

## Article

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## YIELD AND COMPOSITION OF THE ESSENTIAL OIL OF *Tetradenia riparia* (HOCHST) CODD (LAMIACEAE) CULTIVATED UNDER DIFFERENT SHADING LEVELS

*Rendimento e Composição do Óleo Essencial de Tetradenia riparia (Hochst) Codd (Lamiaceae) Cultivada sob Diferentes Níveis de Sombreamento*

**ABSTRACT** - Light has direct influence on growth and development by altering the morphophysiology of the plant and the content and composition of secondary metabolites. The present study aimed to evaluate the yield and composition of the essential oil of *Tetradenia riparia* cultivated under different shading levels. Plants were propagated by cuttings and cultivated either under full sunlight and in environment completely covered by black polyethylene sheeting to obtain shading levels of 30%, 50%, and 80%. Plants were grown under experimental conditions for 150 days. The essential oil was extracted from fresh leaves in triplicate for each treatment through steam distillation with a modified Clevenger apparatus. The components of the essential oil were identified using a gas chromatograph coupled with a mass spectrometer (GC/MS), and compared to retention indices and authentic mass. The largest yields of essential oil came from plants cultivated under 30% and 50% shading, followed by plants cultivated under full sunlight and under 80% shading. The main component found was the sesquiterpene hydrocarbon 14-hydroxy-9-*epi*-(*E*)-caryophyllene. Some components, such as verbenone, were only found in the treatment under full sunlight, while numerous others were exclusive to the different shading treatments. The yield and chemical composition of the essential oil of *T. riparia* is influenced by the level of shading.

**Keywords:** 14-hydroxy-9-*epi*-(*E*)-caryophyllene, medicinal plant, secondary metabolites, shading levels.

**RESUMO** - A luz tem influência direta no crescimento e desenvolvimento, alterando a morfofisiologia da planta e o conteúdo e composição dos metabólitos secundários. O presente estudo teve como objetivo avaliar o rendimento e composição do óleo essencial de *Tetradenia riparia* cultivada em diferentes níveis de sombreamento. As plantas foram propagadas por estaquia e cultivadas tanto em pleno sol como em minicasas de vegetação completamente cobertas com telas de polietileno pretas, a fim de proporcionar diferentes níveis de sombreamento: 30%, 50% e 80%. As plantas foram cultivadas sob condições experimentais de 150 dias. O óleo essencial foi extraído a partir de folhas frescas em triplicata para cada tratamento pelo método de hidrodestilação por arraste de vapor d'água, utilizando-se aparelho Clevenger modificado. Os componentes de óleo essencial foram identificados utilizando-se um cromatógrafo gasoso acoplado a espectrômetro de massas (CG/EM) e por comparação com seus índices de retenção e espectros de massa autênticos. Os maiores rendimentos de óleo essencial foram observados em plantas cultivadas sob 30% e 50% de sombreamento, seguidas por plantas cultivadas em pleno sol e sob 80% de sombreamento. O principal componente encontrado foi de

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*hidrocarboneto sesquiterpênico 14-hidroxi-9-epi-(E)-cariofileno. Alguns componentes, como a verbenona, foram encontrados somente no tratamento em pleno sol, enquanto numerosos outros foram exclusivos dos tratamentos de sombreamento. O rendimento e a composição química do óleo essencial de T. riparia são influenciados pelo nível de sombreamento.*

**Palavras-chave:** 14-hidroxi-9-epi-(E)-cariofileno, metabólitos secundários, níveis de sombreamento, planta medicinal.

## INTRODUCTION

The Lamiaceae is the largest family of the order Lamiales, including about 20 to 30 families. The family currently contains about 240 genera and 7200 species, occurring in tropical and temperate climates in all areas of the world (Harley, 2012). In Brazil there are 46 genera and about 524 species (Harley et al., 2015).

*Tetradenia riparia* (Hochst) Codd, a species of the Lamiaceae, is popularly used as a medicinal plant to treat malaria, angina, tropical skin disease, gastroenteritis, gonorrhoea, diarrhea, dental abscesses, headaches, bronchitis, coughs, ulcers, female sterility, kidney diseases, and fever (van Puyvelde et al., 1986). Laboratory studies confirmed the antimicrobial properties of this plant against *Candida albicans*, *Shigella dysenteriae*, and *Streptococcus pyogenes* (Dunkel et al., 1990), antioxidant and cytotoxic activity compounds (Gazim et al., 2014) and acaricidal effect (Gazim et al., 2011). In Brazil, *T. riparia* is used as an exotic ornamental plant in parks, residential gardens, and botanical gardens, and it is popularly known as incenso, lavândula, lemonete, pluma-de-névoa, or falsa mirra. The plant exudes an intense aroma derived from the presence of essential oils (Martins et al., 2008).

Essential oils form a very important class of secondary metabolites. They are usually extracted from plants through steam distillation, but may also be extracted by pressing the pericarp of citrus fruits, which dominate the Brazilian export market. Essential oils are mostly composed of monoterpenes, sesquiterpenes and phenylpropanoids, metabolites that are responsible for their organoleptic properties (Bizzo, 2009).

Such oils are considered to be one of the potential sources for the screening of antimicrobial and antioxidant agents in Lamiaceae species such as rosemary and basil (Celiktaş et al., 2007; Hussain et al., 2008).

Light has a direct influence on growth and development by altering the morphophysiology of the plant and the content and composition of secondary metabolites, which are the purpose of cultivating plants for pharmacological applications (Szakiel et al., 2011; Vasanthaiyah and Kambiranda, 2011). White light is estimated to modify the expression of 20% of the genome in rice and *Arabidopsis* seedlings (Jiao et al., 2005), which may then affect different metabolic routes in plants, including those involved in secondary metabolism. Light may affect yield and composition of essential oils in some Lamiaceae species. Li et al. (1996) registered higher essential oil yield in thyme (*Thymus vulgaris* L.) cultivated under the sun. But in the other hand, in *Plectranthus neochilus* Schlechter, the yield was higher when the plants were grown under shading conditions (Rosal, 2008).

Although these effects are well known, few studies to date have shown the broad effects of different light intensities on the induction or inhibition of the synthesis of different chemical components of essential oils, as proposed here. Some studies explored only the role of light in the quantitative production of essential oils, and some of them inferred that light may modulate the relative content of a given chemical component depending on treatment. Therefore, the goal of the present study was to evaluate the effect of different levels of shading during the cultivation of *T. riparia* on the yield and composition of essential oils.

## MATERIAL AND METHODS

Saplings of *T. riparia* were obtained from the medicinal plant garden of the Agência Goiana de Assistência Técnica, Extensão Rural e Pesquisa Agropecuária (EMATER-GO), propagated by

cuttings, and cultivated in an experimental area near the Instituto de Ciências Biológicas of Universidade Federal de Goiás, Goiânia, GO, located at 16°36' S and 49°13' W, altitude of approximately 800 m. After the saplings had three ripe leaves, they were cultivated under full sunlight or in environment completely covered by black polyethylene sheeting to obtain shading levels of 30%, 50%, and 80%. Each light level was considered a different treatment.

Twenty plants per treatment were cultivated from January to July 2013 in 3 liter plastic bags filled with a commercial substrate (Plantmax®). Plants were watered daily. The substrate was supplemented with 5 g of NPK 4-14-8 after 30 and 60 days of growth.

A porometer LI-1600 was used to measure the level of photosynthetically active radiation at noon at the median level of the crown of plants in full sunlight and in the mini-greenhouses, relative to the external environment. The 30% shading sheet retained 38% of the radiation; the 50% sheet, 45%, and the 80% sheet, 68%. The experimental design was completely randomized.

The extraction of the essential oil of *T. riparia* was made at the Natural Products Laboratory of the Faculdade de Farmácia of Universidade Federal de Goiás. Oil was extracted from 45 g of fresh leaves collected at 7 AM from plants in the vegetative state after 150 days of treatment. Extractions were made in triplicate and it was performed by hydrodistillation method using a modified Clevenger apparatus with a 1000 mL round-bottom flask was used for hydrodistillation. Each run used 500 mL of distilled water (Simões et al., 2007). The hydrolate was subjected to liquid-liquid partition in a separatory funnel using three 25 mL portions of dichloromethane for 20 min each. The organic fractions from each run were combined and dried with anhydrous sodium sulphate. Salt was removed by simple filtration, and the solvent was evaporated in an exhaust hood at room temperature until it reached a constant weight, to obtain a purified essential oil. Yield results for the essential oil were expressed as the essential oil content in relation to fresh mass, in percentages (% v/w). Aliquots of one to two drops per treatment were taken to identify chemical compounds. Quantitative yield data were analyzed through an ANOVA, and the means were compared using Tukey's multiple comparison test and a 0.05 probability.

The chemical components of the essential oil were identified at the Organic Chemistry Laboratory of the Faculdade de Química of Universidade Federal de Goiás. We used a Shimadzu QP 5050 gas chromatograph coupled with a mass spectrometer (GC/MS) with a CBP-5 capillary column (30 cm x 0.25 mm x 0.25 µm) and helium flow of 1.0 mL min<sup>-1</sup>. The initial programmed temperature was 60 °C for two min, followed by heating at a rate of 3 °C per min up to 240 °C and 10 °C per min up to 280 °C, which was maintained for 10 minutes (van Den Dool and Kratz, 1963). Identification of the compounds relied on a digital database, the mass spectral library NIST11/2011/EPA/NIH, and on a comparison to retention indices and authentic mass spectra in Adams (2007).

Retention indices were calculated by co-injecting of a mixture of Sigma-Aldrich hydrocarbons (C8–C32) and applying the van Den Dool and Kratz (1963) equation. The relative proportions of the main compounds obtained by peak area normalization.

## RESULTS AND DISCUSSION

Essential oil yield of *T. riparia* varied according to the level of shading. Treatments with 30% and 50% of shading presented the highest yields, followed by full sunlight and 80% shade (Table 1).

**Table 1** - Percent yield (v/w) of the essential oil of *Tetradenia riparia* (Hochst) Codd cultivated under different levels of shading

Full sunlight	Levels of shading		
	30%	50%	80%
0.17 <sup>(2)</sup> ± 0.05	0.26 <sup>(1)</sup> ± 0.07	0.22 <sup>(1)</sup> ± 0.06	0.09 <sup>(3)</sup> ± 0.04

<sup>(1)</sup> Mean values obtained from triplicate measurements. <sup>(2)</sup> Means followed by the same letter are not significantly different according to Tukey test, P ≤ 0.05. <sup>(3)</sup> Mean values are followed by the percent coefficient of variation.

For all treatments, the main component of the essential oil of *T. riparia* was the sesquiterpene hydrocarbon 14-hydroxy-9-*epi*-(*E*)-caryophyllene, with a relative content of 16.03%, 16.48%, 16.41%, and 16.42% for plants cultivated under full sunlight, 30%, 50%, and 80% shading, respectively. Plants cultivated in full sunlight and under 30% and 50% shading also had high levels of the monoterpeneoid fenchone (7.90%, 9.93% and 7.78%, respectively), which was only 3.59% of the oil from the 80% shading treatment (Table 2).

The chemical compounds tricyclene, verbenone,  $\alpha$ -humulene, chamigrene and *epi*- $\alpha$ -cadinol were found exclusively in individuals of *T. riparia* cultivated in full sunlight. In addition,  $\gamma$ -himachalene and  $\alpha$ -muurolene were only identified in plants cultivated under 30% shading. There were also chemical compounds found exclusively in plants grown under 50% shading, such as  $\alpha$ -pinene,  $\beta$ -phellandrene, longifolene, widdra-2,4(14)-diene, and 1,7-diepi- $\alpha$ -cedrene, and in plants grown under 80% shading, such as  $\beta$ -selinene e  $\alpha$ -muurolol (Table 2).

Essential oil production is directly correlated to the metabolic capacity of the plant under ideal conditions of radiation intensity and quality (Meira et al., 2012), and varies considerably depending on the species. An increased yield of essential oil in plants cultivated under shade when compared to individuals in full sunlight, as found in the present study for the 30% and 50% shading treatments (Table 1), was also observed for *T. riparia*, *Pothomorphe umbellata* L. and *Melissa officinalis* L. (Mattana et al., 2010; Meira et al., 2012). Essential oil production is usually associated with higher irradiance levels, higher photosynthetic rates, and increased biomass accumulation (Ramos et al., 2005; Pinto et al., 2007; Souza et al., 2007; Gomes et al., 2009). However, in scyophytes and in plants that grow well under low levels of irradiation, such as *T. riparia*, high irradiance levels may trigger photoinhibitory processes that lead to a reduced yield of essential oils, as observed here (Table 1). In species that only grow well in high irradiance environments, on the other hand, shading may reduce essential oil content due to reduced photosynthetic rates and lower biomass production (Mattana et al., 2010).

Regarding Lamiaceae species, Costa et al. (2014) registered decreased growth and essential oil yield for peppermint (*Mentha piperita* L.) cultivated under shading levels, leading the authors to suggest its growth under full sunlight. Pegoraro et al. (2010) point out that intense sunlight influences the diversity of essential oil compounds of peppermint, as in this study and improves its yield per plant. Some other species outside the family, e.g. *Aloysia gratissim* (Gillies and Hook) Tronc. (Verbenaceae), may be subjected to 80% shading without a reduction in essential oil production, unlike what was found for *T. riparia* in this present study (Table 1), although these individuals have thinner leaf blades and lower leaf dry mass (Pinto et al., 2007). Such different responses demonstrate the strong influence of the genotype on light regulation of the synthesis of essential oils, which may vary depending on the adaptation or tolerance of different plant species to different light patterns.

In *T. riparia*, the main component of the essential oil was the sesquiterpene hydrocarbon 14-hydroxy-9-*epi*-(*E*)-caryophyllene, regardless of the irradiance level under which plants were grown (Table 2). Similar results were obtained for all irradiance levels for *Hyptis marrubioides* Epl., where the major component of the essential oil was the oxygenated monoterpene *cis*-thujone (Sales et al., 2009), and for *Mentha x piperita* L., where menthol was the main component (Pegoraro et al., 2010), regardless of the irradiance level. In contrast, a sharp reduction in phenolic content, particularly of monoterpene hydrocarbons, was observed for thyme cultivated under shade; similarly, the content of thymol, the main essential oil component from plants in full sunlight, was drastically lower for plants in the shade (Letchamo et al., 1994; Letchamo and Gosselin, 1995). Thus, the influence of radiance on molecule biosynthesis depends on their origin or specific chemical properties.

The content of chemical compounds did vary in the present study based on radiance levels. For instance, we found lower content of sabinene, limonene, camphor, isoborneol, (*E*)-caryophyllene and  $\alpha$ -terpineol for plants cultivated under 80% shading, and higher content of  $\alpha$ -*trans*-bergamotene and cubenol under 50% shading compared to other treatments (Table 2). Likewise, the composition of the essential oil of *Hyptis marrubioides* varied depending on the radiance level of the environment where plants were grown (Sales et al., 2009). Shading basil plants (*Ocimum basilicum* L.) did not decrease the oil content but affected the relative composition of volatile compounds (Chang et al., 2008).



**Table 2** - Chemical composition of the essential oil of *Tetradenia riparia* (Hochst) Codd cultivated under different levels of shading

Levels of shading	Component	Kovats index (KI)	Retention index (RI)	Relative content (%)
Full sunlight	Tricyclene	926	926.04	0.91
	Camphene	954	940.47	0.69
	Verbenone	967	964.69	1.46
	Sabinene	975	968.91	0.66
	Limonene	1029	1021.35	0.97
	(Z)- $\beta$ -Ocimene	1037	1028.72	0.55
	Fenchone	1086	1082.11	7.9
	Endo-fenchol	1110	1107.65	0.86
	Camphor	1146	1136.70	1.45
	Isoborneol	1160	1159.13	0.53
	Terpinen-4-ol	1177	1170.32	0.23
	$\alpha$ -Terpineol	1188	1184.08	0.35
	$\alpha$ -Ylangene	1375	1369.32	0.44
	$\alpha$ -Gurjunene	1409	1403.02	0.61
	(E)-Caryophyllene	1419	1413.55	3.33
	Trans- $\alpha$ -Bergamotene	1434	1428.68	0.46
	$\alpha$ -Humulene	1454	1446.47	0.16
	$\alpha$ -Chamigrene	1503	1481.52	0.22
	$\alpha$ -Zingiberene	1493	1487.08	0.36
	Bicyclogermacrene	1500	1490.15	1.98
	$\alpha$ -Amorphene	1484	1516.16	1.14
	Epi- $\alpha$ -Cadinol	1640	1635.75	0.86
	Vulgarone B	1651	1643.63	1.58
Cubenol	1646	1651.61	2.7	
14-Hydroxy-9-Epi-(E)-Caryophyllene	1669	1665.54	16.03	
14-Hydroxy- $\alpha$ -Humulene	1714	1706.05	0.35	
30%	$\alpha$ -Thujene	930	925.51	0.79
	Camphene	954	939.94	0.60
	Sabinene	975	964.78	1.56
	$\beta$ -Pinene	979	969.07	0.62
	Limonene	1029	1020.92	0.88
	(Z)- $\beta$ -Ocimene	1037	1028.30	0.48
	Fenchone	1086	1081.45	9.93
	Camphor	1146	1136.33	1.49
	Isoborneol	1160	1158.92	0.53
	Terpinen-4-ol	1177	1170.16	0.23
	$\alpha$ -Terpineol	1188	1183.92	0.40
	$\alpha$ -Copaene	1376	1369.23	0.40
	$\alpha$ -Gurjunene	1409	1402.93	0.82
	(E)-Caryophyllene	1419	1413.04	3.36
	Trans- $\alpha$ -Bergamotene	1434	1428.58	0.60
	$\gamma$ -Himachalene	1482	1481.48	0.27
	Bicyclogermacrene	1500	1489.02	3.02
	$\alpha$ -Muurolene	1500	1493.34	0.18
	$\alpha$ -Amorphene	1484	1515.89	1.32
	Cubenol	1646	1635.18	0.95
14-Hydroxy-9-Epi-(E)-Caryophyllene	1669	1663.43	16.48	
14-Hydroxy- $\alpha$ -Humulene	1714	1705.19	0.31	

To be continued ...

Table 2, cont.

Levels of shading	Component	Kovats index (KI)	Retention index (RI)	Relative content (%)
50%	$\alpha$ -Pinene	939	925.03	0.33
	Sabinene	975	963.51	0.92
	$\beta$ -Pinene	979	967.86	0.42
	Limonene	1029	1020.48	0.75
	$\beta$ -Phellandrene	1029	1027.87	0.42
	Fenchone	1086	1079.87	7.78
	Endo-fenchol	1110	1106.95	0.85
	Camphor	1146	1136.12	1.20
	Isoborneol	1160	1158.78	0.50
	$\alpha$ -Terpineol	1188	1183.78	0.34
	$\alpha$ -Copaene	1376	1368.87	0.42
	Longifolene	1407	1402.46	0.94
	(E)-Caryophyllene	1419	1412.12	4.02
	Trans- $\alpha$ -Bergamotene	1434	1428.16	0.70
	Widdra-2,4(14)-Diene	1482	1480.84	0.25
	$\alpha$ -Zingiberene	1493	1486.31	0.59
	Bicyclogermacrene	1500	1488.96	3.00
	$\delta$ -Cadinene	1523	1515.18	1.86
	Guaiol	1600	1595.28	0.40
	1,7-diepi- $\alpha$ -cedrene	1641	1642.18	1.68
Cubenol	1646	1647.51	4.24	
14-Hydroxy-9-Epi-(E)-Caryophyllene	1669	1660.06	16.41	
80%	$\alpha$ -Thujene	930	926.04	0.17
	Sabinene	975	964.45	0.45
	$\beta$ -Pinene	979	968.77	0.21
	Limonene	1029	1021.14	0.32
	(Z)- $\beta$ -Ocimene	1037	1028.53	0.21
	Fenchone	1086	1080.53	3.59
	Endo-fenchol	1110	1107.53	0.37
	Camphor	1146	1136.63	0.60
	Isoborneol	1160	1159.29	0.24
	$\alpha$ -Terpineol	1188	1184.27	0.18
	$\alpha$ -Copaene	1376	1369.39	0.28
	$\alpha$ -Gurjunene	1409	1403.02	0.78
	(E)-Caryophyllene	1419	1412.80	3.07
	Trans- $\alpha$ -Bergamotene	1434	1428.72	0.57
	$\beta$ -Selinene	1490	1481.66	0.28
	$\alpha$ -Zingiberene	1493	1486.89	0.54
	$\delta$ -Cadinene	1523	1515.79	1.15
	Guaiol	1600	1595.90	0.32
	Cubenol	1646	1634.97	0.80
	Vulgarone B	1651	1642.96	1.49
$\alpha$ -Muurolol	1646	1648.62	2.87	
14-Hydroxy-9-Epi-(E)-Caryophyllene	1669	1661.89	16.42	
14-Hydroxy- $\alpha$ -Humulene	1714	1704.97	0.29	

In our study, we found exclusive presence of numerous essential oil compounds based on the level of shading in the plant cultivation environment (Table 2) underscoring the role of light stimulation and inhibition of specific biosynthetic routes even when the variation in light intensity is not drastic. In addition, the variation in essential oil content and in the relative content of specific chemical compounds depending on incident irradiance levels, as found here, reveals the regulatory role of light over different biosynthetic routes of secondary metabolism in plants. Although this high variability in plant response to different irradiance patterns is little studied or explored commercially, it is to be expected, because the synthesis of many secondary metabolites are induced to prevent damage to the plant by different stress agents. For instance, high levels of solar radiation may induce the synthesis of reactive oxygen species and lead to oxidative stress of plant cells (Gill and Tuteja, 2010; Vahdati and Leslie, 2013), especially in plants that require low radiation levels during cultivation. Another example is the positive red light and negative far-red light modulation, mediated by the phytochrome photoreceptor, of the synthesis of phenylalanine ammonia-lyase, a key enzyme in the metabolism of phenylpropanoids (Zucker, 1972; Camm and Towers, 1977; Guo and Wang, 2008), which are important components of essential oils (Bizzo, 2009). Considering that approximately 20% of the genome of rice and *Arabidopsis* seedlings is influenced by light (Jiao et al., 2005), light modulation of gene expression in plants, in addition to affecting secondary metabolism, can be assumed to have a great impact on metabolism as a whole, as observed in plant development.

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## DECLARATION OF INTEREST

The authors report no declarations of interest.

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