

Light impact assessment in planting and production of *Curcuma longa* in the Amazon, based on the analysis of its essential oils from leaves and rhizomes

Avaliação da incidência de luz no plantio e produção de *Curcuma longa*, com base na análise de seus óleos essenciais de folhas e rizomas

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Resumo

Curcuma longa L. produz rizomas tradicionalmente vendidos no mercado internacional como “Turmeric”. É um arbusto da Ásia, extensivamente cultivado na Índia, China, Japão e Indonésia. No Brasil, é conhecido como “Cúrcuma” ou “Açafrão” e utilizado principalmente em alimentos. O objetivo deste estudo foi avaliar o seu crescimento, dependendo da incidência de luz em cultivo experimental na região do Baixo Rio Amazonas, na cidade de Santarém, tendo por base a análise dos óleos essenciais de folhas e rizomas. As plantas obtidas no tratamento T1 (cultivo com 50% de sombreamento), após completar o ciclo de crescimento, mostraram melhor qualidade dos rizomas do que no tratamento T2 (cultivo a pleno sol). Estes resultados se refletiram na produtividade das plantas do tratamento T1 (rizomas frescos = 14,86 ± 0,20 tons/ha), que foi maior do que no tratamento T2 (rizomas frescos = 3,97 ± 1,05 tons/ha). O rendimento em óleo foi 200% maior nas folhas jovens (3,19%) do que nas folhas adultas (1,17%). Nos rizomas, o rendimento em óleo foi maior no tratamento a pleno sol (3,00%) do que no tratamento com sombreamento de 50% (1,90%). Os principais constituintes do óleo das folhas jovens e adultas foram os monoterpenos α -felandreno (33,6% e 26,2%), terpinoleno (22,0% e 18,3%), 1,8-cineol (16,0% e 15,8%), *p*-cimeno (3,8% e 9,8%), and β -pineno (4,9% e 7,2%). Nos óleos dos rizomas foram as cetonas sesquiterpênicas turmerona (T1, 37,5%; T2, 38,5%), curlona (T1, 17,4%; T2, 30,4%), and ar-turmerona (T1, 9,3%; T2, 8,8%).

Palavras-chave: Cúrcuma. Zingiberaceae. Amazônia. Plantas Medicinais. Arranjos produtivos locais. Óleos essenciais.

Abstract

Curcuma longa L. produces rhizomes traditionally sold on the international market as "Turmeric". It is a shrub from Asia, extensively cultivated in India, China, Japan and Indonesia. In Brazil, it is known as "Cúrcuma" or "Açafrão" and mainly used for food. The aim of this study was to evaluate the plant growth, depending on the incidence of light on an experimental cultivation in the region of Lower Amazon River, city of Santarém, based on the analysis of the essential oils from leaves and rhizomes. The plants obtained in treatment T1 (growing 50% shading), after completing the growth cycle, have showed better quality of rhizomes than in the treatment T2 (full sun cultivation). The results are reflected in the productivity of plants of the T1 treatment (fresh rhizomes = 14.86 ± 0.20 tonnes/ha), which was higher than T2 treatment (fresh rhizomes = 3.97 ± 1.5 tonnes/ha). The yield of oil was 200% higher in young leaves (3.19%) than in mature leaves (1.71%). In the rhizomes, the oil yield was greater in the treatment at full sunlight (T2, 3.00%) than in treatment with shading 50% (T1, 1.90%). The main constituents of the oil from young and mature leaves were the monoterpenes, α -phellandrene (33.6% and 26.2%), terpinolene (22.0% and 18.3%), 1,8-cineole (16.0% and 15.8%), p-cymene (3.8% and 9.8%), and β -pinene (4.9% and 7.2%). In the oils of rhizomes were the sesquiterpene ketones, turmerone (T1, 37.5%; T2, 38.5%), curlone (T1, 17.4%; T2, 30.4%), and ar-turmerone (T1, 9.3%; T2, 8.8%).

Keywords: Turmeric. Zingiberaceae. Amazon. Medicinal plants. Local productive arrangements. Essential oils.

Introduction

Curcuma longa L. (Zingiberaceae) produces rhizomes traditionally sold on the international market as "Turmeric". It is an evergreen shrub, native to South and Southeast Asia, which is extensively cultivated in India, China, Taiwan, Japan, Burma, Indonesia, beyond the African continent (SCARTEZZINI and SPERONI, 2000). In Brazil, the plant is known as "Açafrão", "Açafrão-da-terra" and "Cúrcuma". The use of Turmeric in foods such as dried soups, sauces, meat products and baking and desserts, among others, comes from the flavoring substances and responsible for its characteristic odor, in addition to its use as a dye (MAGDA, 1994; MARTINS and RUSIG, 1992; OLIVEIRA, GHIRALDINI and SACRAMENTO, 1992).

The *C. longa* culture was introduced in Brazil in the 80s, resulting in real productivity. It's grown in the states of Goiás, Mato Grosso and São Paulo, with an average production 8-12 tons of rhizomes per hectare and about 3.5% curcumin content (OLIVEIRA, GHIRALDINI and SACRAMENTO, 1992). In addition to the dye curcumin, the plant produces essential oil of excellent organoleptic quality that, together, can extend its use to the cosmetics markets, medicinal and textiles (CECÍLIO FILHO et al., 2004). The volatiles of rhizomes and leaves are also responsible for various biological activities found in the plant (ALMEIDA, 2006).

The stimuli from the environment in which the plant is located may redirect the metabolic pathways in the biosynthesis of the major compounds. Among these, the constituents of essential oils can provide qualitative and quantitative changes, due to abiotic factors such as temperature, rainfall, brightness,

etc. Solar radiation is a factor that can directly influence the growth of plants resulting in morphological and physiological changes, as well as the content and composition of secondary metabolites (SIMÕES et al., 2007; TAIZ and ZEIGER, 2004).

The essential oils of *C. longa* are reported as products with different physicochemical properties. Some of these differences are attributed to variations in the feedstock and the process of obtaining the oil, such as the degree of maturation of samples and the distillation time (GOVINDARAJAN, 1980). The oil composition of *C. longa*, mainly from specimens grown in India, has been described in the literature. In the leaves oil, the principal constituents were monoterpenes, such as α -phellandrene, 1,8-cineole, *p*-cymene, myrcene, terpinolene and camphor (SHARMA et al., 1997; CHANE-MING et al., 2002; BEHURA and SRIVASTAVA, 2004; RAINA, SRIVASTAVA and SYAMSUNDAR, 2005). In the rhizomes oil have predominated sesquiterpenes from tumerone, zingiberene and curcumene types (SHARMA et al., 1997; CHANE-MING et al., 2002; RAINA, SRIVASTAVA and SYAMSUNDAR, 2005; SINGH et al., 2010).

In Brazil, few studies describe the volatile composition of "Cúrcuma" (turmeric) and, in particular, have not been reported crops conducted in the Amazon region (BRAGA et al., 2003). The objective of this study was to evaluate the growth of *C. longa* depending on the incidence of light in an experimental planting in the region of the Lower Amazon River (State of Pará West). The experiment was conducted as a way to add value to local specimens used as colorants for food and as part of a family farming project. It was used as parameters, the percentage of sprouting, and the productivity of rhizomes, the essential oil content and volatile composition of leaves, throughout the plant life cycle.

Materials and Methods

Installation and cultivation conditions

The experiment was conducted between January and October, in the "Farmácia Viva" area, belonging to the Universidade Federal do Oeste do Pará, located on Highway Everaldo Martins (PA-457), Caranazal community, Km 26, Santarém, Pará state, with the following coordinates: 02°30'49.9" S and 54°56'06.8" W. Rhizomes from specimens of the rural area of Santarém and without particular denomination of germplasm were washed and cut into pieces of 2.0 cm and weighing an average of 2.0 g. Then, were planted in beds of 6.0 x 1.0 m and 0.20 m high, using black soil and cattle and chicken manure (ratio of 5:1:1) as the substrate, in the spacing of 0.20 x 0.20 m, with a piece of rhizome per hole. Plant samples were deposited in the Herbarium of Embrapa Amazônia Oriental (Belém city, Pará State, Brazil) under the code IAN 188089.

To evaluate the influence of solar radiation on the productivity of rhizomes, based on the variation of the contents and composition of the essential oil of the leaves and rhizomes, the cultivation was done in two treatments with three replicates (beds) for each selected condition, which were: T1 (growing under a partially shady screen allowing the passage of 50% of sunlight) and T2 (growing under 100% of sunlight). Cultural practices necessary to the culture were performed: the manual control of weeds, permanently, and irrigation every other day using drip next to the holes.

After the stability of planting, it was held the collection of the leaves in each luminosity condition, intending to determine the content and analysis of essential oil composition, in different stages of the plant life cycle.

The samples were taken at three, five and eight months, corresponding to rain, transition, and dry periods, according to the meteorological data. At the end of the plant life cycle (8 months), beyond the leaves, were collected the rhizomes of the T1 and T2 treatments, to estimate their productivity, and evaluation of content and the oil composition. (TABLE 1) summarizes the conditions for collecting leaves and rhizomes of the *C. longa*. For the present study were analyzed the seasonal variation in rainfall for each month, and the monthly average values of temperature and relative humidity.

TABLE 1 – Collection data of *Curcuma longa*

Ambient conditions	Sample 1	Sample 2	Sample 3
Month of collection	May	July	October
Seasonal period	Rainy period (December/June)	Transitional period (July)	Dry period (August/November)
Plant age	Three months	Five months	Eight months
Vegetative stage	Fertile	Infertile	Infertile
Appearance of leaves	Green	Green	Semi-dry
Plant part	Leaves	Leaves	Leaves and rhizomes

Climatic Data

Climatic factors such as temperature (°C), solar radiation (W/m²), relative humidity (%) and rain precipitation (mm) were measured monthly from January to December 2010. Data were obtained from equipment used at the meteorological station of LBA Project (Large Scale Biosphere-Atmosphere Experiment in Amazonia), located in the region of Santarém, Pará state, Brazil.

Extraction and quantification oil

In different times of collection of leaves and rhizomes of *C. longa*, to evaluate the essential oil yield, it was used hydrodistillation method in a Clevenger-type apparatus. The extraction time was 3h, with three replicates for each sample. The essential oils obtained were packed in amber glass vials and stored in a refrigerator at 5°C. The calculation of oil yield was achieved through the relationship between the volume and dry weight of plant material. The humidity was calculated on the balance of moisture determiner (Celtac, DHS-16 model). Analyzes were performed in triplicate.

Oil composition analysis

The oils were analyzed on a GC-MS Thermo Focus DSQ II, under the following conditions: DB-5ms (30 m x 0.25 mm; 0.25 µm film thickness) fused-silica capillary column; programmed temperature: 60–240°C (3°C/min); injector temperature: 250°C; carrier gas: helium, adjusted to a linear velocity of 32 cm/s (measured at 100°C); injection type: split (2 µl of a 1:1000 hexane solution); split flow was adjusted

to yield a 20:1 ratio; septum sweep was a constant 10 ml/min; EIMS: electron energy, 70 eV; temperature of ion source and connection parts: 200°C. The quantitative data regarding the volatile constituents were obtained by peak area normalization using a GC-FID Thermo Focus operated under similar conditions to the GC-MS, except for the carrier gas, which was nitrogen and detector temperature at 250 °C. The retention index was calculated for all the volatiles constituents using an *n*-alkane (C8-C40, Sigma/Aldrich) homologous series. Individual components were identified by comparison of both mass spectrum and GC retention data with authentic compounds which were previously analyzed and stored in a private library, as well as with the aid of commercial libraries containing retention indices and mass spectra of volatile compounds commonly found in the essential oils (NIST, 2005; ADAMS, 2007).

Statistical analysis

Quantitative data were submitted to analysis of variance (ANOVA) and, when observed a significant difference, it was performed the Student t test (two means) or Tukey test (more than two means), at 5% probability, using the Assisat software (version 7.6). The experiment was conducted completely randomized (in blocks).

Results and Discussion

Budding and productivity, and yield of essential oil from rhizomes

The percentage of budding plants and productivity, and essential oil yield of rhizomes, concerning the treatments T1 (50% luminosity) and T2 (100% luminosity), are summarized in Table 2. In both treatments, the budding began between 20-30 days after planting, with higher productivity and budding index ($p \leq 0.05$) for plants grown in T1 (**TABLE 2**). The plants obtained in T1 treatment, after completing the growth cycle, have showed better quality of rhizomes compared with the T2 treatment, where rhizomes were smaller and with fewer clumps. These results were reflected in the productivity of plants belonging to treatment T1 (fresh rhizomes = 14.86 ± 0.20 tons/ha), which was higher than in treatment T2 (fresh rhizomes = 3.97 ± 1.05 tons/ha). Based on these data, it became apparent that the luminosity has exerted an effect on productivity and budding of rhizomes.

TABLE 2 - Percentage of budding and productivity for the *Curcuma longa* rhizomes, in T1 and T2 treatments

Treatments	Budding (%)	Productivity (tons/ha)	
		Fresh rhizomes	Dry rhizomes
T1 (luminosity 50%)	89.67 ± 6.07^a	14.86 ± 0.20^a	4.38 ± 0.06^a
T2 (luminosity 100%)	64.90 ± 10.69^b	3.97 ± 1.05^b	0.71 ± 0.19^b

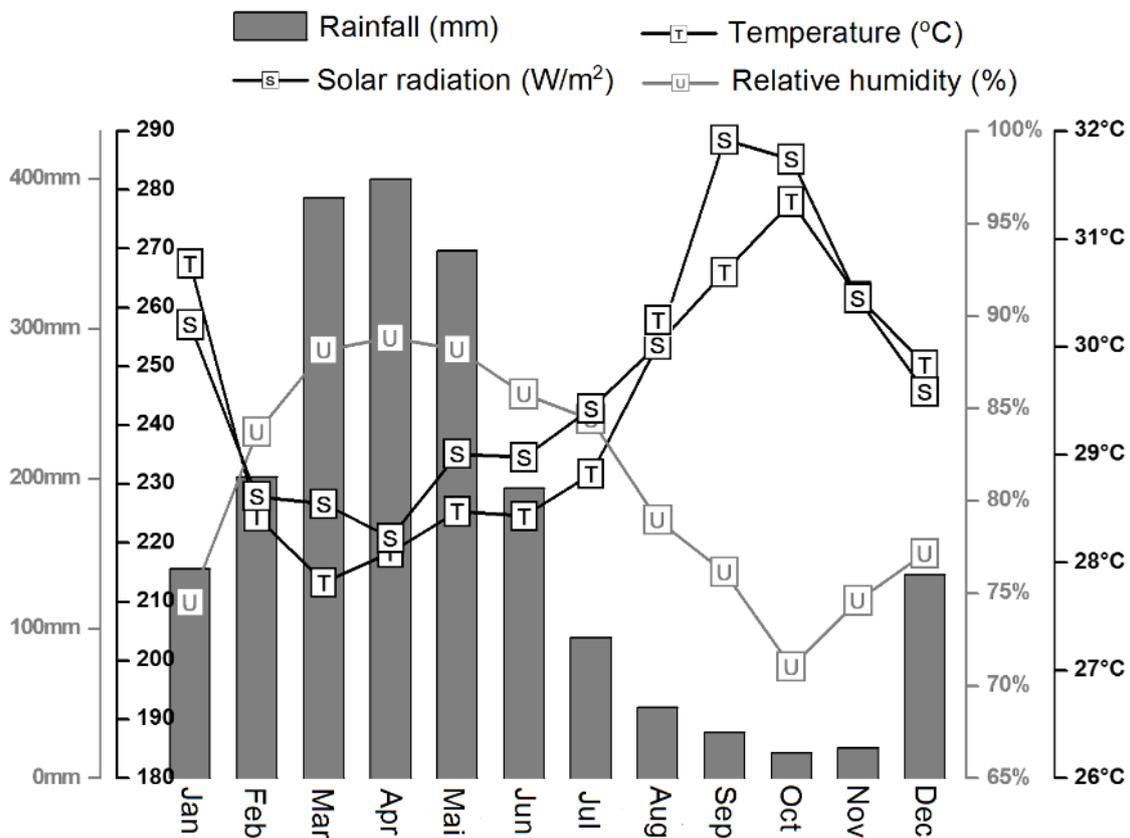
Values represent the mean \pm standard deviation, with three replicates for each treatment. Means followed by different letters in the column differ significantly by Student's t test ($p \leq 0.05$).

Shaded planting can present better results for species that have the ability to grow in lower lighting conditions, due have photosynthetic mechanisms best adapted to these circumstances (PORTELA, SILVA and PINÂ-RODRIGUES, 2001). That's what probably happened with the *C. longa*, which develops best in an experiment with 50% shading.

Climatic conditions

The *C. longa* cultivation was carried out at reduced cost, to be replicated by small farmers in their local productive arrangements with medicinal plants. During the stabilization and growing of the plant, some climate variables were monitored to intending to its adaptation to soil and climate conditions of the region. These variables were rainfall precipitation, temperature, relative humidity and solar radiation (FIGURE 1). As can be seen, the study area is characterized by two distinct seasons. The rainy season extends from December to June with rainfall above 100 mm per month, being March, April and May the wettest months. July is a passing month. The dry season covers the months of August to November, with October and November months the driest and hottest of the year. The rains content (rainfall) in 2010 was 2,019 mm. In the dry season of 2010, the average air temperature was 30°C, with a relative humidity of 77% and an average insolation flow of 266 W/m². In the wet season of 2010, the values of these variables were 28°C, 83% and 235 W/m², respectively.

FIGURE 1 - Monthly averages of temperature (T, °C), humidity (%), solar radiation (W/m²) and precipitation (mm) in the region of Santarém, Pará, Brazil, during the trial period (January-December 2010).



Yield and composition of essential oil from leaves and rhizomes

With the growth of *C. longa*, comparing the average of the oil content in the leaves of the T1 and T2 treatments, it was observed that there was a reduction of about 200% in oil yield of adult leaves (T1, $1.17 \pm 0.15\%$; T2, $1.00 \pm 0.10\%$, eight months), in relation to young leaves (T1, $3.19 \pm 0.14\%$; T2, $3.50 \pm 0.23\%$, three months). These results can be seen in Table 3, demonstrating that the young leaves can produce a higher amount of oil or can have mechanisms to storage oil that prevent its evaporation. For plants of treatment T2, the maximum production of oil from leaves has occurred in the fifth month, whereas in plants of treatment T1, the reduction in the oil content was continued until the eighth month. Despite this observation, by the end of the life cycle of the plant, at eight months of age, there was no significant difference ($p \leq 0.05$) in yield of oil from leaves, between the two treatments.

It was observed a reversal in T1 and T2 treatments, concerning the yield of oil from rhizomes, with higher yield ($p \leq 0.05$) for the T2 treatment ($3.00 \pm 0.22\%$) in comparison to T1 treatment ($1.90 \pm 0.13\%$) (TABLE 3). The increased production of secondary metabolites in a high level of sunlight can occur in some plants, because the light intensity increases the formation of oil-producing glands, with a consequent rise in the content of essential oil (SILVA et al., 2006). For *C. longa*, it is very likely that planting in full sun was a stress condition, whose initial propagation occurred in the rainy season, with little sun exposition. In the plant survival mechanism, an attempt to reduce water loss by transpiration can lead to the production of higher essential oil content, as the present case of *C. longa*.

TABLE 3 - Essential oil yields of leaves and rhizomes of *Curcuma longa*, based on the treatments T1 and T2

Treatments	Oil %		
	May (3 months)	July (5 months)	October (8 months)
T1 - Leaves	3.19 ± 0.14	2.36 ± 0.18	1.17 ± 0.15
T2 - Leaves	3.50 ± 0.23	5.25 ± 0.09	1.00 ± 0.10
T1 - Rhizomes	wr	yr	1.90 ± 0.13
T2 - Rhizomes	wr	yr	3.00 ± 0.22

Values represent the mean \pm standard deviation, with three replicates for each treatment; wr = plant without rhizome; yr = plant with young rhizome.

The constituents of the essential oils of young and adults leaves of *C. longa*, resulting from the treatments T1 and T2, were analyzed by GC and GC-MS (TABLE 4). The primary identified components (above 5%) were the monoterpenes α -phellandrene (24.2-33.8%), terpinolene (16.5-22.6%), 1,8-cineole (14.7-16.3%), *p*-cymene (2.2-12.4%), and β -pinene (4.9-7.2%), whose sum ranges between 75 and 80%, when it is considered the total content of the constituents in the oil. These compounds were the same previously described in the literature (SHARMA et al., 1997; CHANE-MING et al., 2002; BEHURA and SRIVASTAVA, 2004; RAINA, SRIVASTAVA and SYAMSUNDAR, 2005). There was no significant variation in the percentage of these compounds in young plants harvested three to five months, in both treatments (T1 and T2). On the other hand, in the adult plant collected

after eight months, there was an increase in the percentage of *p*-cymene (T1, 9.8%, T2, 12.4%) and a proportional reduction of their biogenic precursors, α -phellandrene (T1, 26.2%; T2, 24.2%) and terpinolene (T1, 18.3%; T2, 16.5%), as would be expected for a compound with higher oxidation pattern and stability, as the *p*-cymene.

TABLE 4 – Constituents (%) of oils of leaves and rhizomes from *Curcuma longa*, based on the T1 and T2 treatments.

Constituents	RI	May		July		October			
		T1	T2	T1	T2	T1	T2	T1	T2
		Leaves (%)						Rhizomes (%)	
α -Thujene	924	0.1	0.2	0.2	0.2	0.2	0.2		
α -Pinene	934	2.8	3.0	3.0	3.0	4.0	4.3		0.3
α -Fenchene	945	0.1	0.1	0.1	0.1				
Camphene	948	0.1				0.1			
Sabinene	970	0.5	0.6	0.5	0.5	0.4	0.5		
β -Pinene	978	6.3	5.5	5.0	6.7	7.2	6.9		
Myrcene	990	3.1	3.4	3.4	3.2	2.8	3.1	0.2	0.1
α -Phellandrene	1003	31.5	31.5	33.6	33.8	26.2	24.2	12.0	4.4
δ -3-Carene	1008						2.5		
α -Terpinene	1016	1.8	1.9	2.1	2.0	1.4	1.3	0.2	0.1
<i>p</i> -Cymene	1022	4.5	4.6	3.8	2.2	9.8	12.4		
1,8-Cineole	1032	15.2	16.3	16.0	15.4	15.8	14.7	6.7	5.7
(<i>E</i>)- β -Ocimene	1045	0.6	0.7	0.7	0.6	0.5	0.5		
γ -Terpinene	1056	2.4	2.5	2.7	2.4	1.9	1.8	0.3	0.2
Terpinolene	1088	22.5	21.7	22.0	22.6	18.3	16.5	2.1	1.0
Linalool	1095	1.4	1.5	1.4	1.6	1.3	1.1		
(<i>E</i>)-pinocarveol	1135					0.1	0.5		
δ -Terpineol	1160	0.2	0.2	0.2	0.2	0.2	0.2		
Pinocarvone	1164					0.1	0.1		

Terpinen-4-ol	1174	0.6	0.5	0.5	0.5	1.0	1.0	0.1	
<i>p</i> -Cymen-8-ol	1181					0.5	0.6		
α -Terpineol	1187	0.7	0.8	0.8	0.8	0.9	0.8	0.2	0.1
Myrtenol	1194					0.6	0.8		
Undecan-2-one	1293	0.1	0.1	0.1	0.1	0.1			
(<i>E</i>)-Caryophyllene	1416	0.1	0.1	0.1	0.1				0.2
α -Humulene	1445								0.1
(<i>E</i>)- β -Farnesene	1457	0.6	0.5	0.3	0.8				
<i>ar</i> -Curcumene	1477							0.3	0.4
α -Zingiberene	1493		0.1	0.1	0.1	0.1	0.2	0.6	0.6
(<i>Z</i>)- α -Bisabolene	1504							0.1	0.2
(<i>E,E</i>)- α -farnesene	1506	0.3	0.3	0.1	0.4				
β -Sesquiphellandrene	1523					0.1	0.2	0.7	0.8
(<i>E</i>)-Nerolidol	1563	0.1	0.1	0.1	0.1	0.1	0.1	0.5	0.2
<i>ar</i> -Turmerol	1574							0.5	0.2
Caryophyllene oxide	1582					0.1	0.1		
<i>ar</i> -dihydro-Turmerone	1594							0.1	0.1
β -Atlantol	1606								0.3
1- <i>epi</i> -Cubenol	1628								0.1
<i>ar</i> -Turmerone	1662							9.3	8.8
Turmerone	1668	0.8	1.0	0.7	0.8	0.8	0.9	37.5	38.5
Curlone	1681	0.3	0.4	0.3	0.3	0.3	0.4	17.4	30.4
Curcuphenol	1706							0.2	0.1
(6 <i>R</i> , 7 <i>R</i>)-Bisabolene	1737							0.6	1.2
(<i>Z</i>)- α -Atlantone	1741							1.3	0.7
(<i>E</i>)- α -Atlantone	1751							0.2	0.1

(E)- γ -Atlantone	1764							0.3	0.9
Oxygenated sesquiterpenes unidentified	1599-1777							5.9	1.8
Total		96.6	97.5	97.6	98.3	94.9	95.8	96,8	97,4

RI = Retention Time on DB-5ms capillary column, using homologous series of n-alkanes.

The constituents of the essential oils of rhizomes, resulting from the T1 and T2 treatments, (**TABLE 4**). The main components were the sesquiterpene ketones turmerone (T1, 37.5%; T2, 38.5%), curlone (T1, 17.4%; T2, 30.4%), and ar-turmerone (T1, 9.3%; T2, 8.8%), followed by α -phellandrene (T1, 12.0%; T2, 4.4%) and 1,8-cineole (T1, 6.7%; T2, 5.7%), as previously reported (LEELA et al., 2002; RAINA, SRIVASTAVA and SYAMSUNDAR, 2005; SINGH et al., 2010). The total percentage of sesquiterpene ketones was higher in the treatment T2 (77.7%, full sun) than in treatment T1 (64.2%, 50% shading). Therefore, in the production of turmeric, if the interest is the volatile fraction containing the sesquiterpene ketones with biological activity, the ideal cultivation will be in full sun for eight months.

Conclusion

Based on the results, this work recommends that the best cultivation condition for rhizomes of *Curcuma longa* occurs with 50% of shading, which will reflect higher productivity. Therefore, it is apparent that the luminosity exerts an effect on productivity and budding of rhizomes. Comparing the average of the oil content in the leaves of both treatments (50% shading and full sun), it was observed that there was a reduction in the yield of adult leaves oil, in relation to young leaves oil, demonstrating that this latter can produce a higher amount of oil or can have mechanisms to storage oil that prevent its evaporation. The oil of leaves is rich in monoterpenes while in the oil of rhizomes predominates the ketones sesquiterpenes with biological activity.

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