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Influence of Organic and Mineral Soil Fertilization on Essential Oil of *Spilanthes oleracea* cv. Jambuarana

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ABSTRACT

In the present study, the composition of essential oil of leaves and inflorescences of jambu (*Spilanthes oleracea* . Jambuarana), under organic manuring and mineral fertilization, was studied. Jambu plants show important chemical properties and their production has been addressed for the extraction of the essential oils for cosmetics industries, due to their pharmacological properties. The experimental area of treatments contained urea as mineral fertilizer (120 g m^2), applied twice and organic fertilizer (8 kg m^2), applied at the planting. Jambu leaves and flowers were harvested twice: the first at 90 days after seedling transplantation and at the opening of the flower buds. Branches were cut at 7 cm from the soil, thus new branches can bud for the accomplishment of the second crop which happened 40 days after the re-budding. The essential oil was analyzed by gas chromatography coupled with mass-spectrometry. According to our results the most representative compounds were trans-caryophyllene, germacrene-D, 1-dodecene, spathulenol and spilanthol (a compound presenting anesthetic properties) occurring in inflorescences. Fertilization procedure does not affect the content and the quality of the essential oil in Jambu plants.

Key words: Composition of essential oil, trans-caryophyllene, germacrene-D, spilanthol, Asteraceae

INTRODUCTION

Jambu plants (*Spilanthes oleracea* cv. Jambuarana) (Asteraceae), also known as “cresson du Pará”, belong to the Asteraceae family, native of Amazonian region, living in tropical climate and widely consumed in the Northern area of Brazil, mainly in Para State. The plant presents important chemical properties, waking up the interest of pharmaceutical industries, mainly for the presence of its active compound, spilanthol. Others properties, such as a larvicidal action, of the powder of *Spilanthes mauritiana* (Ohaga *et al*., 2007a), on two mosquito species (*Anopheles gambiae* and *Culex quinquefasciatus*) were described (Ohaga *et al*., 2007b).

Early phytochemical studies on *Spilanthes* were carried out by Jacobson (1957), reporting spilanthol as an amide (an N-isobutylamide), occurring also in other species of *Spilanthes* genre (Ramsewak *et al.*, 1999). Studies showed that flowers and leaves of *Spilanthes* contain amino acids (Mondal *et al.*, 1998; Peiris *et al.*, 2001), alkaloids (Peiris *et al.*, 2001) and flavonoids (Makambila-Koubemba *et al.*, 2011).

Some reports considered the effects of soil conditions, determined by agricultural history and crop management, on plants production of primary and secondary metabolites (Upadhyay and Patra, 2011). The knowledge of how soil conditions and essential oil production are related may contribute not only to understand the way plants respond to environmental stresses but may also provide useful information to design sustainable agricultural strategies, since sustainable production could be achieved by decreasing the amount of inputs needed to sustain high yields in poor environments (De la Fuente *et al.*, 2003).

The intensive use of chemical fertilizers has side effects in polluting underground water, destroying microorganisms and insects, making plants more susceptible to the attack of diseases and reducing soil fertility (Malik *et al.*, 2009). Soil fertilization, among several other factors, represents the main aspect affecting plant chemical composition. External factors can affect the content of active molecules and many researchers reported the influence of agriculture procedure on plant content of many substances. Altitude, photoperiod, temperature, incidence of solar light and conditions related to soil composition are examples, beyond the effects of environmental cultivation conditions. For instance, variations of these factors can influence the biomass and the quality of essential oils in aromatic plants, as already verified, studying the effect of nitrogen addition in the production of alkaloids. Ren *et al.* (2001) studied antioxidant activity in vegetable species produced following two different cultivation procedures (organic manuring and mineral fertilization), observing significant differences between treatments, demonstrating that plant nutrition can alter secondary metabolism. Concerning the production of essential oil in lemongrass (*Cymbopogon citratus*), several papers reported that the build-up of the essential oil was promoted by organic cultivation and in relation to biomass, this kind of cultivation procedure increased the production of the aerial and radicular plant parts (Costa *et al.*, 2008).

Companies using natural products, such as pharmaceutical and cosmetics industries, have been opting for plants cultivated without mineral addition (organic production), in particular because of consumers take increasing care about health and it is clear the effect of fertilization procedure on environment conservation.

Thus, we asked if the influence of fertilization can increase the phytochemicals, as the volatile substances of *Spilanthes oleracea* cv. Jambuarana. Therefore, the objective of this work is to determine the chemical composition of essential oil in jambu leaves and inflorescence, under organic manuring and mineral fertilization.

MATERIALS AND METHODS

Experimental: Jambu plants (*Spilanthes oleracea* cv. Jambuarana) were cultivated during the spring (January/June) with an average temperature of 21 °C, under organic manuring and mineral fertilization, within the approximate area corresponding to Greenwich geographical coordinates latitude 22° 44' 50" S and longitude 48° 34' 00" W, at an altitude around 765 m. The mean annual rainfall is 1534 mm, the mean rainfall during the wettest month (January) is around 240 mm and 38 mm is observed during the driest period (July and August). A sprinkler irrigation device was used for water supply. Irrigation was carried out twice a day.

The seeding was performed in January 2008, in polystyrene trays of 128 cells containing the commercial substrate Plantimax®. The seeds of jambu jambuarana were obtained from producers in the state of Para (Brazil) with good features phytosanitary field production. The germination occurred at seven days after the seeding and transplanting to 40 days after seeding.

The planting was done manually in 6 m^2 (for each experiment: organic and mineral), placing 18 plants per row and each plot consisted of five lines. The spacing used was 20×25 cm which allowed the cultivation of 90 plants. The experiment presented as doses of mineral fertilizer treatment (120 g m^{-2} urea) applied in two times (15 days after planting) and the doses of organic fertilizer (8 kg m^{-2} castor pomace) applied at planting.

The first crop of jambu was collected in the morning, 90 days after seedling transplantation (seedling was cultivated on Plantimax® substrate), at the opening of the flower buds. Branches were cut at 7 cm from the soil, thus new branches can bud for the accomplishment of the second crop which happened 40 days after the re-budding.

Picked plants (leaves and flowers) were transferred in the laboratory and washed for the elimination of sludge. The first wash was accomplished with clean water in order to eliminate residues from the field. The second wash was carried out with a solution containing 150 mg L^{-1} of sodium hypochlorite and in order to reduce microbial contamination, plant material was further immersed in this solution for 5 min. The third and the fourth washes were in distilled water. Leaves and inflorescences were separated, weighted and transferred in a greenhouse, under forced circulation at 40°C, until a constant weight was reached.

Isolation of essential oils: In order to obtain essential oils, leaves and inflorescences were finely ground and separately subjected to distillation in a Clevenger apparatus for 2 h. Obtained oils were separated from the aqueous phase by liquid-liquid extraction with dichloromethane.

Separation and quantification: Separation and quantification (normalization area method) of the substances were carried out by gas chromatography (Shimadzu, GC-2010), equipped with a flame ionization detector, using a DB-5 (J and W Scientific; 30 m×0.25 mm×0.25 μm) capillary column. Substance identification was accomplished by gas chromatography coupled to a mass spectrometer (Shimadzu, QP-5000), operating by electron impact (70 eV), equipped with a capillary column OV-5 (Ohio Valley Specialty Chemical, Inc., 30.0 m x 0.25 mm x 0.25 μm). CG analyses in were carried out as follows: injector and detector temperatures were 220 and 230°C, respectively. Helium was used as carrier gas at a flow rate of 1.0 mL min^{-1} with 1:20 split ratio. Samples were diluted (1 μL mL^{-1}) in ethyl acetate and the injection volume was 1 μL. Temperature program was: 60-120°C at 6°C min^{-1}, 120-195°C at 3°C min^{-1} and 195-240°C at 8°C min^{-1}.

Identification of volatile components: Compound identification was carried out by comparison of the mass spectra with the system database CG-MS (Nist. 62 lib.) and retention times according to Kortvelyesi *et al.* (1995). Substance retention times were obtained by co-injection of the essential oil with a mixture of hydrocarbons (C_9-C_{24}), using the equation of Van Den Dool and Kratz (1963).

Statistics: Data were subjected to statistical analysis using SAS program package. The one-way analysis of variance (ANOVA) followed by Duncan multiple range test were used and the differences between individual means were deemed to be significant at $p<0.05$.

RESULTS AND DISCUSSION

The CG-MS analysis of essential oil of *Spilanthes oleracea* coming from the first crop resulted in the identification of twelve compounds (Table 1, Fig. 1). Total identified substances were 89.05% in inflorescences and 85.04% in leaves. As regards the second crop (cultivar), in plants cultivated under mineral fertilization, 90.82% were identified in inflorescences and 94.90% in leaves. Regarding plants from organic manuring it was noticed that total identified substances in essential oil of the first crop were 87.13 % in inflorescences and 99.56% in leaves while 86.30 and 86.33% in inflorescences and in leaves of the second, respectively.

Although there was no significant difference between the methods of cultivation, was observed higher levels of trans-caryophyllene in relation to others components of the oil analyzed. In plants coming from mineral fertilizer cultivation, the average content of trans-caryophyllene was 48.64 and 47.42% in inflorescences of the first and the second crop, respectively. In leaves it was found at 45.39 and 33.61% in the first and in the second crop, respectively. Regarding organic manure cultivation, the average content of trans-caryophyllene varied from 47.83% in inflorescences of the first crop to 43.85% of the second. For leaves the contents found was 59.29 and 42.13% in the first and in the second crop, respectively. The analysis of essential oil in *Micromeria biflora* ssp. *arabica* also showed that the major sesquiterpene component was trans-caryophyllene (43.7%) (Al-Rehaily, 2006).

Another compound found at high concentration *Spilanthes oleracea* was germacrene-D. In inflorescences of plants grown under mineral fertilization it was 20.75 and 19.57% of the total essential oil in the first and the second crop, respectively. While in leaves it was found at a concentration of 20.06% in the first and 17.46% on the second crop. On the other hand, organically cultivated plants showed a content of germacrene-D of 19.87 and 19.86% in inflorescences of the first and the second crop, respectively. In leaves, the contents were of 22.36 and 23.40%, in the first and the second crop, respectively. These germacrene-D contents were higher than those reported for *Salvia spinosa* (10.66%) (Salehi Sourmaghi *et al*., 2006) and *Melissa officinalis* L. cultivated in two sites of Turkey (2.04% and 1.89%) (Ayanoglu *et al*., 2005) and lower than that found in *Marrubium astracanicum* Jacq. (23.4%) (Teimori *et al*., 2008).

Table 1: Chemical composition of essential oil of Jambu (*S. oleracea* cv. Jambuarana), grown under organic and mineral manuring

	Organic manuring						Mineral manuring					
	Inflorescence		Leave				Inflorescence		Leave			
Compound	(average %)		(average %)		IK exp.	IK lit.	(average %)		(average %)		IK exp.	IK lit.
Cultivar	1°	2°	1°	2°			1°	2°	1°	2°		
Sabinene	tr	tr	tr	tr	972	976	- 0.00	0.46	tr	tr	972	976
β-pinene	tr	0.50	tr	tr	980	980	0.34[b]	0.95[a]	tr	tr	980	980
β-phellandrene	tr	0.00	tr	tr	1024	1031	0.00	0.35	tr	tr	1024	1031
α-gurjunene	1.00[b]	2.09[a]	tr	tr	1395	1409	1.02[b]	2.43[a]	tr	tr	1395	1409
Trans-caryophyllene	47.83[a]	43.85[a]	59.29[a]	42.13[b]	1448	1458	48.64[a]	47.42[a]	45.39[a]	33.61[b]	1416	1418
α-humulene	2.50[a]	2.33[a]	1.73[a]	1.76[a]	1476	1480	2.63[a]	2.52[a]	2.12[a]	1.54[b]	1448	1458
Germacrene D	19.87[a]	19.86[a]	22.36[a]	23.40[a]	1448	1458	20.75[a]	19.57[a]	20.06[a]	17.46[b]	1476	1480
L-dodecene	6.11[b]	7.83[a]	9.75[a]	10.97[a]	1490	-	8.59[a]	6.58[b]	9.45[b]	11.09[a]	1490	-
Germacrene B	tr	tr	1.15	tr	1551	1556	tr	tr	1.00[b]	1.88[a]	1551	1556
Spathulenol	3.87[a]	3.80[a]	5.28[b]	8.07[a]	1575	1576	2.93[b]	4.07[a]	7.02[b]	29.32[a]	1575	1576
Spilanthol	3.70[a]	4.09[a]	nd	nd	1877	-	2.53[b]	3.84[a]	nd	nd	1877	-
Ethyl hexadecanoate	2.25[a]	1.95[b]	nd	nd	1989	1993	1.62[b]	2.63[a]	nd	nd	1989	1993
Total identified	87.13[a]	86.30[b]	99.56[a]	86.33[b]			89.05[a]	90.82[a]	85.04[b]	94.90[a]		

IK exp.: Experimental Kovats index, IK lit: Kovats Index from bibliography, tr: ≤ 0.2 and nd: Not detectable

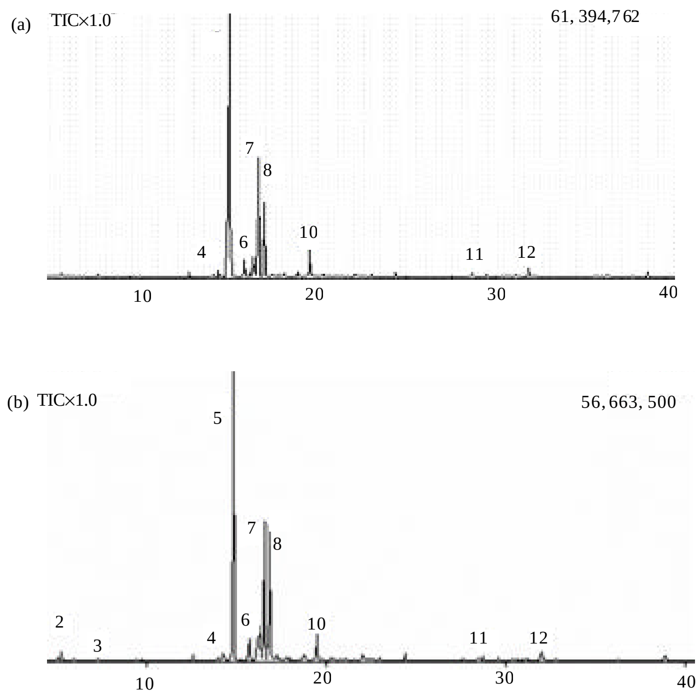

Fig. 1 (a-b): Chromatogram of total ions in essential oil of jambu (*Spilanthes oleracea* cv. Jambuarana) inflorescence, cultivated under (a) Mineral manuring and (b) Organic, Peaks 5, 7, 8 and 10 are trans-caryophyllene, germacrene-D, 1-dodecene and spathulenol, respectively

We detected spilanthol between 3.70% and 4.09% in plants under organic manuring and 2.53 and 3.84% in those under mineral fertilization, in the first and second crop, respectively (Table 1). Other important compounds of *Spilanthes oleracea* were also observed during the chemical analysis of the oil, such as 1-dodecene and spathulenol, mainly in leaves, showing an average content of 29.32% in the second crop, against 7.02% of the first crop of plants grown under mineral fertilization. While in *Spilanthes oleracea* cultivated under organic manuring, these compounds appeared in lower amount in leaves (5.28% in the first crop and 8.07% in the second), when compared to plants coming from mineral fertilized fields.

The quality and the yield of essential oils have been reported previously to be influenced by fertilizers and soil pH (Alvarez-Castellanos and Pascual-Villalobos, 2003; Upadhyay and Patra, 2011). Fertilizers have been found to increase the yield of essential oil from established crops, like *Valeriana officinalis* (Letchamo *et al*., 2004), *Artemisia annua* (Malik *et al*., 2009) and *Matricaria recutita* (Upadhyay and Patra, 2011). The results reported in the present work, regarding the analysis of essential oil, showed that fertilization procedure was not influenced essential oil composition of *Spilanthes oleracea* (Table 1). From our analysis, trans-caryophyllene content is well above that reported by Almeida *et al*. (2005), finding 33.43% trans-caryophyllene in leaves of *Leonurus sibiricus*. The presence of trans-caryophyllene was previously observed in essential oil of *Piper duckei* (Abraham, 2001) and this substances, trans-caryophyllene and germacrene-D, have been studied for having an anti-fungal and anti-microbial activity

(Duarte *et al.*, 2005). Thus, the use of essential oil of jambu (*Spilanthes oleracea*), due to its content of trans-caryophyllene and germacrene-D, may find an application in crop biological control.

According to our results, germacrene-D was not influenced by the fertilization procedure and its content was generally lower than that reported by others authors. As a comparison, Almeida *et al.* (2005) obtained 24.95% germacrene-D content in leaves of *Leonurus sibiricus*. This substance (peak 7 of Fig. 1) was also found in *Artemisia parviflora* (Rana *et al.*, 2003), *Piper friedrichsthalii* and *P. pseudollindeni* (Vila *et al.*, 2003), *Juniperus turbinata* (Cosentino *et al.*, 2003) and *Lippia javanica*, showing, *in vitro*, an antibacterial and fungicidal activity (Ngassapa *et al.*, 2003). Literature reports showed that others plants containing high percentages of germacrene D present eminent anti-microbial activity, as well (Azimi *et al.*, 2011). The increasing interest on plants containing high amount of this genre of substances is certainly due to the biological properties of their molecular structure (sesquiterpenes), showing a wide antibacterial and anti-fungal activity and acting as enzymatic inhibitors (Abraham, 2001).

The content of spilanthol observed in the present study was lower than that described in literature. The analysis of essential oil of jambu performed by Vulpi *et al.* (2007) reported an average content of spilanthol of 15.16% in inflorescences: value well above that obtained in the present work. The same authors verified its presence at an average content of 1.46% in essential oil from jambu leaves. However, the presence of this substance was not detected in our study, in the essential oil of jambu leaves cultivated under organic manuring and mineral fertilization, neither in the first nor in the second crop (Table 1). Although, Vulpi *et al.* (2007) reported in leaves a higher content of germacrene-D (38.51%), β-farnesene (36.04%) and 3-tridecene (2.97%). While in inflorescences, 15.38% 3-7-dimethyl-1,3,6-octatriene, 15.16% spilanthol and 15.02% β-farnesene were found. In the present study, the data reveal a higher average content of trans-caryophyllene, germacrene-D, 1-dodecene and spathulenol was observed, regardless of fertilization.

According to the literature, the component of jambu essential oil that causes the sensation of anesthesia provided by spilanthol, which influences its cultivation for medicinal or culinary purposes. However, we detected low spilanthol content found in jambu plants, which can be attributed to the period in which the experimental study was carried out, i.e., the temperature was quite unstable, showing temperatures below the recommended values (16-18°C). Has been suggested that climatic factors influence the concentration of active molecules (secondary metabolites) in plants and, among climatic factors, temperature affects very deeply the survival of vegetables, being linked to plant growth and development. Species showing scarce adaptation to temperature variation in a specific area will present serious problems in biomass and active molecule (secondary metabolites) production, because it influences primary metabolism (breathing and photosynthesis) and, as a consequence, secondary metabolism. According to Malik *et al.* (2009) among those that can affect the chemical composition of a plant, is nutrition. The deficiency, or the excess, of nutrients can promote a lower or a higher secondary metabolite production in plants. For this reason, organic manuring and mineral fertilization used in the present work could interfere with the content and the quality of essential oil of jambu plants, but this effect did not occur during this experiment. Several studies pointed out that environmental, genetic and agronomic factor can cause changes in the chemical composition of products from vegetable origin. For this reason periodic evaluations are so important.

CONCLUSION

In the present study, results reported allow to give several answers about the effect of organic and mineral manuring on jambu plant cultivation. From the results neither of the cropping system (organic or conventional) changed significantly the composition of essential oil of jambu in relation to spilanthol and the others components of essential oil. In this study, the benefit from organic agriculture is the absence of pesticides. Furthermore, these results allow to conclude that the most abundant compounds found in this study were trans-caryophyllene, germacrene-D, 1-dodecene and spathulenol and spilanthol, mainly in inflorescences.

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