

INFLUENCE OF ALTITUDE, AGE AND DIAMETER ON YIELD AND ALPHA-BISABOLOL CONTENT OF CANDEIA TREES (*Eremanthus erythropappus*)

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(received: June 25, 2008; accepted: June 26, 2009)

ABSTRACT: The heartwood of candeia tree is a source of essential oil rich in alpha-bisabolol, a substance widely used in the cosmetic and pharmaceutical industry. Bearing in mind the economic importance of alpha-bisabolol, this work aimed to evaluate the influence of tree age on the yield and content of alpha-bisabolol present in essential oil from candeia, considering two distinct reliefs and three diameter classes, in Aiuruoca region, south Minas Gerais state. The two distinct reliefs correspond respectively to one section of the stand growing at 1,000m of altitude (Area 1) and another section growing at 1,100m of altitude (Area 2). In each section, 15 trees were felled from among 3 different diameter classes. Discs were removed from the base of each tree to estimate their age by doing growth ring count. Soil samples were taken and subjected to physical and chemical analysis. The logs were reduced into chips and random samples were taken for distillation to extract essential oil. The method used was steam distillation at a pressure of 2 kgf/cm²/2.5 h. The chemical analysis was performed in a gas chromatograph (GC) based on the alpha-bisabolol standard reference. The yield of essential oil from trees in Area 1 was higher than that from trees in Area 2, with the same pattern of influence for older trees. In Area 2, the alpha-bisabolol content was higher in younger trees. No differences were found between the relevant parameters in relation to diameter classes.

Key words: Essential oil, tree age, *Eremanthus erythropappus*, alpha-bisabolol.

INFLUÊNCIA DA ALTITUDE, IDADE E DIÂMETRO DE ÁRVORES DE CANDEIA (*Eremanthus erythropappus*) SOBRE O RENDIMENTO E TEOR DE ALFA-BISABOLOL

RESUMO: O cerne da madeira de candeia é um fornecedor de óleo essencial rico em alfa-bisabolol, muito utilizado na indústria. Em virtude da importância econômica desse componente, neste trabalho, objetivou-se avaliar a influência da idade da árvore no rendimento e teor de alfa-bisabolol no óleo essencial obtido da madeira de candeia, considerando duas condições distintas de relevo e três classes diamétricas, na região de Aiuruoca, Sul de Minas Gerais. As duas condições distintas de relevo correspondiam a uma parte do povoamento crescendo a 1000m (Área 1) e outra a 1.100m de altitude (Área 2). Em cada uma delas foram abatidas 15 árvores distribuídas em 3 classes diamétricas. Da base das árvores, foram retirados discos para a estimativa da idade a partir da contagem dos anéis de crescimento. Amostras do solo foram tomadas para análise física e química. Os troncos das árvores foram picados e dos cavacos obtidos retiradas amostras aleatórias para condução de destilações das madeiras e obtenção de óleo essencial. O método utilizado na destilação foi arraste à vapor d'água com pressão de 2 kgf/cm²/2,5 h. A análise química foi feita em cromatógrafo gasoso (CG) com base no padrão de alfa-bisabolol. O rendimento em óleo essencial da madeira de candeia das árvores da Área 1 foi maior que na Área 2, havendo o mesmo tipo de influência, para árvores de maior idade. Na Área 2, o teor de alfa-bisabolol, foi maior nas árvores mais jovens. Não houve diferença entre os parâmetros estudados, considerando a variação de classes diamétricas.

Palavras-chave: Óleo essencial, idade das árvores, *Eremanthus erythropappus*, alfa-bisabolol.

1 INTRODUCTION

Essential oils are constituted by many volatile compounds that have a high vapor pressure and are insoluble in water but soluble in several water-immiscible solvents and in ethanol (COSTA 1975). These oils are classified according to their consistency (viscosity) and

to the origin and chemical nature of their main constituents (BRAGA 2002). Essential oils are present in various plant organs and contain a blend of solid, liquid and volatile substances which are chemically complex and vary in composition (CARDOSO et al. 2000). The characteristics of an essential oil depend on soil conditions, climate and cultivation practices

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involving the plant from which they are extracted (CARNEIRO & FERNANDES 1996).

Candeia (*Eremanthus erythropappus* (DC.) McLeish) is thought by Lorenzi (1992) to come from a special habitat, since in native form it is found in altitudes of around 1,000 meters in areas of cambisol and litosol, with rock outcrops. The alpha-bisabolol contained in the essential oil of candeia is commonly used as a substitute for azulene in hypoallergenic cosmetic preparations (PEREZ 2001, TEIXEIRA et al. 1996). And due to the importance of performing studies on this species, we conducted this study to try and detect differences in the yield and content of alpha-bisabolol between two different sample areas and different diameter classes of a native candeia stand, under specific soil conditions, and also to detect any influence of tree age on the values found.

2 MATERIAL AND METHODS

2.1 Tree selection and wood sampling

The material was collected in Aiuroca, Minas Gerais state. The study site had no history records of previous crops, and the tree stands were located in two areas where the soil is characterized as cambisol. Five trees were felled from each diameter class (class I: 5-10 cm; class II: 10.1-15.0 cm, and class III: 15.1-20 cm) per type of terrain, to a total of 30 trees. Tree height was set as the distance between ground and first bifurcation. The two areas differed as to conditions of terrain, one at 1,000 m and the other at 1,100 m of altitude.

2.2 Soil sampling and analysis

Once the areas were identified, 15 samples were collected in each area from a depth of 0-20 cm, then blended together into one compound sample of each area and submitted to chemical and physical tests. Analyses were conducted according to soil analysis methodologies described by Ferreira et al. (2003) and Silva et al. (1998), including determination of pH, soil fertility and soil-water retention gradient at 0.02 atm and at 15 atm.

2.3 Determination of age

Discs were obtained from each tree base and sanded using sandpaper 100, 150, 220, 320 and 400 grit sizes, and growth rings were counted by drawing a straight line across the diameter segment of the disc, and using a magnifying glass in order to obtain the estimated age of each tree. This methodology was adapted by Baruso (1977), as cited by Perez (2001).

2.4 Essential oil extraction

Trees were felled and logs reduced into chips to a granulometry of 1 inch, then submitted to ESALQ College of Agriculture, in Piracicaba (SP), for distillation procedures. To isolate the essential oil, the steam distillation method was used. In the process, samples were placed and distilled in an autoclave subject to a pressure of 2.0 kg/cm² for 2.5 hours. The oil and water vapor originating from the autoclave were collected by condensation at a temperature of 50-60 °C. Each material being assessed was subjected to 3 distillations.

At the end of each extraction, the distillate was transferred to a separatory funnel and 12mL of dichloromethane added to the hydrolate to cause the oil to separate, along with the solvent. After some agitation and vapor release, the water-oil-dichloromethane mixture was left to rest for 24 hours. The solvent-oil mixture was then transferred to a pre-weighed glass flask and the solvent was left to evaporate at 45 °C (air circulation oven) for 4 hours, after which time the yield of essential oil from candeia was gravimetrically determined.

2.5 Assessment of oil quality (GC)

The oil characterization procedure was performed in ESALQ using a gas chromatograph (GC) with alpha-bisabolol as standard reference. Oil injection followed its dilution in ethyl ether at a rate of 250 mg/L of solvent. An HP Ultra-2 capillary column was used (5% phenyl methyl siloxane, 25 m x 0.2 mm x 0.33 mm). The column flow rate was 1 mL/min, the injector temperature was 250 °C, and the oven temperature started at 45 °C for 2 min and increased at a heating rate of 5 °C/min until 280 °C. The injected volume was 1.0 ml, the split ratio was 1:20, and the carrier gas was helium.

2.6 Statistical analysis

The experiment followed a completely randomized design, with five replications per diameter class. The data showed homogeneity of variance according to Cochran test. An analysis of covariance was performed to fit tree age values, and the Tukey test at the 5% probability level was used for the relevant variables. The statistical model is given by:

$$Y_{ij} = \mu + t_i + \beta(x_{ij} - \bar{X}) + e_{ij},$$

Where: Y_{ij} = observation for i-th treatment in j-th replication; μ = general mean; t_i = effect of i-th treatment;

β = coefficient of linear regression; x_{ij} = covariate (age) as measured in i-th treatment in j-th replication; e_{ij} = experimental error.

3 RESULTS AND DISCUSSION

3.1 Soil analysis

Results of soil analysis are given in Table 1.

The sampled areas presented low fertility soils. The soil in Area 2 presented lower P content and higher K content. Phosphorus (P) is essential for plant growth, acting on photosynthesis, respiration, energy storage and transfer, cellular division and growth, and on various other plant processes (VENTURIM et al. 2005). Potassium (K) is also an important nutrient in that it influences plant metabolism, as in small amounts it reduces the supply of carbohydrates in plants (LOPES 1989).

As for secondary nutrients (Ca, Mg and S), no difference was detected in their content from Area 1 to

Area 2. As for micronutrients (B, Cu, Fe, Mn, Mo and Zn), no trace of these elements was found in either soil. Many nutrients, including Zn, Mn, Cu, Fe, Mo, Co, present low or no mobility in the soil, and that prevents their absorption by plants, particularly during the development stage which is when they have a high demand for nutrients, therefore lacking nutrients can potentially affect productivity (BRAKEMEIER 1999). B (boron) activates the starch phosphorylase enzyme which is responsible for the synthesis of starch, a reserve food stored by seeds, roots and stems. Boron deficiency may result in reduced flowering and seed formation as well as dieback with dead terminal buds, leading to concentration of growth hormone (IAA) in the leaves and branches, and contributing to lateral shoot growth and stunted roots (FAVARIN & MARINI 2000).

The cation exchange capacity values (CEC) ranged between 1.6 (Area 1) and 2.4 (Area 2). According to Lopes (1989), soils with CEC values between 1 and 10 have a high

Table 1 – Fertility and soil-water retention.

Tabela 1 – Fertilidade e Retenção de água no solo.

Fertility	Area 1 (1,000 m)	Area 2 (1,100 m)
pH (H ₂ O)	5.2	4.9
P (mg/dm ³)	1.2	0.6
K (mg/dm ³)	25	42
Ca ²⁺ (cmol _c /dm ³)	0.4	0.4
Mg ²⁺ (cmol _c /dm ³)	0.2	0.2
Al ³⁺ (cmol _c /dm ³)	0.9	1.7
H + Al (cmol _c /dm ³)	6.3	9.8
SB (cmol _c /dm ³)	0.7	0.7
CEC (t) (cmol _c /dm ³)	1.6	2.4
CEC (T) (cmol _c /dm ³)	7.0	10.5
V (%)	9.5	6.8
M (%)	58	71
OM dag/kg	4.0	4.3
res-P (mg/L)	10.9	9.9
Moisture Retention Curve	Area 1	Area 2
15 atm (%)	10.70	11.19
0.02 atm (%)	53.03	40.56

pH in water, KCL and CaCl₂ – Ratio 1:2.5; P–Na–K –Zn –Mn –Cu – Extractant Mehlich 1; Ca–Mg–Al – Extractant KCl 1N; H + Al Extractant: SMP; SB=Sum of Exchangeable Bases; CEC (t) – actual cation exchange capacity; CEC (T) cation exchange capacity; V=base saturation ratio; M=aluminum saturation ratio; Organic Matter (OM) oxidation: Na₂Cr₂O₇ 4N + H₂SO₄ 10N; res-P= residual phosphorus.

content of sandy material, being more prone to nitrogen and potassium leaching and having low water retention capacity. This can be confirmed by the sandy-silty texture of the areas studied.

The organic matter values were 4.0 in Area 1 and 4.3 in Area 2 and were associated to CEC and pH values, leading to conclude that overall both areas have a weathered soil. According to Schumacher et al. (1999), organic matter has a positive correlation with P, K, Ca, Mg, CEC and V, as verified in soils with high organic matter content and low pH in areas of high altitude. Thus the higher the concentration of organic matter, the higher the concentration of the above elements in the soil. We also noted a predominance of Al^{3+} in the soils, with higher concentrations in the soil of Area 2. Here, the percentage of calcium, magnesium and potassium saturation was also found to be low.

Observing the soil-water retention curve, we noted the soil in Area 1 to present a higher value than the soil in Area 2, in particular with water retention at 0.02 atm. According to Lima (1986), on the topic of soil-water

retention in forest regions, it can be said that declivity influences surface runoff and young stands with a scanty leaf litter coat will favor a greater surface runoff. Sandy soils allow greater infiltration of water in comparison to clayey soils, while land clearing fires directly influence surface runoff by eliminating residue and organic matter and producing hydrophobic substances that form an impervious layer to water at a shallow depth.

3.2 Determination of age

Table 2 presents a summary of analysis of variance for tree age and Table 3 presents mean values per area.

We noted that the age difference in candeia trees between different areas and diameter classes was statistically significant at the 5% probability level.

The Tukey test to compare age means in each diameter class showed that trees with larger diameter (class 3) were also older. In statistical terms, trees in class 1 and class 2 in Area 2 can be considered similar. Lower altitude Area 1 presented older trees, comparatively to higher altitude Area 2.

Table 2 – Analysis of variance for tree age in candeia.

Tabela 2 – Análise de variância da idade das árvores de candeia.

Source of Variation	DF	MS
Diameter class	2	120.73 *
Area	1	1,385.66*
Residual	23	20.21

DF: degree of freedom; MS: mean square; CV (%): coefficient of variation; *significant at the 5% probability level by the F test.

Table 3 – Mean values of tree age per diameter class.

Tabela 3 – Valores médios de idade das árvores por classe de diâmetro.

Area	DC	Age/DC		Age/Area	
		M	CV	M	CV
1	1	34 c	20.64	39.8 a	18.75
	2	40 b	12.73		
	3	45 a	9.26		
2	1	24 b	11.41	25 b	11.21
	2	24 b	12.03		
	3	27 a	6.04		

Area 1 = 1,000 m and Area 2 = 1,100 m; DC: diameter classes: 1 (5.0-10.0 cm), 2 (10.1-15.0 cm), 3 (15.1-20.0 cm); CV = coefficient of variation (%); M = mean. Means followed by the same letter in the same column do not significantly differ by the Tukey test at the 5% probability level.

3.3 Essential oil yield and alpha-bisabolol content

Mean and statistical values of essential oil yield and its alpha-bisabolol content are illustrated in Tables 4 and 5.

We noted that differences in yield were statistically significant for area and age and non-significant for diameter

class, at the 5% probability level. The alpha-bisabolol content presented significant differences from one area to the other, while in considering diameter classes the results were not statistically different. Results should be considered with some caution, however, given the high coefficients of variation of means which indicates the existence of important variability among trees.

Table 4 – Summary of analysis of variance for essential oil yield and alpha-bisabolol content.

Tabela 4 – Resumo da análise de variância do rendimento em óleo essencial e teor de alfa-bisabolol.

	Source of Variation	DF	MS
Yield	Diameter class	2	0.62x10 ⁻²
	Area	1	0.12 *
	Age	1	0.75 *
	Residual	22	0.82x10 ⁻¹
	CV	56.47	
Alpha-bisabolol content	Diameter class	2	130.30
	Area	1	164.78 *
	Age	1	2.65
	Residual	22	154.49
	CV	19.56	

Area 1 = 1,000 m and 2 = 1,100 m; Classes: 1 (5.0-10.0 cm), 2 (10.1-15.0 cm), 3 (15.1-20.0 cm); CV: coefficient of variation (%); DF: degree of freedom; MS: mean square; *significant at the 5% probability level by the F test.

Table 5 – Essential oil yield and alpha-bisabolol content - mean values per area and diameter class (%).

Tabela 5 – Rendimento em óleo essencial e teor de alfa-bisabolol - Valores médios por área e classe de diâmetro (%).

	Area	DC	Mean/DC		Mean/Area	
			M	CV	M	CV
Yield	1	1	0.32 a	38.03	0.67 a	56.47
		2	0.74 a	56.06		
		3	1.05 a	50.88		
	2	1	0.49 a	37.27	0.37 b	19.56
		2	0.35 a	50.68		
		3	0.30 a	28.38		
Alpha-bisabolol	1	1	63.4 a	10.57	58.88 b	27.46
		2	50.1 a	31.99		
		3	59.8 a	16.89		
	2	1	71.6 a	11.35	67.23 a	23.70
		2	69.7 a	12.93		
		3	60.4 a	31.43		

Area 1 = 1,000 m and 2 = 1,100 m; DC: diameter classes 1 (5.0-10.0 cm), 2 (10.1-15.0 cm), 3 (15.1-20.0 cm); M: mean; CV: coefficient of variation (%); Means followed by the same letter in the same column do not significantly differ by the Tukey test at the 5% probability level.

The yield of essential oil from candeia trees in Area 1 was higher than the yield in Area 2 by the Tukey test at the 5% probability level, there being no statistical differences in results among diameter classes. The essential oil content ranged between 0.67% (Area 1) and 0.37% (Area 2). As to alpha-bisabolol, younger trees in Area 2 presented higher alpha-bisabolol content than trees in Area 1, yet results were statistically similar in regard to diameter classes.

Siqueira (2002) observed that the oil component presenting the highest concentration was alpha-bisabolol (66.11%). On the same topic, Galdino et al. (2003) observed important differences in alpha-bisabolol content while studying variations in samples of yellow candeia, which ranged between 63.10% and 72%.

3.4 Influence of age on essential oil yield and alpha-bisabolol content

The influence of tree age on the yield of essential oil from candeia and its alpha-bisabolol content are illustrated in Table 6.

Table 6 – Pearson correlation coefficient between age and yield of essential oil from candeia wood and its alpha-bisabolol content.

Tabela 6 – Coeficiente de correlações de Pearson entre a idade com o rendimento em óleo essencial da madeira de candeia e seu teor de alfa-bisabolol.

Variable	Age
Yield of essential oil	0.652*
Alpha-bisabolol content	-0.347*

* significant at the 5% probability level by the t-test respectively.

From results in Table 6, we noted that, in the candeia trees studied, age had a significant correlation with the yield of essential oil and its alpha-bisabolol content. The yield of essential oil had a positive correlation with age, in other words, the older the trees the higher the yield of essential oil. The alpha-bisabolol content had a negative correlation with age, meaning that the older the trees the lower the alpha-bisabolol content in the essential oil. This can be explained by plant physiology: younger trees have a higher metabolic rate and therefore greater ability to produce and store reserve material for plant protection and growth, while in adult plants this metabolism tends to stabilize until senescence.

4 CONCLUSION

Qualitatively, the soils sampled have low fertility and present low pH and low water retention capacity, which affect the development of the plant. The yield of oil and its alpha-bisabolol content did not present differences from one diameter class to another, with higher yields of essential oil in the lower altitude area and among older trees. The alpha-bisabolol content was higher in the higher altitude area and among younger trees.

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