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Original Article

Growth study and essential oil analysis of *Piper aduncum* from two sites of Cerrado biome of Minas Gerais State, Brazil

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ABSTRACT

Piper aduncum L., Piperaceae, stands out due to its biological activities, however, it is still found in the wild and little is known about its agronomic point of view. The aim of this study was to evaluate the growth and to analyze the chemical composition of essential oils from leaves of *P. aduncum* collected in two different sites of Cerrado as well as in cultivated plants. The cultivation was installed out in a greenhouse using cuttings of adult specimens. Essential oils were obtained from fresh leaves. Plants from the two studied locations showed erect growth habit and behavior of linear growth. The essential oils composition of *P. aduncum* from Bocaiuva did not differ between wild and cultivated plants, as the major substance identified as 1,8-cineole. The plants from Montes Claros site showed a distinct concentration for the two samples, being the major substance characterized as *trans*-ocimene (13.4%) for wild and 1,8-cineole (31.3%) for cultivated plants. Samples from both locations showed a similar essential oil composition in cultivars. Our results showed that *P. aduncum* cultivation is feasible and the variation in chemical composition of the two sites may indicate an environmental influence, since chemical and isoenzyme analysis did not show great differences.

Introduction

The Piperaceae family has a pantropical distribution and is composed of approximately 2,500 species that are mainly of the genus *Piper*. The Piperacae family is important because of its aromatic, ornamental and medicinal applications (Parmar et al., 1997; Ramos et al., 1986; Sumathykutty et al., 1999). *Piper nigrum* L. is popularly known as black or white pepper, depending on the time of harvest and the method of processing. This species is a Piperaceae family member with high economic value and is widely used as a condiment throughout the world. (Bandyopadhyay et al., 1990; Orav et al., 2004; Sumathykutty et al., 1999). *P. longum* is also used as

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condiment and for medicinal purposes (Huang et al., 2010; Vedhanayaki et al., 2003). Many other species of Piperaceae are recognized for having biological and pharmacological activities. These species include *Piper betle* (antidiabetic, antioxidant, antimicrobial activities), *P. methysticum* (anxiety treatment), *Peperomia blanda* (antifungal), *P. pellucida* (anti-inflammatory and analgesic), *P. sui* (cytotoxic) and *Pothomorphe umbellate* (anti-inflammatory and analgesic) (Arrigoni-Blank et al., 2004; Cheng and Chen, 2008; Cordeiro et al., 2005; Lei et al., 2003; Perazzo et al., 2005; Sacchetti et al., 2004).

P. aduncum is a shrub that is native to tropical regions of the Americas and was introduced in Asia during the 19th century (Hartemink, 2001). This plant is considered to be a weed and grows spontaneously in sandy-clay soils in pastures and at the edges of forests in Southeastern Brazil. In contrast, *P. aduncum* occurs mainly in wetter areas, such as in the Forest of Galleries in the North of Minas Gerais State, Brazil. This plant is popularly known in the Amazon region as "pimenta-de-macaco" (monkey pepper) and "aperta-ruão", and in the North of Minas Gerais as "jaborandi" or "falso-jaborandi" (Lorenzi and Matos, 2002; Sousa et al., 2008). This Piperaceae family member has been used and reported as medicinal for the treatment of many diseases (Berg, 1993; Coimbra, 1994; Moreira et al., 1998) and has been highlighted, especially, by showing an insecticidal, larvicidal, leishmanicidal, molluscicide, antibacterial and antifungal activities (Bernard et al., 1995; Lara Júnior et al., 2012; Moreira et al., 1998; Orjala et al., 1993; Orjala et al., 1994; Torres-Santos et al., 1999). These biological activities are related to the arylpropanoid dillapiole, which is a substance that has been reported by some authors to be the major essential oil compound found in the leaves of *P. aduncum*. This compound is therefore considered to be responsible for the biological activities that are associated with this species (Gottlieb et al., 1981; Maia et al., 1998). However, literature data have shown that there is a chemical diversity for the essential oil composition of *P. aduncum* (Lara Júnior et al., 2012; Maia et al., 1998; Potzernheim et al., 2012; Vila et al., 2005).

P. aduncum has small seeds and fruits that are mainly dispersed by wind and birds (Hartemink, 2001). From an agricultural point of view, however, little is known about this species and other species of the genus Piper that remain in the wild. To the best of our knowledge, there have been no studies on vegetative propagation of this species, and only a few studies examining biomass have been published (Hartemink, 2001). In Brazil, for instance, most of the medicinal plant species that are used by the population are obtained from natural habitats. The ability to sustain production of raw vegetable materials that are of high quality under controlled conditions is important because of the need to produce standardized herbal medicines (Jannuzzi et al., 2010). There are a number of advantages of using controlled cultivation instead of herbal collection from natural habitats. As highlighted by Palevitch (Palevitch, 1998), the standardized raw materials have an increased availability, are relatively free of tampering, have a process for quality control, provide a more stable supply and allow for the correct botanical identification and proper post harvest handling.

The analysis of plant growth is also very important and useful in studies of plant behavior under different environmental conditions and when there are physical and/or chemical variables (Benincasa, 1988; Paiva and Oliveira, 2006). Moreover, plant growth analysis is an indispensable tool for understanding the biological complexity of plants. Plant growth analyses involves the cultivation of the species of interest and the study of their growth by measuring several characteristics, including plant height, stem diameter, number of inflorescences and number of leaves (Benincasa, 1988).

A number of studies have been carried out to obtain information about the chemical profiles of food and medicinal species (Manzan et al., 2003; Sacchetti et al., 2004). In this context, it is known that plants grown under conditions that are different from those of their natural habitat may display qualitative and/or quantitative changes in their composition with regard to special metabolites. The chemical composition of essential oils, for example, is known to be altered depending on the exposure to different environmental conditions (Maia et al., 2009; Taiz and Zeiger, 2009).

The aim of this study was to evaluate the growth and to analyze the chemical composition of the essential oils in leaves of *Piper aduncum* L. obtained from two different locations in the Cerrado biome of North of Minas Gerais State and from cultivars.

Materials and methods

Plant material

Leaves and cuttings of adult specimens of *Piper aduncum* L., Piperaceae, for growth and chemical studies were collected from two locations in the Cerrado (Savanna) of Northern region of Minas Gerais State (Table 1). The plant material was identified by Dr. Elsie Franklin Guimarães and samples of each specimen were deposited in the Herbarium RB (Botanical Gardem of Rio de Janeiro) (Table 1).

Table 1
Data of each site of collection of *Piper aduncum* in the North of Minas Gerais/ Brazil.

Sample	Vegetation	Date	City	Latitude/ LongitudeRB	Height	Record in the Herbarium
1	Forest of Gallery of the River Angico	19/10/2011	Bocaiuva	S 16° 57,582' W 43° 51,912'	954 m	RB 501.330
2	Forest of Gallery of the River Boi	25/10/2011	Montes Claros	S 16° 42,501' W 43° 56,480'	712 m	RB 501.332

Growth study

Growth experiments were carried out in the greenhouse of the Agricultural Sciences Institute, Federal University of Minas Gerais (ICA/UFMG), Regional Campus of Montes Claros, located in the geographic coordinates: latitude 16°40'50,92"S and longitude 43°50'22,36"W, at a height of 646 m. The cuttings of *P. aduncum* were planted in styrofoam trays, one per cell, using the Bioplant® commercial substrate and were kept in the rooting beds with automatic irrigation for sixty days, until root system was formed and could be transplanted.

Obtained the seedlings were transplanted to individual pots of 14 l in February 2011. The cultivation was installed out in a greenhouse and was adopted the completely randomized design, with nine replicates and two treatments each consisting of two different sites of collection of *P. aduncum* (1. Bocaiuva and 2. Montes Claros). Assessment of growth was non-destructively and began fifteen days after transplantation. The following variables were evaluated, ever in 15 to 15 days, for a period of six months: *diameter of the stem* (mm) - determined from the growth medium of the stem of the individuals, measuring the stem with a caliper, 10 cm above ground; *plant height* (cm) - obtained from the growth medium of the individual, measured of the stem base to the apex of branch; *number of leaves* - obtained from the medium growth of the individuals, by counting the total number of leaves per plant; *number of inflorescences* - considered biweekly from the emission of the buds.

The collected date were subjected to regression analysis using the software SAEG - System for Statistical Analysis and Genetic (Ribeiro Júnior, 2001).

Chemical study of the essential oils

Chemical composition analyses essential oil from leaves of *P. aduncum* were performed with plants of two different sites of collection in both environments, cultivated and wild, in order to evaluate qualitative and/or quantitative variations. Two analyzes were performed with plants grown for two months consecutively (September 22th, 2011 and October 25th, 2011). For the second analysis, the same day (October 25th, 2011), fresh leaves of each environment (wild and cultivated) were collected for extraction. Plants from all locations were at the flowering stage. Plant materials was subjected to hydrodistillation for 2 h in a modified Clevenger type apparatus in the Laboratory of Medicinal and Aromatic Plants, Agricultural Sciences Institute, Federal University of Minas Gerais, Montes Claros, Minas Gerais, Brazil.

Obtained the essential oils were packed in an amber vial and storage in freezer at -20 ºC. The samples were subjected to analysis by gas chromatography coupled to flame ionization detector (HP-Agilent 6890 GC-FID) and by gas chromatography coupled to mass spectrometry (HP Agilent GC 6890 - MS 5973), in the Analytical Platform of Farmanguinhos, Fiocruz, Rio de Janeiro. Initially, the essential oils were diluted in dichloromethane (1 mg.ml^{-1}) and analyzed by GC-MS to obtain the mass spectra and to performer chemical characterization. Concomitantly, the diluted samples of essential oils (0.5 mg.ml^{-1}) were analyzed by GC-FID for quantification of chemical constituents and to determination the retention index (RI). The substances in the essential oil were identified by comparing their mass spectra with database registration (WILEY7n) and by comparison of Retention Indices (RI) calculated with those from literature records (Adams, 2001). RI were calculated using GC data of a homologous series of saturated aliphatic hydrocarbons within C8 to C20 (Sigma-Aldrich), performed at the same column and the conditions used in the GC analysis for the essential oils, and using the equation proposed by Vandendool and Kratz (Vandendool and Kratz, 1963).

GC-FID parameters: HP-5ms column (30 m x 0.32 mm x 0.25 µm), temperature programming from 60 to 240 ºC, with increase of 3 ºC.min^{-1}, using the hydrogen and synthetic air carrier gas, with a flow rate of 1.0 ml.min^{-1}. GC-MS parameters: HP-5ms column (30 m x 0.32 mm x 0.25 µm), temperature programming from 60 to 240 ºC, with increase of 3 ºC.min^{-1}, using the helium carrier gas, with a flow rate of 1.0 ml.min^{-1}.

Soil analysis

In order to characterize the soils of the two environments, soil samples were collected in the two sampling areas (Table 2) and of the cultivation substrate. Chemical and granulometric analyses were performed by Laboratory of Soil of Agricultural Sciences Institute, Federal University of Minas Gerais, Montes Claros, Minas Gerais, according

Table 2
Soil parameters from the two locations of occurrence of *P. aduncum* and cultivation soil.

Soil parameters	Soil samples		
	Natural Habitat		Cultivation
	Bocaiuva	Montes Claros	
pH in water	6.50	8.00	7.50
P Mehlich (mg.g^{-1})	0.61	0.61	14.43
P remaining (mg.l^{-1})	22.84	22.28	36.21
K (mg.kg^{-1})	247.00	26.00	61.00
Ca (cmolc.dm^{-3})	6.20	8.40	12.60
Mg (cmolc.dm^{-3})	1.80	1.50	1.70
Al (cmolc.dm^{-3})	0.00	0.00	0.00
H + Al (cmolc.dm^{-3})	1.76	0.68	0.76
SB (cmolc.dm^{-3})	8.63	10.07	14.46
t (cmolc.dm^{-3})	8.63	10.07	14.46
m (%)	0.00	0.00	0.00
T (cmolc.dm^{-3})	10.39	10.75	15.22
V (%)	83.00	94.00	95.00
Org. Mat. (dag.g^{-1})	2.50	2.37	9.96
Grit (dag.g^{-1})	8.40	6.00	7.60
Fine sand (dag.g^{-1})	45.60	76.00	44.40
Silt (dag.g^{-1})	28.00	10.00	30.00
Clay (dag.g^{-1})	18.00	8.00	18.00
Texture	Met	Sd	Met

Org. Mat., organic matter; Sd, sandy; Met, medium texture.

to Embrapa (Embrapa, 1999). Briefly, the pH was determined in water using soil and water in the ratio 1:2.5. The content of organic matter was determined from the organic carbon content of which was oxidized with potassium dichromate. Phosphorus was extracted with sulfuric acid, and then quantified by spectrophotometry. The exchangeable potassium was determined by flame photometry. The calcium and magnesium contents were determined by titration complexometric in the presence of murexide and eriochrome indicators, after extraction with potassium chloride (KCl). The exchangeable aluminum was determined by volumetric after extraction with KCl. The Pipette Method was used in particle size analysis (Embrapa, 1999).

Isozyme Analysis

Isoenzyme analysis of two accessions of *P. aduncum* was performed at the Laboratory of Medicinal and Aromatic Plants of the Institute of Agricultural Sciences of the Federal University of Minas Gerais, according to the methodology of Alfenas (Alfenas, 2006). Horizontal electrophoresis on starch gel (14%) it was used to determine the isozyme phenotypes of malate dehydrogenase (MDH), isocitrate dehydrogenase (IDH) and shikimate dehydrogenase (SKDH).

The samples, consisting of young leaves, were crushed in porcelain mortar previously cooled and kept at low temperature. The crushed was absorbed into rectangles of chromatographic paper (Whatman 3M) and applied to the gels. It was the pre-race performed at 4 °C with constant current at 150 V, 15 mA and 5W for 30 min, and then, withdrew the rectangles of paper and set the voltage to 300 V, 33 mA and 10W, remaining so until the end of the race (Alfenas, 2006).

Previously, it was prepared the Electrode Solution according to Shaw & Prasad (1970), consisting of 16.35 g Tris (hydroxymethyl), 8.5 g citric acid in 1 liter of distilled water (pH = 7,0), which was stored in amber glass container. Also it was prepared the Extraction Solution (0.3 g bibasic sodium phosphate, 3.5 g sucrose, 50 mg ascorbic acid, 50 mg of diethyldithiocarbamate, 25 mg sodium bisulfite solution, 25 mg of sodium borate, 0.5 g of polyethylene glycol 6000, 50 ml of distilled water, 1.28 g of polivinilpirrolodona-PVP-40 and 0.1 ml β-mercaptoethanol) and the starch gel (40 ml of electrode and the solution completed to volume with distilled water up to 600 ml, 18 g of sucrose, 84 g of corn starch) (Alfenas, 2006).

For the revelation gel samples were kept in the dark and it was used for each specific developing solution. IDH and SKDH systems were kept at room temperature and the system MDH at 37 ºC for 5 h approximately until the observation of bands (Alfenas, 2006).

Results and discussion

Growth study

Plants from the two locations studied showed erect growth and linear growth behavior during the evaluation period for the following four analyzed variables: plant height, stem diameter, number of leaves and number of inflorescences. The most likely observed linear behavior occurred as a result of the restriction of the evaluation period to only 180 days.

At the end of the evaluation period, plants from the site Bocaiuva showed average branch lengths of 52 cm. Plants from the Montes Claros site had average branch lengths of 60 cm (Fig. 1). The variable stem diameters showed a similar pattern for the two treatments (Fig. 2).

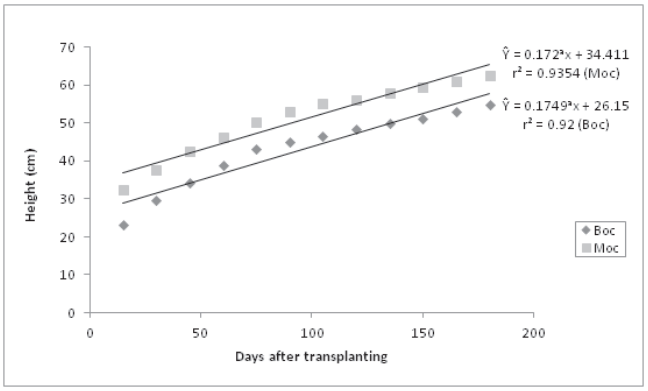

Fig. 1 - Variation in the height of three accessions of *P. aduncum* throughout 180 days of cultivation in a greenhouse. ICA/UFMG, 2011. Boc, Bocaiuva access; Moc, Montes Claros access. a Significant at 5% of the test T.

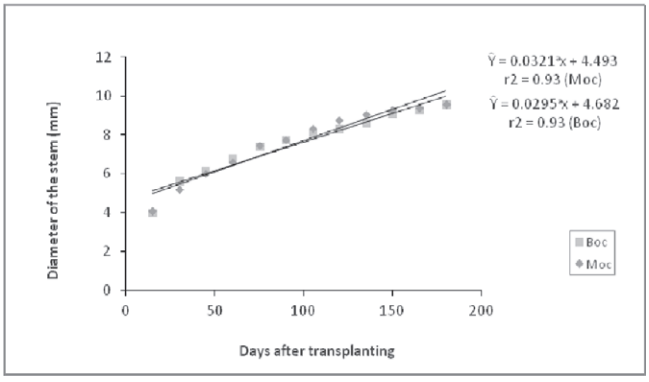

Fig. 2 - Variation in the diameter of the stem of three accessions of *P. aduncum* throughout 180 days of cultivation in a greenhouse. ICA/UFMG, 2011. Boc, Bocaiuva access; Moc, Montes Claros access.a Significant at 5% of the test T.

Because this species has relatively large leaves, an evaluation of the number of leaves revealed that a significant number of leaves were found on the plants from the two locations (Fig. 3). *P. aduncum* is to erect shrub, branched, evergreen, with articulated rods and gnarled. This species shows leaves simple, entireand opaque on both sides are that within the range of 10-17 cm in length (Lorenzi and Matos, 2002; Sousa et al., 2008). This leaf development is expected because many species prioritize the formation of leaves during the early stages of development to accelerate and increase photosynthetic activity. After strengthening, leaf development is supported by the lignified tissues of the stem (Cutter, 1986).

The plants from Bocaiuva and Montes Claros showed differences in the production of inflorescences. The reproductive structures began to appear in the Bocaiuva plants 45 days after transplantation. In contrast, reproductive structures did not appear in the Montes Claros plants until the 90th day.Both sites showed plants from the low number of inflorescences during the 180-day evaluation period, and this observation was true particularly for plants from the Montes Claros location (Fig. 4).

Plants from the two locations showed a rapid rate of development. The fast rate of *P. aduncum* development also was observed by (Hartemink, 2001), who studied the accumulation of biomass and nutrients in this plant in Papua New Guinea. According to Hartemink (Hartemink, 2001), this species showed significant increases in above ground biomass after fourteen months of cultivation, which was primarily due to the growth of the main stems. During the first fourteen months, the rods formed less than 50% of the total biomass that was above the ground because the plants were rich in leaves. However, after 23 months, more than three quarters of the t tal biomass consisted of woody stems. According to the author (Hartemink, 2001), the seedling reached a mean height of 90 cm during the first five months after planting, but this mean height increased to 2.5 m by fourteen months and to 4.5 m by 23 months. These results show that the species is fast-growing. The larger growth of *P. aduncum* in that study relative to the growth observed in the present study may be a consequence of different environmental conditions and genetic factors.

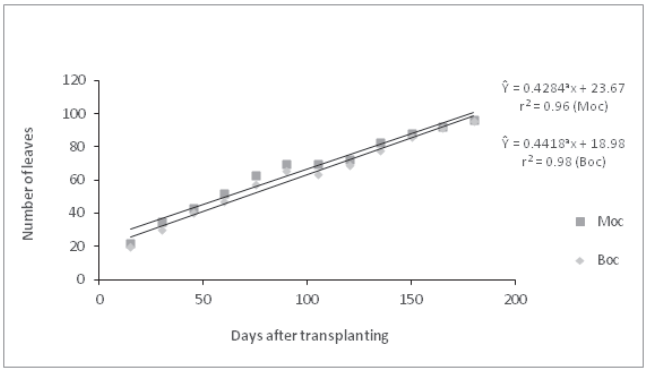

Fig. 3 - Variation in the number of leaves of three accessions of *P. aduncum* throughout 180 days of cultivation in a greenhouse. ICA/UFMG, 2011. Boc, Bocaiuva access; Moc, Montes Claros access. a Significant at 5% of the test T.

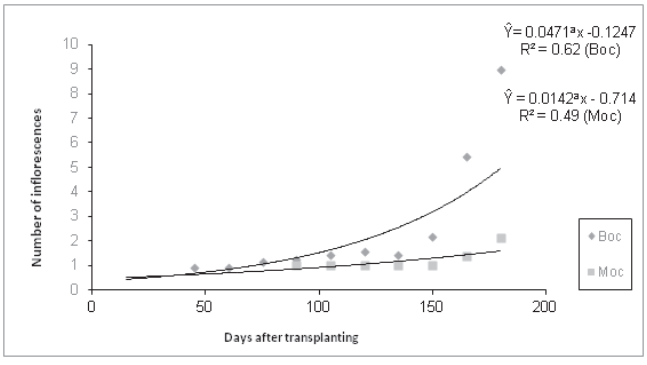

Fig. 4 - Variation in the number of inflorescences of three accessions of *P. aduncum* throughout 180 days of cultivation in a greenhouse. ICA/UFMG, 2011. Boc, Bocaiuva access; Moc, Montes Claros access.a Significant at 5% of the test T.

The study carried out in Papua New Guinea was motivated by the rapid growth of *P. aduncum* that occurs in that region (Oceania). The rapid growth is most likely associated with the high fertility of the soil and with high humidity because the species consumes a large quantity of water (Hartemink, 2001; Hartemink and O'Sullivan, 2000; Stohlgren et al., 1999). Though this species may be locally abundant in the Neotropics, it rarely dominates the vegetation and is rarely found in areas where the vegetation is ripe. However, in the Amazon region this species has been reported to be a weed that arises after wood exploration (Maia et al., 1998). Research of the secondary growth properties of *P. aduncum* also in the Amazon region failed to show a high rate of biomass accumulation compared to those obtained from Papua New Guinea, possibly because though the lowlands are humid, the region is limited in nutrients (Gehring et al., 1999).

Chemical study of the essential oil

The chemical composition of the essential oils of *P. aduncum* showed few differences related to the origin of the plants when the cultivars were compared with plants from the wild habitat. Furthermore, the chemical profiles of the essential oils of the plants that were cultivated that were similar to those of the plants found in the wild for each location, and the variation was primarily that did occur in the concentrations of the substances. The sample yields were determined to be approximately 0.7% for the plants from the Bocaiuva location (wild and cultivated/two months) and 0.5% and 0.3% for plants from the Montes Claros that location were either cultivated (two months) or found in the wild, respectively. It was possible to characterize 52 different substances in the essential oils of the plants from the two locations.

Monoterpenes were the main fraction identified in the essential oils from the plants were obtained from the Bocaiuva site (wild habitat and cultivar). The major substance was determined to be 1,8-cineole (wild habitat: 57.2%; cultivated: 35.2-37.9%), followed by a-pinene (14.2%) in the wild habitat and by *trans*-ocimene (13.5-13.8%) in the cultivated plants. The essential oil chemical composition for the plants from the Bocaiuva site was very similar to what was previously published. The presence of arylpropanoids in the essential oils from the plants of wild habitat was not observed. In contrast, it was possible to identify small amounts of safrole (0.5-0.9%) and sarisan (0.2%), which is also known as asaricin, in the essential oils of the cultivated plants (Table 3).

The high percentage of 1,8-cineole relative to the arylpropanoids in the essential oils from the wild Bocaiuva plants can be associated with environmental adaptation because 1,8-cineole has a high allelopathic activity (Koitabashi et al., 1997; Müller, 1965). For example, previous work on 1,8-cineole allelopathic activity demonstrated that this compound inhibited the growth of seedlings of *Brassica campestris*. Furthermore, its inhibitory effects were more severe than in root growth than in the hypocotyl (Koitabashi et al.,

Table 3
Chemical constitution of the essential oil from leaves of three accesses of *P. aduncum* (natural habitat and cultivar).

Constituents	Ri_{lit}	RI	Relative percentage (%)					
			Boc wild	Boc cult/sep	Boc cult/oct	Moc wild	Moc cult/sep	Moc cult/oct
Monoterpenes	-	-	92.7	64.5	69.5	28.6	53.0	64.5
α-pinene	939	923-924	14.2	8.0	4.3	2.1	5.0	8.0
β-pinene	980	967-968	9,0	7.8	4.0	4.8	5.9	7.8
β-mircene	991	977-978	2.2	2.2	1.8	0.5	1.2	2.2
limonene	1031	1025-1026	1.7	1.6	1.9	1.1	1.5	1.6
1,8-cineole	1033	1031-1033	57.2	31.3	37.9	-	23.3	31.3
cis-ocimene	1040	1032	-	-	-	4.1	-	-
trans-ocimene	1050	1035-1037	4.0	8.9	13.5	13.4	13.5	8.9
γ-terpinene	1062	1050-1052	0.3	0.5	0.5	-	-	0.5
isoterpenol	1087	1074	-	-	0.5	-	-	-
linalol	1098	1088-1090	-	1.7	0.8	0.6	0.7	1.7
perilene	1102	1103	-	-	-	1.0	-	-
δ-terpineol	1166	1159-1168	0.7	-	0.6	-	-	-
4-terpineol	1177	1169-1179	0.6	-	0.5	-	-	-
α-terpineol	1189	1183-1197	3.0	2.3	3.2	0.2	1.8	2.3
unidentified MT	-	1270-1271	-	0.3	-	0.9	0.2	0.3
Sesquiterpenes	_	_	5.9	26.3	21.2	57.5	32.9	26.3
α-copaene	1376	1360-1362	-	0.4	-	1.1	0.5	0.4
β-elemene	1391	1374-1375	-	0.7	-	0.9	0.6	0.7
E-caryophyllene	1418	1403-1408	0.2	3.5	0.4	4.0	2.8	3.5
β-cedrene	1419	1413-1415	-	0.2	-	0.3	0.2	0.2
aromadendrene	1439	1422-1423	0.6	0.3	1.1	0.3	0.3	0.3
α-humulene	1454	1439-1440	-	2.9	0.5	4.1	2.8	2.9
seychelene	1460	1444-1446	-	-	-	0.6	0.3	-
γ-muurolene	1477	1460-1464	-	0.3	-	0.5	0.3	0.3
germacrene D	1480	1464-1466	-	3.2	1.6	4.5	3.7	3.2
b-selinene	1489	1473-1476	-	0.3	1.6	0.4	0.3	0.3
guaieno	1490	1475	-	-	0.3	-	-	-
valencene	1496	1480-1487	-	4.2	-	6.9	4.5	4.2
biciclogermacrene	1500	1486-1490	0.4	-	8.6	-	-	-
γ-cadinene	1513	1496-1500	0.2	0.3	0.1	0.2	0.2	0.3
cubebol	1514	1499-1502	0.6	0.8	1.4	3.1	1.6	0.8
7-*epi*-a-selinene	1520	1502-1503	-	1.0	-	2.0	1.3	1.0
trans-calamenene	1521	1505	-	-	-	0.3	-	-
δ-cadinene	1524	1508-1510	1.5	-	1.0	-	-	-
unidentified ST	_	1524-1525	-	0.2	-	0.7	0.4	0.2
germacrene B	1556	1539-1541	-	0.1	0.2	0.1	0.2	0.1
nerolidol	1564	1547-1548	0.6	2.0	-	5.9	2.7	2.0
unidentified ST	_	1551-1557	0.2	-	0.7	-	-	-
germacrenol	1575	1559-1560	-	0.4	-	1.0	0.5	0.4
caryophyllene oxide	1581	1563-1565	0.3	1.0	-	4.7	2.5	1.0
unidentified ST	-	1565-1568	-	-	0.8	-	-	-
globulol	1590	1585-1591	0.4	-	0.2	-	-	-
viridiflorol	1592	1586-1587	-	0.4	-	1.5	0.3	0.4
guaiol	1600	1603	-	-	0.6	-	-	-
humulene epoxide II	1606	1587-1593	0.4	0.7	-	4.1	0.8	0.7
1,10 di-*epi*-cubenol	1618	1597-1609	-	0.3	0.2	-	0.9	0.3
10-*epi*-γ-eudesmol	1622	1603	-	-	-	-	0.5	-
epi-cubenol	1627	1609	-	0.5	-	1.7	0.3	0.5
epi-α-cadinol	1638	1617-1624	0.2	1.5	-	4.7	2.6	1.5
hinesol	1639	1621-1623	0.3	-	-	-	-	-
τ-cadinol	1640	1622	-	-	0.3	-	-	-
epi-α-muurolol	1641	1629	0.2	-	-	-	-	-
τ-muurolol	1642	1624-1628	-	-	0.1	-	-	-
α-muurolol	1645	1625-1628	-	0.3	0.9	1.0	0.5	0.3
α-cadinol	1653	1635-1636	-	0.9	0.3	2.6	1.5	0.9
unidentified ST	-	1650-1651	-	-	0.6	0.5	-	-
Arylpropanoids	-	-	-	1.6	1.1	0.5	0.3	1.6
safrole	1285	1276-1279	-	1.6	0.9	0.5	0.3	1.6
sarisan	1495	1481-1497	-	-	0.2	-	-	-

RI_{lit}, retention index from literaturea; IR, retention index (variation); Boc wild, access Bocaiuva/wild; Boc cult, access Bocaiuva/cultivar; Moc wild, access Montes Claros/wild; Moc cult, access Montes Claros/cultivar; Pat wild, access Patis/wild; Pat cult, access Patis/cultivar; ST, sesquiterpene; MT, monoterpene.
[a] (Adams, 2001).

1997). Allelopathy is a biological phenomenon by which an organism produces one or more compounds that influence the growth, survival and reproduction of another organism (Nishida et al., 2005). Once the soil of the Cerrado biome shows nutrient limitations and low humidity (see discussion bellow), 1,8-cineole may assist *P. aduncum* to settle in the area.

Despite containing the same qualitative pattern of the substances as that found in the essential oils from the Bocaiuva location, the essential oils from the plants from the Montes Claros location showed differences in the relative percentages of compounds for the two samples. The major fraction was characterized containing the sesquiterpenes (57.5%) in the essential oils from the plants from the wild habitat. In contrast, the substance with the highest relative percentage was determined to be the monoterpene *trans*-ocimene (13.4%). The monoterpenes (53.0-64.5%) were found to constitute the major fraction of the essential oils from the plants cultivated, and 1,8-cineole (23.3-31.3%) was the major compound. This oxygenated monoterpene was not identified in the sample from the wild habitat. Safrole was the single arylpropanoid that was identified in the two samples from the Montes Claros location, but it constituted a lower percentage of the essential oils (Table 3).

The essential oils in the plants grown from the Montes Claros location were similar to those of plants from the Bocaiuva location. The differences between the chemical compositions of the samples from the natural habitat of Montes Claros in comparison with the other samples may be due to the influence of the microclimate or to the soil composition at the collection area. Also It is interesting to note that dillapiole was not identified in any of the analyzed samples. According to the literature, this arylpropanoid is the main compound that is found in the essential oils of *P. aduncum*, and it is responsible for several biological activities that are attributed to the oil from the leaves of this species (Gottlieb et al., 1981; Maia et al., 1998). These factors have led some authors to associate the biological activity of *P. aduncum* essential oil with the presence of dillapiole (Gottlieb et al., 1981; Lara Jr et al., 2012; Maia et al., 1998; Navickiene et al., 2006; Vila et al., 2005).

Also observed differences were in the major essential oil components between the wild and cultivated populations of *Baccharis trimera* (Less.) DC. While the sesquiterpene germacrene D was the major component between the months of September and November in both populations, the sesquiterpene ledol showed a wide variation within the wild population. In the cultivated samples, the sesquiterpene ledol was found in greater amounts, and this was independent of the time of year. The sesquiterpene bicyclogermacrene was the major component between July and February in the wild population and from December to February in the cultivated plants (Silva et al., 2007).

In the present study, plants from the Montes Claros location were shown to be more susceptible to the influence of environmental factors. The essential oils compositions in these plants were found to have limited qualitative but extensive quantitative variations, even when considering the major fractions and substances. According to Trap and Croteau (Trapp and Croteau, 2001), the special metabolites that are observed are controlled at the genetic level. However, the amount and concentration of the metabolites vary depending on biotic and abiotic environmental factors (Palevitch, 1987), including light intensity, latitude, average temperature, soil type, wind strength, water availability and also interactions between these factors. However, each species has a different response to environmental factors. Moreover, intraspecific genetic variability may also influence the content and chemical composition of the essential oils (Hay and Svoboda, 1993).

The soil is one of the main factors that influence plant development (Palevitch, 1987). However, analyses of the soil (Table 2) at the two locations showed that the soil does not appear to be a limiting factor for *P. aduncum* growth. Soil analyses showed an absence of aluminum (Al) in both the natural habitat and at the cultivation sites, despite the fact that this element is common in the Cerrado (Savannah) biome. Even at low concentrations, Al is known to limit the growth of many plants and is considered the toxic element in high concentration. Aluminum toxicity occurs when the pH is less than 5.3, in poor soils and when there is a low content of organic matter (Haridasan, 2006; Kamprath and Foy, 1985; Silva et al., 2002). The soil pH greater than 5.3 and the presence of organic matter may explain the absence of this element in the analyzed soils. These results suggest that Al content is not interfering with the growth of the *P. aduncum* samples that were studied.

The pH value appears to be within a satisfactory range (Taiz and Zeiger, 2009) in the soils of Bocaiuva (pH 6.5). The high pH value (8.0) registered for the Montes Claros soil (Table 2) is most likely due to the presence of high concentration of limestone in the region (Miranda et al., 2011). However, the pH value that was obtained for the cultivation soil (pH 7.5) was higher than at Bocaiuva also. Indeed, although there were differences in the pH of the soils in the wild and cultivated samples, the essential oil chemical composition from the Bocaiuva plants did not show great variation. These results are different than what was observed at the Montes Claros site. These results indicate further that the pH parameter may not be the primary factor that is responsible for the differences in *P. aduncum* secondary metabolism.

The analyzed soils are poor in phosphorus (P), is very common in tropical soils (Fardeau, 1996; Rolim Neto et al., 2004). However, the measured phosphorus levels are sufficient for strong plant growth. The phosphorus concentration of 0.2 $mg.l^{-1}$ was established by Beckwith (Beckwith, 1965) and by Fox and Kamprath (Kamprath and Fox, 1989) to be the equilibrium concentration for the maximum growth of most plants. Regardless of the type of tropical soil, the phosphorus content is considered to be much lower than this optimum level. The soils of the Cerrado biome (Savannah) show chemical limitations. However, despite the low levels of nutrients, the soil has a high phosphate adsorption capacity (Costa et al., 2008; Furtini Neto et al., 1999; Lana et al., 2004). When P is found in high concentrations, it may indicate that the area has been under the influence of animals and/or humans in the past (Rolim Neto et al., 2004). In natural ecosystems undisturbed by man, several biological and chemical processes allow plants to use the resources efficiently, even in conditions of low availability. In such cases, there may be direct absorption of

organic forms of P without the passage of the nutrient to the mineral phase in the soil (Novais and Smyth, 1999).

Variation in the concentration of potassium (K) was observed among the soils of the two locations and cultivation samples (Table 2). However, all of the K values are in the proper range to support plant growth. The K concentration that is required to support plant growth in optimal conditions is in the range of 20-50 $mg.kg^{-1}$. Plants are capable of absorbing amounts of K that exceed their needs, which is commonly called *luxury consumption* of K (Meurer, 2006).

Other features that also influence plant growth are deficiencies in calcium and magnesium (Tan and Keltjens, 1995; Vale et al., 1996); however, these elements were found in suitable levels in the analyzed soils.

In an attempt to correlate the chemical composition analyses with the growth studies, we observed that plants from the Bocaiuva and Montes Claros locations, which exhibited less inflorescence and greater numbers of leaves, produced the compound 1,8-cineole to the major substance in the cultivated samples. Indeed, this was oxygenated monoterpene also determined to be the major compound in the essential oils from the plants of the natural habitat in Bocaiuva. It is interesting to note that the *trans*-ocimene contents that were identified in the essential oils of plants from the Bocaiuva locations and Montes Claros (wild and cultivar) are quite similar. The pattern of variation in the essential oils from different locations may also indicate chemical variation and the existence of chemotypes (Silva et al., 2007) that have never been described for Piperaceae species.

The chemical and phenotypic differences, including the differing numbers of inflorescences and leaves that were found in *P. aduncum* from the different locations, were also observed in a study with *Lippia alba* (Mill) N. E. Brown. In that study, the analysis of plants at sixteen locations also showed phenotypic variations and different chemical components in the essential oils. Data in the literature show that great genetic variability is characteristic of native plants that have not been domesticated (Jannuzzi et al., 2010). These factors are very important and raise concerns about the use of plants for medicinal or their biological properties. The quality and activities of the essential oils are associated with their chemical composition (Jannuzzi et al., 2010; Martins et al., 2006). Thus, to address these issues, agronomy and botany studies include chemotype identification that should be encouraged and disseminated.

Isoenzymes analysis

Differences in isoenzyme mobility in an electric field are the results of differences in the DNA sequences encoding the enzymes. Thus, if the banding patterns of two individual enzymes differ, it is believed that the differences have a genetic basis and are inherited (Murphy et al., 1990).

Three bands were monomorphic identified for the MDH system from the plants from the two locations from within the same area of activity. The bands showed the same isoenzyme pattern (Fig. 5). However, the intensities of bands showed different coloration and thickness. Through the characterization of *Polygonum punctatum* Ell. from eight locations, Lopes and colleagues (Lopes et al., 2003) also detected multiple bands for the MDH system, but their results indicated isoenzyme polymorphisms. According to the authors, the degree of kinship is close suggested when the bands between the two locations are similar (monomorphic), and the variations in the number, intensity and thickness of the bands can be related to the degree of ploidy in the species.

The MDH isoenzyme system phenotypes of *Eclipta alba* (L.) Hassk. plants from eight locations observed by (Bizão, 2002) also showed the presence of several bands and the absence of polymorphism in this species. For some authors, the study of inheritance of the MDH isoenzymes in plants has been considered complex because these isoenzymes are associated with a variety of structures, including mitochondria. For this reason, overlapping bands may occur (Arulsekar et al., 1986; Harry, 1983). However, verification of the existence of MDH enzyme polymorphism is interesting and important because the enzyme plays a significant role in the Krebs cycle, which catalyzes the conversion of malate to oxaloacetate and produces NADH. NADH is a key product in ATP production and has intermediates that are critical to cell function (Taiz and Zeiger, 2009).

Only one monomorphic band was identified during the analyses of the SKDH and IDH systems (Figs. 6 and 7, respectively). These analyses again revealed a similar pattern of bands for both isoenzymes from the two plant locations. In a

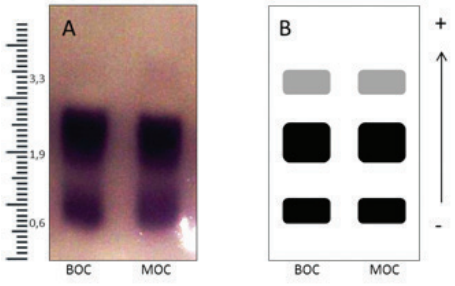

Fig. 5 - Gel image with revelation of bands on the enzymatic activity (A) and schematic representation of isoenzyme phenotypes of the enzyme malate dehydrogenase (MDH), from Bocaiuva (BOC) and Montes Claros (MOC) sites, with three monomorphic bands (B).

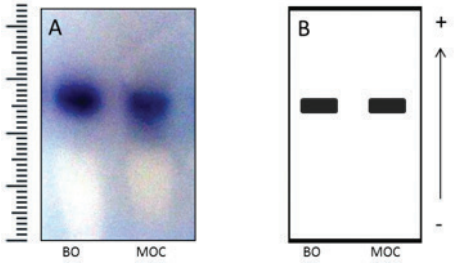

Fig. 6 - Gel image with revelation of bands on the enzymatic activity (A) and schematic representation of isoenzyme phenotypes of the shikimate dehydrogenase enzyme (SKDH), from Bocaiuva (BOC) and Montes Claros (MOC) sites, with only one monomorphic band (B).

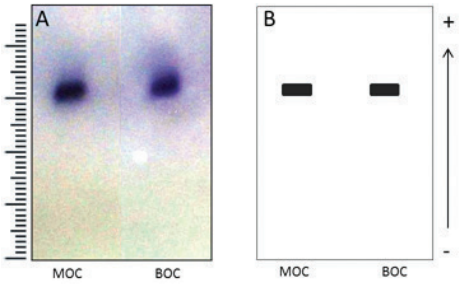

Fig. 7 - Gel with revelation of bands on the enzymatic activity (A) and schematic representation of isoenzyme phenotypes of the enzyme isocitrate dehydrogenase (IDH), from Bocaiuva (BOC) and Montes Claros (MOC) sites, with only one monomorphic band (B).

previous study with *Polygonum punctatum* Elliott., both systems showed multiple bands with wide polymorphisms (Lopes et al., 2003). The presence of more than one monomorphic band for the system was SKDH also observed during the characterization of *Eclipta alba (L.)* Hassk. from eight locations by Bizão (Bizão, 2002). This monomorphic SKDH feature of the system can be adaptive because this enzyme is involved in secondary metabolism (shikimate biosynthetic pathway) and the synthesis of chemical defenses (Taiz and Zeiger, 2009).

The isozyme analyses of the plants from the two sites did not show polymorphisms for the three systems that following studied were: malate dehydrogenase (MDH), isocitrate dehydrogenase (IDH) and shikimate dehydrogenase (SKDH). These data are consistent with those observed in the analysis of the chemical components of the essential oils of cultivated plants. The similarities of the chemical compositions and isozyme and when cultivated in the environment suggest same that samples of the two populations of *P. aduncum* (Bocaiuva and Montes Claros) are of the same chemotype, which is characterized by its 1,8-cineole and *trans*-ocimene monoterpene content. The results of the isozyme analyses reinforce what has already been discussed in this article. In other words, environmental influences can produce changes in the chemical composition of the essential oils of *P. aduncum*.

Our results showed that the cultivation of *P. aduncum* is feasible, once this species has rapid growth and development, as well as it produces large amounts of essential oils rich in monoterpenes, and this is mainly observed in cultivars. The observed variation in the essential oil chemical composition in *P. aduncum* plants from the different locations may be due to environmental influences, because similar results were obtained with the cultivated plants. The identification of 1,8-cineole as the major substance, the identification of *trans*-ocimene in lower amounts and the similar pattern of isoenzymes may suggest a new chemotype of *P. aduncum* that has not previously been reported for this species.

Authorship

GLO (PhD student) contributed in collecting plant samples, confection of herbarium, running the laboratory work regarding to essential oil extraction, plant cultivation, and essential oil analysis data. DLM contributed to essential oil analysis using GC-FID and GC-MS as well as supervising all data of essential oil analysis and organizing the manuscript. ADRM contributed to isoenzime analysis. EFG was responsible to plant identification. LSF and ERM were responsible for supervising the plant cultivation and analysis of the plant cultivation date. MACK contributed to critical reading of the manuscript and the final results of the supervision . All the authors have read the final manuscript and approved the submission.

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