

## Full Length Research Paper

## Increased phenylpropanoid accumulation in essential oils of *Petroselinum crispum* at different Sulphur dilutions

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This study aimed to evaluate the influence of different doses of *Sulphur* on yield and chemical composition of parsley essential oil. *Sulphur* was applied at different dilutions in the centesimal scale ranging from 0c (control), 6, 12, 18, 24 to 30c. The experiment was carried out in the field, and for each treatment there were five replications, that is, five vases with one plant each. The evaluated parameters were: plant height (cm), fresh biomass of the aerial parts and root (g), yield and chemical composition of the essential oil. The oil was obtained by hydrodistillation and analyzed by gas chromatography and mass spectrometry (GC/MS). The results indicated that 12c dilution caused aerial part inhibition; there was an inhibitory effect on roots at 12 and 30c dilutions, and 6, 12 and 30 c dilutions inhibited fresh biomass when compared to the control (0c) and the other dilutions. Regarding EO yield, an increase in yield (%) at 18c (0.150 ± 0.01) and 30c (0.180 ± 0.01) dilutions occurred when compared to control (0c) (0.017 ± 0.01). The essential oil presented phenylpropanoids as the main class in its composition and apiole and myristicin as major compounds in all evaluated treatments. The 12c dilution allowed an increase in apiole (96.24%) and decrease in myristicin (3.76%). However, myristicin has an increase in treatments 6c (14.65%), 18c (10.46%), 24c (13.66%) and 30c (14.80) when compared to the control 0c (5.99%). In conclusion, a stimulating or inhibitory response occurred in the evaluated parameters depending on the utilized dilutions. The increase in apiole and myristicin is considered an economically important factor because they are substances utilized by the pharmaceutical, food and agricultural industries.

**Key words:** Parsley, apiole, myristicin, phenylpropanoids, yield, dilutions.

## INTRODUCTION

*Petroselinum crispum* (Mill.), (Apiaceae) popularly known as parsley, garden parsley, chopped greens or rock parsley, stands out as one of the most consumed herbs worldwide. In Brazil, parsley is cultivated mainly by small rural producers to be sold as herbs, it can be used fresh or dehydrated, and its most consumed parts are leaves, petioles and seeds (Petropoulos et al., 2009). The plant reproduces better in sandy-clayey soil with high content of organic matter, good fertility and pH between 5.8 and 6.8 (Heredia et al., 2003). Some cultures are highly perishable, have short cycle (three months), and are susceptible to several pathogens (Rodrigues et al., 2010). Besides, its *in natura* utilization, it is used to obtain essential oil (EO) which has two main phenylpropanoids: apiole and myristicin in its composition. Almost 90% of the EO may consist of apiole; however, EO's chemical composition can be altered due to several factors like plant genotype, location of plant cultivation, type of utilized soil, harvesting time, luminosity, altitude, temperature and water management (Kurowska and Galaska, 2004; Morais, 2009; Borges et al., 2016). Parsley EO is highly valued in the international market and broadly used in the food industry to aromatize meats, canned foods and processed vegetables. In agriculture, studies have shown its potential to control bovine tick (Camilotti et al., 2015).

It is estimated that 3% of the world production of essential oil is utilized by the pharmaceutical industry, 34% by the beverage industry and 63% by food and cosmetic industries. Brazil is one of the four greatest world producers of EO alongside India, China and Indonesia, but it suffers from chronic problems such as the absence of maintenance of oil quality standard (Bizzo et al., 2009). In the production of EO, cultivation type is one of the factors that directly interfere in the quality since the industries in the market of aromatic and culinary plants have greater interest in acquiring a product that has a standard in its chemical composition, which is the case of the cosmetic industry where the alterations in the oil may interfere in the perfume aroma (Craveiro and Queiroz, 1993). According to Bastos (2007), the utilization of agrochemicals (chemical fertilizers and agrotoxic products) directly affects the chemical composition of essential oils, and then alters their quality, making their utilization unviable. Therefore, organic fertilization has been recommended for medicinal, aromatic and culinary plants. According to Corrêa Junior and Scheffer (2009), medicinal plants from organic cultivation are more resistant to pests and diseases, reducing the need of phytosanitary control.

In agriculture, the indiscriminate utilization of chemical

products has increased the resistance of insects, pests, phytopathogenic fungi, weeds and environmental contamination. Natural products (EO, plant vegetal extracts and substances obtained from high dilutions) have been used as an alternate method within organic agriculture, and can be utilized as bioherbicides (Isman, 2006; Mapeli et al., 2010; Otoni et al., 2013). Thus, the Brazilian Ministry of Agriculture and Supply recommends the utilization of high dilutions in the production of organic foods (Brazil, 1999). These substances have been utilized by several segments in agriculture, including germination (Hamman et al., 2003), seedling production (Bonfim et al., 2008), pest control (Almeida et al., 2003), and has been promoting general improvement of the plant, production of more vigorous seeds, production variation, yield of active principles (phytochemicals), adaptation to adverse conditions and productivity, reducing the use of fertilizers and chemical pesticides, and resulting in greater safety to the product and the producer (Andrade et al., 2001; Shah-Rossi et al., 2009). Among these substances, *Sulphur* stands out because it presents a broad action spectrum to improve the general aspect of a plant in order to strengthen natural defenses, increasing resistance under nutritional, unfavorable climatic conditions to its development; it also shows development of essential oil and aerial parts (Bonato and Silva, 2003; Oliveira et al., 2014).

*Sulphur* is an essential element for plant development and is found in the macronutrient group like nitrogen, potassium, calcium and magnesium (Bonato and Silva, 2003). This element is a key nutrient for plant development because it participates in the synthesis of amino acids such as cysteine, cysteine and methionine which are needed for protein formation and are also fundamental in the development of certain vitamins, glutathione and co-enzyme (Coleman, 1966). In soils, 90% of sulfur is found as organic form but most of the cultivable soils present deficiency of this element, mainly the soils with little organic matter and those submitted to burning, which causes the loss of this element by volatilization. Throughout time, this deficiency also occurred due to the substitution of sulfur as fungicide and insecticide (Coleman, 1966; Oliveira et al., 2014). The objective of several producers and the aim of researchers (Toledo et al., 2015) has been to search for alternatives in the production of healthy foods without agrochemical residues and smallest possible impact on the environment, produced in an economically and socially sustainable manner. Thus, the goal of this study was to evaluate the utilization of different *Sulphur* dilutions in the development of parsley and its EO yield and chemical

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**Table 1.** Analysis of pH, macro and micronutrients of soil utilized in parsley cultivation.

pH and Macronutrients									
pH (CaCl <sub>2</sub> )	Al <sup>3+</sup>	H <sup>+</sup> + Al <sup>3+</sup>	cmoldm <sup>-3</sup>			mg dm <sup>-3</sup>			
			Ca <sup>2+</sup>	Mg <sup>2+</sup>	K <sup>+</sup>	SB	CTC	P	C
5.57	0	2.63	5.00		0.32	13.46	15.35	199.70	13.25
Micronutrients									
V	Ca	Mg (%)	K	Ca/Mg	Ca/K	Mg/K			
87.68	57.02	21.94	1.34	1.94	42.66	21.94			

**Ca, Mg, Al:** extracted with KCl 1 mol L<sup>-1</sup>; **P, K:** extracted with Mehlich 1; **H + Al:** SMP method; **C:** Walkley and balckmethod; **SB:** Sum of Bases. **Source:** Laboratório Solo Fértil (Umuarama-PR).

composition.

## MATERIALS AND METHODS

### Botanical Identification

The experiment was carried out in the Medicinal Garden of Paranaense University UNIPAR Campus II, in the city of Umuarama, northwestern region of Paraná State, Brazil S23° 46,225' and WO 53° 16,730', 391 m of altitude, from October 2012 to February 2013. The plant was identified by Professor Ezilda Jacomasi of the Department of Pharmacy of Paranaense Univeresity (UNIPAR), Paraná State, Brazil. A voucher specimen is deposited at the UNIPAR Herbarium (code number 192).

### Culture implementation

The soil utilized in the experiment was collected at 20 cm of depth from experimental beds and homogenized; its chemical and physical properties were determined (Table 1). The soil was poured into 30 10 L polypropylene vases (27 cm of height and 25.5 cm of diameter), without any nutritional correction or addition of fertilizers or agricultural pesticides. A 180 cell seed tray (3.5 cm<sup>2</sup>) was used with four seeds per cell. After 30 days, three cells with four seedlings each were transplanted equidistantly to each vase. After 40 days, trimming was done and one plant per vase remained.

### Preparation of treatments

Each treatment consisted of a *Sulphur* dilution in the centesimal scale (c) (10<sup>-2</sup> g/mL). Five dilutions were tested: 0c (control), 6c (10<sup>-12</sup>), 12c (10<sup>-24</sup>), 24c (10<sup>-48</sup>) and 30c (10<sup>-60</sup>) (Bonato et al., 2009). For the control, distilled water was used. The dilutions were obtained according to the phamacotechnique for insoluble drugs in the centesimal scale whose technique is described in the Brazilian Homeopathic Pharmacopoeia (Brasil, 2011). After dilution preparation (6, 12, 18, 24 and 30c), they were diluted in distilled water at the proportion of 0.1/100mL. The diluted treatments were applied to the seedlings in the seed tray and to the plants in the vases. 2 mL of dilutions were weekly applied to each cell of the seed tray for four weeks until transplantation to vases. 250 mL of dilutions were applied weekly to the vases until the end of the plant cultivation (Bonato and Silva, 2003). The experiment was carried out in the field, and for each treatment there were five replications,

that is, five vases with one plant each. The evaluated parameters were: plant height (cm), fresh biomass of the aerial part and root (g), yield and chemical composition of the essential oil. The plant height was calculated by measuring the distance between the basis and the stem apex using a measuring tape.

### Obtention and yield of parsley essential oil

At the end of the vegetative cycle, during flowering, which was characterized by the emergence of panicles (Lorenzi and Matos, 2008), the aerial parts at 2 to 3 cm above the soil were removed, fresh mass (g) was measured and submitted to the extraction of essential oil by hydrodistillation for 2 h (Petropoulos et al., 2008; Petropoulos et al., 2010). The oil was removed from the equipment using *n*-hexane, filtered in anhydrous sodium sulfate and stored under refrigeration (3°C). The essential oil was measured in (g/g) of fresh weight.

### Chemical identification of parsley essential oil by GC/MS

The essential oil were analysis were carried out in a gas chromatograph (Agilent 7890 B) coupled to mass spectrometer (Agilent 5977 A), equipped with a DB-5 capillary column (30 m x 0.25 mm x 0.25 µm, Agilent, PA, USA) using the following conditions: injector temperature of 250°C, injection volume 1 µL at injector (split 2:1; 2.1 mL min<sup>-1</sup>), initial column temperature of 40°C, and gradually heated to 300°C at 6°C min<sup>-1</sup> rate. The carrier gas (helium) flow was set at 4.8 mL minute<sup>-1</sup>. The temperature transfer line was held at 250 and 320°C, respectively. The mass spectra were obtained in the range of 40 to 500 (*m/z*) provided through scan mode with solvent delay time of 3 min, and the compounds were identified based on comparison of their retention indices (RI) obtained using various *n*-alkanes (C7-C26). Also, their EI-mass spectra were compared with the Wiley library spectra and the literature (Adams, 2007).

### Chemicals and reagents

All used solvents were of analytical grade. Homologous series of C7 to C25 *n*-alkane and *n*-nonadecane reference chemicals used for identification were obtained from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). All other chemicals, all of analytical grade, that is, anhydrous sodium sulfate, *n*-hexane, used in this study were purchased from Merck (Darmstadt, Germany), unless stated

**Table 2.** Influence of *Sulphur* at 6c, 12c, 18c, 24c, 30c and 0c dilutions on *P. crispum* considering the development of aerial parts (cm), root development (cm), fresh mass (g) and the yield of essential oil (%) as parameters.

Treatments	Development of aerial parts(cm)	Root Development (cm)	Aerial part (fresh biomass) (g)	Yield of essential oil (%)
0c	25.58 ± 0.65 <sup>a</sup>	44.00 ± 4.50 <sup>ab</sup>	36.73 ± 4.04 <sup>a</sup>	0.017 ± 0.01 <sup>a</sup>
6c	22.42 ± 0.65 <sup>bd</sup>	46.00 ± 1.73 <sup>a</sup>	21.73 ± 2.10 <sup>b</sup>	0.035 ± 0.01 <sup>b</sup>
12c	18.25 ± 1.04 <sup>c</sup>	34.16 ± 0.60 <sup>c</sup>	24.20 ± 1.13 <sup>bc</sup>	0.024 ± 0.01 <sup>c</sup>
18c	24.29 ± 0.44 <sup>ad</sup>	38.83 ± 2.16 <sup>ac</sup>	24.36 ± 2.33 <sup>bd</sup>	0.150 ± 0.01 <sup>d</sup>
24c	22.29 ± 1.52 <sup>d</sup>	38.33 ± 2.02 <sup>bc</sup>	30.07 ± 1.42 <sup>acd</sup>	0.050 ± 0.01 <sup>e</sup>
30c	25.38 ± 0.85 <sup>a</sup>	34.00 ± 1.52 <sup>c</sup>	12.87 ± 0.55 <sup>b</sup>	0.180 ± 0.01 <sup>f</sup>

\*The values for average and standard deviation were obtained from sextuplicate. Averages followed by the same letters in the column do not differ among themselves by TLSD test,  $p \leq 0.05$ .

otherwise.

### Statistical analysis

The experimental was completely randomized. Prior analysis of variance (ANOVA) excluded outliers on plant fresh mass using the box plot method. Data were subjected to one-way ANOVA using general linear model with mixed-effects and balanced design, considering each *Sulphur* dilution as one treatment, and compared to Duncan's test ( $P \leq 0.05$ ) using SPSS version 16.0 for Windows (SPSS Inc., Chicago, IL, USA). To comply with ANOVA assumptions, data were previously checked by Levene's test.

## RESULTS AND DISCUSSION

The analysis of the soil utilized for the parsley cultivation is found in (Table 1). The soil granulometric analysis indicated that it contains a mixture of coarse sand (28.60%), fine sand (50.60%), silt (1.40%) and clay (19.40%), and it was classified as sandy soil according to the Normative Ruling 2, from October 9, 2008 of the Brazilian Ministry of Agriculture, Livestock and Supply (Brasil, 2008). The soil also presented high fertility with base saturation ( $V=87.68\%$ ). Therefore, the results showed soil with pH within normality, between 5.57 and 6.0, and with appropriate contents of micro and macronutrients for the development of *P. crispum*, which according to Andrade et al. (2010), better develops in soils with pH (5.8 to 6.8), high content of organic matter and good fertility. The choices of 6, 12, 18, 24 and 30c *Sulphur* dilutions are based on the fact that the physiological responses do not depend only on the utilized substance, but on the utilized dilutions. The effect of *Sulphur* treatment with different dilutions on the development of aerial parts (cm), root development, fresh mass of aerial part (g), and EO yield (%) are described in (Table 2). The results indicated that there was significant inhibition at 12c dilution on the development of aerial parts when compared to the control (0c), 6, 24 and 30c dilutions. Regarding root development, dilutions of 12 and 30c presented inhibiting effect on root when compared to

the control (0c); as for fresh biomass, it was observed that dilutions of 6, 12 and 30c inhibited biomass compared to control (0c) and dilution of 24c. The different *sulphur* dilutions significantly influenced essential oil yield (Table 2). The greatest yields were provided by 30c ( $0.180 \pm 0.01$ ) and 18c ( $0.150 \pm 0.01$ ) dilutions when compared to control (0c) ( $0.017 \pm 0.01$ ). The influence of *Sulphur* dilutions on the yield of essential oil was also observed by Bonato et al. (2009) who carried out an experiment with *Mentha arvensis* which resulted in increase of the yield at 6c dilution and a reduction at dilutions 12, 24 and 30c.

The influence of different *Sulphur* dilutions on the chemical composition of parsley essential oil is described in (Table 3), where 48 compounds were identified; the predominant class was phenylpropanoids: (0c) (66.60%), 6c (71.20%), 12c (100.00%), 18c (63.00%), 24c (76.41%) and 30c (75.77%). Apiole was the major component in all treatments: (0c) (60.27%), 6c (56.55%), 12c (96.24%), 18c (51.91%), 24c (62.75%) and 30c (60.97%). The second main compound in quantity in EO was myristicin presented (0c) (5.99%), 6c (14.65%), 12c (3.76%), 18c (10.46%), 24c (13.66%) and 30c (14.80%). These results are in accordance to Borges et al. (2016) who found apiole, followed by myristicin as the main compound of oil from parsley cultivated in the same region of this experiment when the plant was submitted to different water stress management levels. The treatment 12c presented a different behavior from the others, by increasing apiole (96.24%) and decreasing myristicin (3.76%), showing the other compounds just as traces within the chromatograms as it can be observed in (Figure 1). However, myristicin increased in treatments 6c (14.65%), 18c (10.46%), 24c (13.66%) and 30c (14.80%) when compared to the control 0c (5.99%). Similarly, treatments 18c and control (0c) presented, besides apiole and myristicin, *p*-cymene (4.63; 3.70%) and *p*-cimenene (4.78; 5.73%), respectively, as major compounds in their composition; they were also different from treatments 6, 24 and 30c which had myristicin (14.65, 13.66 and 14.80%) and apiole (56.55, 62.75 and

**Table 3.** Chemical composition of *Petroselinum crispum* essential oil under different Sulphur dilutions (0, 6, 12, 24 and 30c).

Peak	<sup>A</sup> Compounds	RI <sup>a</sup>	0c	6c	12c	18c	24c	30c	Methods of identification
1	Sabinene	1038	t	t	t	t	t	t	a,b
2	$\beta$ -pinene	1039	t	t	t	t	t	t	a,b
3	p-cymene	1073	4.63	2.90	t	3.70	1.94	2.66	a,b
4	p-cymenene	1119	4.78	2.50	t	5.73	3.07	2.56	a,b
5	Citronellal	1120	1.79	1.69	t	0.39	1.13	2.97	a,b
6	p-methyl-acetophenone	1124	t	t	t	t	t	t	a,b
7	p-cymen-8-ol	1125	0.68	1.36	t	2.30	t	2.14	a,b
8	Myrtenal	1126	t	t	t	t	t	t	a,b
9	o-cumenol	1129	0.71	0.50	t	0.50	0.43	1.00	a,b
10	Phellandral	1130	t	t	t	0.64	t	t	a,b
11	cis-piperitol	1137	2.40	2.50	t	1.40	1.25	2.39	a,b
12	Cinnamaldehyde	1201	t	t	t	t	t	t	a,b
13	Carveol	1209	1.44	1.30	t	t	1.64	t	a,b
14	Cuminal	1226	0.33	t	t	0.48	t	t	a,b
15	2-bornanone-oxime	1235	t	t	t	t	t	t	a,b
16	Carvenone	1244	0.73	0.76	t	0.76	0.55	t	a,b
17	trans-myrtanol	1258	t	t	t	t	t	t	a,b
18	Cuminol	1260	0.47	t	t	0.75	t	t	a,b
19	Thymol	1263	0.64	0.86	t	0.81	0.53	t	a,b
20	Carvacrol	1272	0.82	0.89	t	1.14	t	t	a,b
21	n.i.	1301	0.69	0.46	t	t	t	t	a,b
22	cis-Citral	1333	0.77	t	t	0.60	0.52	t	a,b
23	Limonene aldehyde	1344	0.70	0.77	t	0.85	0.70	t	a,b
24	$\alpha$ -copaene	1378	t	t	t	t	t	t	a,b
25	$\beta$ -elemene	1395	1.21	1.76	t	1.54	1.81	2.11	a,b
26	$\alpha$ -guaiene	1447	0.74	t	t	1.10	0.55	t	a,b
27	cis- $\beta$ -farnesene	1460	t	2.26	t	0.37	t	t	a,b
28	Germacrene D	1472	2.06	t	t	2.01	2.22	2.61	a,b
29	trans- $\beta$ -ionone	1488	t	t	t	t	t	t	a,b
30	trans- $\alpha$ -farnesene	1501	t	t	t	0.38	t	t	a,b
31	$\beta$ -bisabolene	1504	t	t	t	0.63	t	t	a,b
32	Germacrene A	1507	0.65	0.75	t	0.76	0.76	1.41	a,b
33	Myristicin	1515	5.99	14.65	3.76	10.46	13.66	14.80	a,b
34	$\delta$ -cadinene	1518	t	t	t	t	t	t	a,b
35	trans- $\gamma$ -bisabolene	1527	0.89	1.01	t	0.96	1.00	1.73	a,b

Table 3. Contd.

36	Elimicin	1544	t	t	t	0.34	t	t	a,b
37	5 $\beta$ , 7 $\beta$ H, 10 $\alpha$ – Eudesm-11-en-1 $\alpha$ -ol	1562	t	t	t	0.47	t	t	a,b
38	Caryophyllene oxide	1572	0.36	0.73	t	0.55	t	t	a,b
39	Carotol	1588	t	0.85	t	0.90	t	t	a,b
40	Apiole	1680	60.27	56.55	96.24	51.91	62.75	60.97	a,b
41	Caryophyllene acetate	1681	t	t	t	0.40	t	t	a,b
42	Acethophenone (2,4,6-trimethyl-3-methyl)	1682	0.34	t	t	0.85	0.69	t	a,b
43	Benzyl benzoate	1757	0.52	0.85	t	0.73	0.72	t	a,b
44	$\beta$ -costol	1761	0.67	1.12	t	0.92	0.85	t	a,b
45	$\beta$ -bisabolene	1776	0.84	1.09	t	1.40	0.75	t	a,b
46	n.i.	1792	0.42	t	t	0.47	t	t	a,b
47	n.i.	1817	0.74	0.80	t	0.85	0.59	t	a,b
48	Farnesyl acetate	1828	0.93	0.75	t	1.42	1.89	2.65	a,b
49	Phytol	1922	0.32	t	t	0.39	t	t	a,b
50	Hexadecanoic acid	1937	0.67	t	t	0.65	t	t	a,b
51	4-methoxy-stilbene	1988	0.34	t	t	0.29	t	t	a,b
<b>Compounds groups (%)</b>									
	Monoterpene hydrocarbons	-	9.41	5.20	0.00	9.43	5.01	6.22	
	Oxygenated monoterpenes	-	11.46	12.09	0.00	8.00	6.32	7.50	-
	Sesquiterpene hydrocarbons	-	5.55	5.78	0.00	7.75	5.34	7.86	-
	Oxygenated sesquiterpenes	-	3.03	4.59	0.00	5.96	2.19	0.00	-
	Oxygenated diterpenes	-	0.32	0.00	0.00	0.39	0.00	0.00	-
	Phenylpropanoids	-	66.60	71.20	100.00	63.00	76.41	75.77	-
	Others	-	3.86	2.06	0.00	4.15	3.73	2.65	
	<b>Total Identified (%)</b>	-	<b>98.38</b>	<b>99.66</b>	<b>100.00</b>	<b>98.68</b>	<b>98.41</b>	<b>100.00</b>	

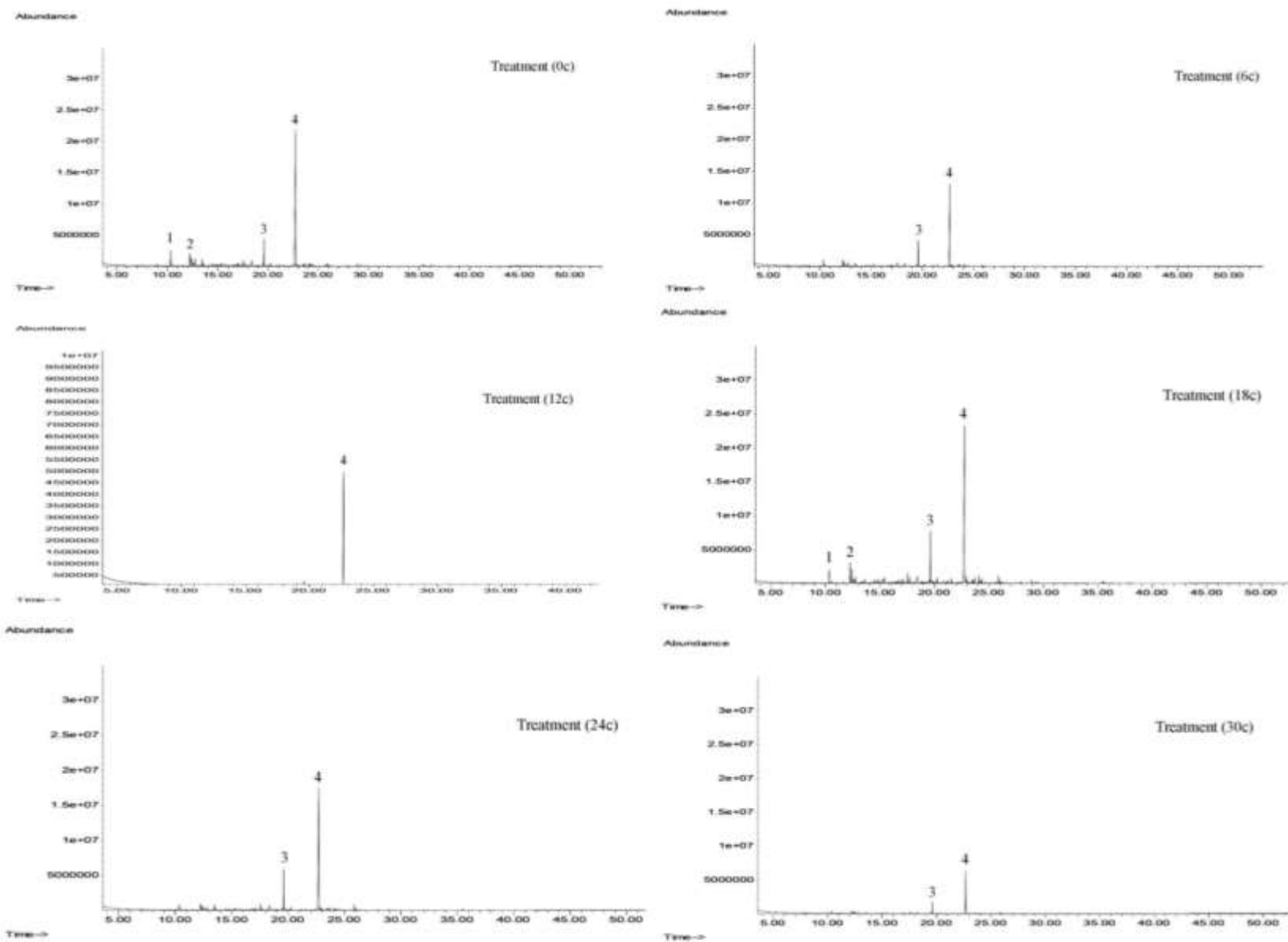
<sup>a</sup>Identification based on retention index; <sup>b</sup>identification based on comparison of mass spectra; n.i.: unidentified compound; t: trace; <sup>A</sup>Compound listed in order of elution from a DB-5 column.

60.97%), respectively, as their major compounds as shown in Figure 1. This oscillation in the chemical composition of the essential oil in function of sulphur dilution was also observed by Oliveira et al. (2014) who utilized the same Sulphur dilutions and the same method utilized in

this experiment in their studies.

The authors found alterations in the chemical composition of *Ocimum basilicum* L. EO. They verified that Sulphur substantially increased the concentration of the major compound, linalool: 12c (33.14%), 6c (30.92%), 30c (27.13%), 24c

(23.86%) and 18c (19.68%) when compared to control (0c) (7.41%). However, the treatments caused decrease of  $\alpha$ -bergamotene: 18c (7.47%), 24c, (6.68%), 6c (5.47%), 30c (5.22%) and 12c (5.06%), when compared to control (0c) (15.45%) According to Dayenas et al. (1988) and Bonato



**Figure 1.** Chromatograms obtained by GS of major compounds of *Petroselinum crispum* essential oil extracted from leaves and submitted to treatments with *Sulphur* under dilutions (0, 6, 12, 18, 24 and 30c). 1 = p-cymene; 2 = p-cymenene; 3 = Myristicin; 4 = Apiole.

and Silva (2003), *Sulphur* frequently causes different effects, depending on the application. In certain cases, an increase may occur, whereas in others, inhibitions may be reported within a specific physiological variable. Such behavior is still not fully explained. One of the hypotheses, based on biodynamic agricultural data, is that such behavior may be related to the existing rhythmic movements in nature. Another hypothesis, based on experimental data, is that such behavior is due to similarity between the applied homeopathic drug and the organism (Bonato, 2007).

The high apiole concentration in 12c dilution and the increase in myristicin in dilutions 6, 18, 24 and 30c makes *Sulphur* utilization at high dilutions extremely important in vegetal nutrition when the objective is to isolate plant compounds. According to the studies done by Reyes-Munguía et al. (2012), apiole presented antioxidant, chemo preventive and antifungal potential. Studies done by Camilotti et al. (2015) on parsley EO and its major compound, apiole, found high activity against bovine tick and *Aedes aegypti* larvae. Regarding myristicin, studies proved its action as anti-depressing, anti-inflammatory and antioxidant (Tisserand and Balacs, 1994); chemo preventive agent (Zheng et al., 1992), larvicide against *A. aegypti* (Marston et al., 1995), insecticide against *Spilarctia obliqua* (Srivastava et al., 2001). The results found in this study demonstrated that *Sulphur* and ultra-dilutions represent an important tool within organic agricultural practices in order to stimulate plant to increase the concentration of active principles that are interesting for several agroindustrial segments.

## Conclusion

The application of different *Sulphur* dilutions to parsley provided alterations in the development of aerial parts, roots, fresh mass, yield and chemical composition of parsley essential oil. Pheynilpropanoids are a predominant class in parsley essential oil; apiole and myristicin were the major compounds in all evaluated treatments. The 12c dilution allowed an increase in apiole (96.24%) and decrease in myristicin which increased in treatments 6, 18, 24 and 30 c. The increase in apiole and myristicin is considered an economically important factor because they are substances utilized by the pharmaceutical, food and agricultural industries.

## Conflict of Interests

The author(s) have not declared any conflict of interests.

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## REFERENCES

- Adams RP (2007). Identification of essential oil components by gas chromatography/mass spectroscopy. *J. Am. Soc. Mass Spectrom.* 6(8):671-672.
- Almeida AA, Galvão JCC, Casali VWD, Lima ER, Miranda GV (2003). Tratamentos homeopáticos e densidade populacional de *Spodoptera frugiperda* (J.E.Smith,1797) (*Lepidoptera: Noctuidae*) em plantas de milho no campo. *Rev. bras. milho sorgo.* 2:1-8.
- Andrade FMC, Casali VWD, Cupertino MC (2010). Seleção de indicadores, monitoramento e sistematização de experiências com homeopatia em unidades agrícolas familiares. *Rev Bras Agroecol.* 5:61-73.
- Andrade FMC, Casali VWD, De Vita B, Cecon PR (2001). Efeito de homeopatia no crescimento e na produção de cumarina em chambá (*Justicia pectoralis* Jacq.). *Rev. Bras. Plant Med.* 4:19-28.
- Bastos CN (2007). Fungitoxicidade *in vitro* e ação protetora e curativa de óleos essenciais contra *Crinipellis pernicioso*. *Braz. J. Agric. Sci.* 47:137-148.
- Bizzo HR, Hovell AMC, Rezende CM (2009). Óleos essenciais no Brasil: aspectos gerais, desenvolvimento e perspectivas. *Quim. Nova.* 32:588-594.
- Bonato CM (2007). Homeopatia em modelos vegetais. *Cult. Homeopat.* 21:24-28.
- Bonato CM, Silva EP (2003). Effect of the homeopathic solution *Sulphur* on the growth and productivity of radish. *Acta Sci. Agron.* 25:259-263.
- Bonato CM, Telles de PG, Reis B (2009). Homeopathic drugs *Arsenicum album* and *Sulphur* affect the growth and essential oil content in mint (*Mentha arvensis* L.). *Acta Sci. Agron.* 31:101-105.
- Bonfim FPG, Martins ER, Rodrigues das DRG, Barbosa CKR, Casali VWD, Honório ICG (2008). Use of homeopathic *Arnica montana* for the issuance of roots of *Rosmarinus officinalis* L. and *Lippia alba* (Mill) N.E.Br. *Int J High Dilution Res.* 7:113-117.
- Borges IB, Cardoso BK, Silva ES, Oliveira JS, Silva RF, Rezende CM, Gazim ZC (2016). Evaluation of performance and chemical composition of *Petroselinum crispum* essential oil under different conditions of water deficit. *Afr. J. Agric. Res.* 11:480-486.
- Brasil (1999). Ministério da Agricultura, Pecuária e Abastecimento. Normas para a produção de produtos orgânicos vegetais e animais. Diário Oficial da República Federativa do Brasil, Brasília-DF, Instrução Normativa nº 007, de 17 de maio.
- Brasil (2008). Ministério da Agricultura, Pecuária e Abastecimento. Classificação dos tipos de solo. Diário Oficial da República Federativa do Brasil, Brasília-DF, Instrução Normativa nº 2, de 9 de outubro.
- BRASIL Farmacopéia homeopática brasileira (2011). *Parte I – Métodos Gerais*. São Paulo: Atheneu.
- Camilotti J, Ferrarese L, Bortolucci WC, Gonçalves JE, Takemura OS, Piau Junior R, Gazim ZC (2015). Essential oil of parsley and fractions to *in vitro* control of cattle ticks and dengue mosquitoes. *J. Med. Plants Res.* 9:1021-1030.
- Corrêa Junior C, Scheffer MC (2009). Boas práticas agrícolas (BPA) de plantas medicinais, aromáticas e condimentares. Instituto Paranaense de Assistência Técnica e Extensão Rural – EMATER.
- Coleman R (1966). The importance of sulfur as a plant nutrient in world crop production. *Soil Sci.* 101:203-239.
- Craveiro AA, Queiroz DC (1993). Óleos essenciais e química fina. *Quim. Nova* 16:224-228.
- Dayenas E, Beauvais F, Amara J, Oberbaum M, Robinzon B, Miadonna A, Tedeschi A, Pomeranz B, Fortner P, Belon P, Sainte-Laudy J, Poitevin B, Benveniste J (1988). Human basophil degranulation triggered by very dilute antiserum against IgE. *Nat.* 333:816-818.
- Hamman B, Konning G, Lok KL (2003). Homeopathically prepared giberellic acid and barley seed germination. *Homeopathy* 92:140-144.
- Heredia ZNA, Vieira MC, Weismann M, Lourenção ALF (2003). Produção e renda bruta de cebolinha e de salsa em cultivo solteiro e consorciado. *Hortic. Bras.* 21:574-577.
- Isman MB (2006). Botanical insecticides, deterrents, and repellents in



- modern agriculture and an increasingly regulated world. *Ann. Rev. Entomol.* 51:45-66.
- Kurowska A, Galaska I (2004). Essential oil composition of the parsley seed of cultivars marketed in Poland. *Flavour Frag. J.* 21:143-147.
- Lorenzi H, Matos FJA (2008). *Plantas medicinais no Brasil – nativas e exóticas*. 2. ed. Instituto Plantarum: Nova Odessa.
- Mapeli NC, Santos RHS, Casali VWD, Cremon C, Long L (2010). Repellency of *Ascia monuste orseis* (Latreille) (Lepidoptera: Pieridae) exposed a homeopathic solutions. *Rev. Agrarian.* 3:119-125.
- Marston A, Hostettmann K, Msonthi JO (1995). Isolation of antifungal and larvicidal constituents of *Diplophium buehanani* by centrifugal partition chromatography. *J. Nat. Prod.* 58:128-130.
- Morais LAS (2009). Influência dos fatores abióticos na composição química dos óleos essenciais. *Hort. Bras.* 27:4050-4063.
- Oliveira AM, Moura GM, Zardeto G, Cardoso BK, Alves AAR, Tsukui A, Gazim ZC (2014). Effect of sulphur on yield and chemical composition of essential oil of *Ocimum basilicum* L. *Afr. J. Agric. Res.* 9:688-694.
- Ootani MA, Aguiar RW, Ramos ACC, Brito DR, Silva JB, Cajazeira JP (2013). Use of essential oils in agriculture. *J. Biotechnol. Biodivers.* 4:162-174.
- Petropoulos SA, Daferera D, Polissiu G, Passam HC (2008). The effect of water deficit stress on the growth, yield and composition of essential oils of parsley. *Sci. Hortic.* 115:393-397.
- Petropoulos SA, Daferera D, Polissiu G, Passam HC (2009). Effect nitrogen-application rate on the biomass, concentration, and composition of essential oils in the leaves and roots of three types of parsley. *J. Plant Nutr. Soil Sci.* 172:210-215.
- Petropoulos SA, Daferera D, Polissiu G, Passam HC (2010). Effect of freezing, drying and the duration of storage on the composition of essential oils of plain-leafed parsley [*Petroselinum crispum* (Mill.) Nym. spp. *neapolitum* darnet] and turnip-rooted parsley [*Petroselinum crispum* (Mill.) Nym. spp. *tuberosum* (bernh.) Crov.]. *Flav. Frag. J.* 25:28-34.
- Reyes-Munguía A, Zavala-Cuevas D, Alonso-Martínez A (2012). Perejil (*Petroselinum crispum*): compuestos químicos y aplicaciones. *Tlatemoani Rev. Acad. de Investigación* 11.
- Rodrigues AAC, Maia CB, Silva GS, Sardinha DHS (2010). Incidência de fungos associados à apiaceas em Imperatriz - MA. *Trop. Plant Pathol.* 35:289-293.
- Shah-Rossi D, Heusser P, Baumgartner S (2009). Homeopathic treatment of *Arabidopsis thaliana* plants infected with *Pseudomonas syringae*. *Sci. World J.* 9:320-330.
- Srivastava S, Gupta MM, Prajapati V, Tripathi AK, Kumar S (2001). Insecticidal Activity of Myristicin from *Piper mullesua*. *Pharm Biol.* 39:226-22.
- Tisserand R, Balacs T (1994). Nutmeg: A review of the toxicology and pharmacology of nutmeg and its essential oil. *Int. J. Aromather Winter.* 6:28-38.
- Toledo MV, Stangarlin JR, Bonato CM (2015). Control of early blight and effect on growth variables of tomato plants by using homeopathic drugs. *Summa Phytopathol.* 41:126-132.
- Zheng GQ, Kenney PM, Lam LKT (1992). Myristicin: A potencial cancer chemopreventive agent from parsley leaf oil. *J. Agric. Food Chem.* 40:107-110.