | - | - | - | - | 1.4 |
| NI | 49.79 | 1790 | - | - | - | - | - | - | - | 1.2 |
| Hexadecanoic Acid | 52.18 | 1957 | 1.3 | - | - | 0.3 | - | - | - | - |
| NI | 62.65 | 1882 | 0.7 | 1.4 | 1.4 | 1.4 | 1.2 | 0.5 | 1.5 | - |
| NI | 62.70 | 2156 | 2.5 | - | - | - | - | - | - | 13.6 |
| NI | 65.93 | 2184 | 0.5 | - | - | - | - | - | - | 4.2 |
| Total | - | - | 95.6 | 98.6 | 98.7 | 97.5 | 98.8 | 98.3 | 98.5 | 76.1 |
| Monoterpenes | - | - | 72.3 | 83.5 | 77.4 | 79.2 | 84.5 | 80.7 | 79.3 | 33.6 |
| Sesquiterpenes | - | - | 4.6 | 1.1 | 2.7 | 2.6 | 2.1 | 2.6 | 2.8 | 12.4 |
| Outhers | - | - | 18.7 | 14.0 | 18.6 | 15.7 | 12.1 | 15.0 | 16.4 | 30.1 |
| Yeld (%) | - | - | 0.03±0.01 | * | 0.02 | 0.04±0.01 | 0.03±0.01 | 0.04±0.00 | 0.03±0.00 | * |

NI: Not identified; Full: complete solution; $-N^{3}$: solution with no nitrogen; $-P^{5+}$: solution with no phosphorus; $K^{+}$: potassium absence solution; $-Mg^{2+}$: absence of magnesium solution; $-Ca^{2+}$: solution absence of calcium; $-S^{2-}$: solution with no sulfur; Control: treatment with no nutrient solution. Values referring to the average of three extractions except $–N^{3-}$, $-P^{5+}$ and witness. Income calculated based on the fresh weight + standard deviation.

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