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Biotransformation of volatile compounds of the leaves of *Schinus terebinthifolius* by caterpillar *Automeris* Hubner

Biotransformação de compostos voláteis das folhas de *Schinus terebinthifolius* por lagarta *Automeris* Hubner

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ABSTRACT
Aromatic plants are rich in essential oils composed mainly of terpenoids, which have great pharmacological potential. Generally, *S. terebinthifolius* trees are preyed upon by a species of caterpillar known as *Automeris* Hubner. The volatile compounds present in the hexane extract of their feces were identified and quantified. Twenty compounds (98.83%), 7 monoterpenes (83.02%) and 13 sesquiterpenes (15.81%) were identified. The main constituents of essential oil in feces are the monoterpenes Perillal (34.51%), D-Limonene (23.31%) and *trans*-Shisool (20.96%). The comparison of the volatile composition of the leaves and feces revealed that some constituents present in the leaves were identified in the feces in lower or higher concentrations than in the feces, such as the *δ*-3-Carene monoterpenes, whose percentage values were reduced from 27.80 % (leaves) to 0.41% (feces) and D-limonene whose concentration increased significantly in leaves (2.23%) compared to feces (23.31%). Analysis of the biosynthetic pathway of most terpenoid hydrocarbons showed that constituents such as *α*-Pinene, D-Limonene and Germacrene D present in the essential oil of the leaves were biotransformed by the caterpillar *Automeris* Hubner in the monoterpenes: Myrtenol, Perillal and *trans*-Shisool and in the sesquiterpenes: Aromadendrene, *β*-Selinene, Valencene, *γ*-Cadinene, *δ*-Cadinene, *α*-Cadinene and α-Eudesmol.

Keywords: *Schinus terebinthifolius*, Biotransformation, *Automeris* sp

RESUMO
As plantas aromáticas são ricas em óleos essenciais compostos principalmente de terpenoides, que apresentam grande potencial farmacológico. Geralmente, as árvores de *S. terebinthifolius* são predadas por uma espécie de lagarta conhecida como *Automeris* Hubner. Os compostos voláteis presentes no extrato hexânico de suas fezes foram identificados e quantificados. Foram identificados 20 compostos (98,83%), 7 monoterpenos (83,02%) e 13 sesquiterpenos (15,81%). Os principais constituintes do óleo essencial das fezes são os monoterpenos Perillal (34,51%), D-Limoneno (23,31%) e *trans*-Shisool (20,96%). A comparação da composição volátil das folhas e das fezes revelou que alguns constituintes presentes nas folhas foram identificados nas fezes em concentrações menores ou mais elevados do que nas fezes, como os monoterpenos δ-3-Carene, cujos valores percentuais foram reduzidos de 27,80% (folhas) para 0,41 % (fezes) e do D-limoneno cuja concentração aumentou significativamente nas folhas (2,23%) em comparação as fezes (23,31%). A análise da via biossintética da maioria dos hidrocarbonetos terpenoides mostrou que constituintes como *α*-Pineno, D-Limoneno e Germacreno D presentes no óleo essencial das folhas foram biotransformados pela lagarta *Automeris* Hubner nos monoterpenos: Myrtenol, Perillal e *trans*-Shisool e nos sesquiterpenos: Aromadendreno, *β*-Selineno, Valenceno, *γ*-Cadineno, *δ*-Cadineno, *α*-Cadineno e *α*-Eudesmol.

Palavras-chave: *Schinus terebinthifolius*, Biotransformação, *Automeris* Hubner

1 INTRODUCTION

The chemical composition of essential oils is determined by genetic factors and other factors. Among these factors, it is possible to highlight the interactions between plant and microorganisms, inset and plant; age and stage of development and abiotic factors.[1-3]

Aromatic plants are rich in essential oils, such as terpenoids, which present great pharmacological potential and are known to have mainly ecological roles in acting as deterrents against feeding by herbivores, as antifungal defenses and attractants for pollinators.[4] *Schinus terebinthifolius* (Figure 1a) popularly known as red-cheek, red pepper, Brazilian pepper is a plant belonging to the family Anacardiaceae, native to Brazil, present in different parts of the world, such as South and Central America, Europe Mediterranean, Africa and United States. Generally, the species blooms between July and September, and fruit maturation occurs from September to October.[5] *S. terebenthifolius* is widely used in folk medicine in the treatment of various pathologies.[6]

The essential oil of the leaves from *S. terebinthifolius* (Figure 1a) presents in its chemical composition high concentrations of monoterpenes, together with some sesquiterpenes.[6] (Silva *et al*., 2019). However, this composition is variable, since it depends on the part of the plant from which the oil is extracted, the place of origin of the collection, circadian cycle, extraction methods and analytical.[7]

Insects play an important ecological role, since they can act as predators, pollinators and phytophagous. Some of these insect-plant interactions are extremely beneficial, such as pollination; however, interactions involving the consumption of plant parts have led to the development of defense mechanisms, including the production or reduction of secondary metabolites, which act as protection substances that is aim to minimize the damage caused by the attack.[8,9] Usually trees of *S. terebinthifolius* are predated by a caterpillar species known as *Automeris* Hubner (Fig. 1b,c). To digest food the digestive tract of *Automeris* Hubner produces several enzymes, which are able to transform macromolecules to be absorbed by the body and making chemical modifications in other compounds. [10] The aim of this work was analyzed the volatile compounds of the feces from caterpillar *Automeris* Hubner to verify which they underwent chemical modifications in the gastrointestinal tract of the insect.

Fig. 1 *S. terebenthifolius* tree (a), caterpillar species *Automeris* sp (b,c)

2 METHODS AND MATERIALS

2.1 COLLECTION AND IDENTIFICATION OF PLANT MATERIAL

Leaves of *S. terebinthifolius* family were collected in the municipality of Eusébio - CE, in the Coaçu neighborhood, in July 2018 at 10:00 am. The collected material was sent to Herbarium Professor Francisco José de Abreu Matos of the State University of Vale do Acaraú (UVA), where the exsicata was deposited and registered, under registration number 21832.

2.2 OBTAINING THE HEXANIC EXTRACT FROM THE FECES OF AUTOMERIS SP.

Feces (1.2 g) of *Automeris* Hubner were placed in a 50 mL beaker and then 10 mL hexane was added. After 5 min 2 mL of the solution was collected and analyzed using GC-FID and GC-MS. Gas.

2.3 GC-MS AND GC-FID

The analysis of the volatile constituents was carried out on a Hewlett-Packard Model 5971 GC/MS using a non-polar DB-5 fused silica capillary column (30 mm x 0.25 mm i.d., 0.25m film thickness); carrier gas helium, flow rate 1 ml/min and with split ratio 1:1. The injector temperature and detector temperature were 250º C and 200º C, respectively. The column temperature was programmed from 35C to 180º C at 4º C/min and then 180C to 250º C at 10º C/min. Mass spectra were recorded from 30 - 450 m/z. Individual components were identified by matching their 70 eV mass spectra with those of the spectrometer using retention indices as a preselection routine, as well as by visual comparison of the fragmentation pattern with those reported in the literature.[11] The quantitative analysis was carried out on a Shimadzu GC-17A gas chromatograph using a dimethylpolysiloxane DB-5 fused silica capillary column (30 mm x 0.25 mm, film thickness 0.25 m). H2 was used as the carrier gas at a flow rate of 1 ml/min and 30 psi inlet pressure; split, 1:30; temperature program: 35-180° C at 4° C/min, then heated at a rate of 17° C/min to 280° C and held isothermal for 10 min; injector temperature, 250° C; detector used FID, detector temperature, 250° C.

3 RESULTS AND DISCUSSIONS

The essential oils present complex chemical composition extracted from diverse parts of plants, giving to the plants adaptive forms in the medium in which they are inserted.[12] The chemical composition of essential oils varies between species and parts of the same plant. The constituents of essential oils are mainly terpene derivatives, such as mono and sesquiterpenes, and phenylpropanoids.[13-14]

The essential oils extracted from the leaves *S. terebinthifolius* were analyzed by GC/MS and GC/FID the constituents identified and quantified (Table 1). A total of 23 constituents (95.7%) were identified. The major components of essential oil were β-Caryophyllene (30.2%) and δ-3-Carene (27.8%). The predatory caterpillar *Automeris* Hubner feeds the leaf of *S. terebinthifolius* and the essential oil extracted from feces were identified and quantified (Table 1). A total of 19 compounds (98.83%) were identified, being 7 monoterpenes (83.02%) and 13 sesquiterpenes (15.81%) (Figure 2). The essential oil of caterpillar *Automeris* Hubner feces showed greater abundance of monoterpenes, being four non-oxygenated (25.45%) and three oxygenates (57,57%). The major constituents essential oil feces were Perillal (34.51%), D-Limonene (23.31%) and *trans*-Shisool (20.96%).

Comparison of leaf and feces volatile composition revealed that some constituents present in the leaves were identified in the feces with percentage values smaller and higher than in the feces (Table 1) like monoterpenes δ-3-Carene whose percentage values decrease from 27.80% (leaves) to 0.41% (feces) and D-Limonene whose concentration increased significantly from leaves (2,23%) to feces (23,31%). The literature reveals that insects play an important ecological role, since they can act as predators, pollinators and phytophagous. Some of these insect-plant interactions are extremely beneficial, such as pollination; however, interactions involving the consumption of plant parts have led to the development of defense mechanisms, including the production or reduction of secondary metabolites.[15]

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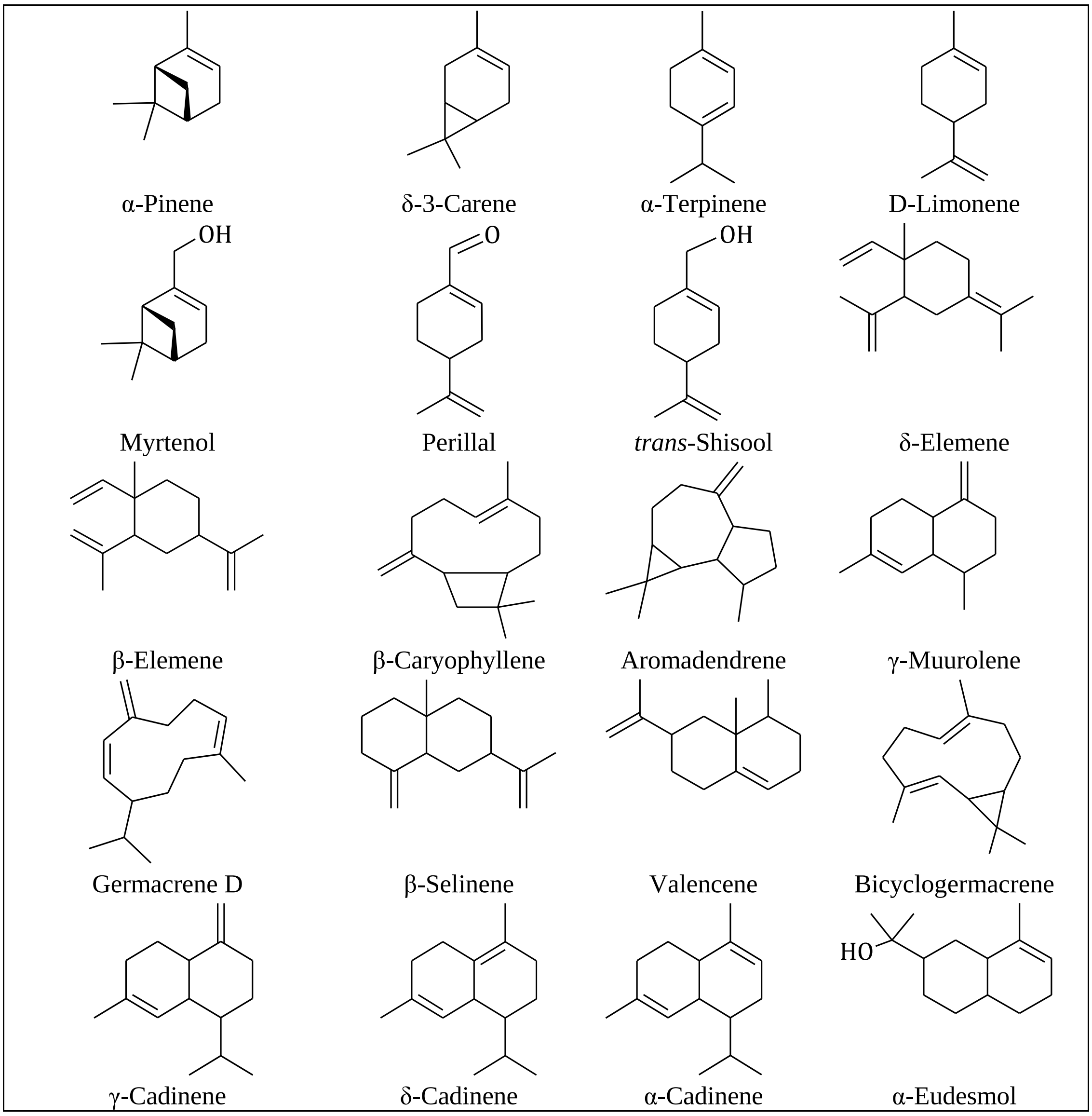
Table 1. Volatile chemical composition of the leaves of *Schinus terebinthifolius* and feces of predatory caterpillar *Automeris* Hubner

Compounds	RI[a]	Percent Composition	
		leaves	feces
β-Tujene	930	0.60	
α-Pinene	932	3.20	1.02
Sabinene	975	1.20	
β-Pinene	979	0.9	
Mircene	990	2.20	
α-Fenlandrene	1002	0.80	
δ-3-Carene	1008	27.80	0.41
α-Terpinene	1014	0.50	0.66
D-Limonene	1024	2.30	23.31
β-Cimene	1024	2.70	
Terpinolene	1088	0.70	
γ–Terpinene	1059	0.90	
Terpinen-4-ol	1177	1.50	
α-Terpineol	1188	1.30	
Myrtenol	1194		2.15
Perillal	1279		34.51
trans-Shisool	1250		20.96
δ-Elemene	1335		0.59
β-Elemene	1389	0.8	0.55
β-Caryophyllene	1417	30.20	1.27
Aromadendrene	1439		0.50
γ-Muurolene	1478		1.04
Germacrene D	1484	1.80	1.87
β-Selinene	1489		2.08
Valencene	1496		0.48
Bicyclogermacrene	1500	1.50	2.74
γ-Cadinene	1513		0.92
δ-Cadinene	1522	0.20	1.52
α-Cadinene	1537		0.48
Espatulenol	1578	5.70	
Caryophyllene oxide	1583	5.80	
Viridiflorol	1592	0.90	
α-Eudesmol	1662		1.78
	Total	97.7%	98.83%

[a]Retention indices on DB-5 column

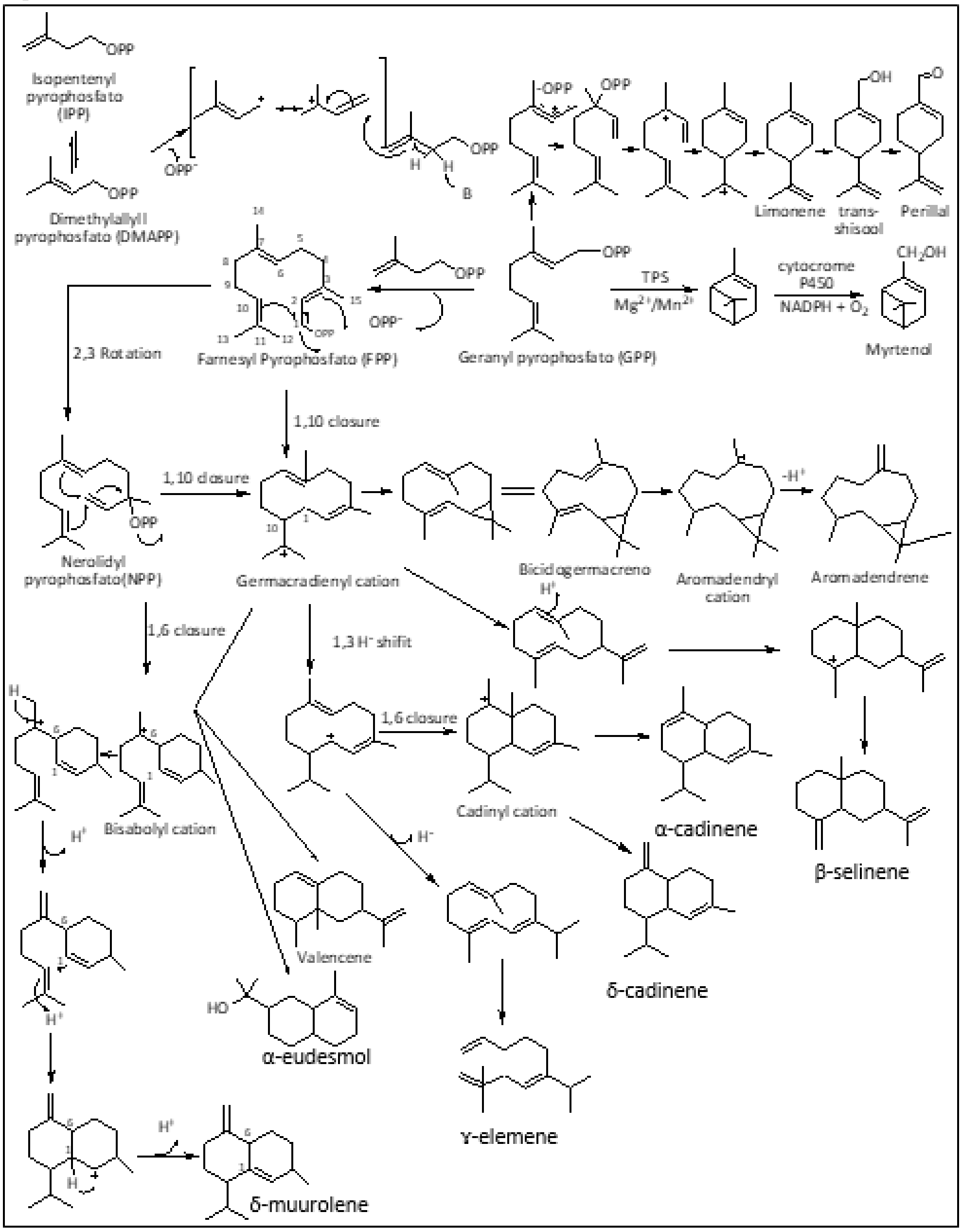
Figure 2. Chemical constituents of the feces essential oil from predatory caterpillar *Automeris* Hubner



The analysis of biosynthetic route of most terpenoids hydrocarbons showed that constituents like α-Pinene, D-Limonene and Germacrene D presentes at leaves essential oil were biotransformed by the coupling of isopentenyl pyrophosphate (IPP) to give polyenyl pyrophosphates which are then transformed to the terpenes by terpene synthases, following condensation of multiple of IPP and DMADP units by synthase enzymes which catalyze the dissociation of pyrophosphate to form polyenyl carbocations which can undergo complex rearrangements that are controlled by the enzyme active site resulting in coupling of two IPP molecules to gives geranyl pyrophosphate (GPP), which is the precursor of the structures of the monoterpenes found in the feces essential oil of *Automeris* sp like Myrtenol, Perillal and *trans*-Shisool. Similarly, addition of another isoprene unit to GPP gives farnesyl pyrophosphate (FPP), from which all sesquiterpenes observed in the feces essential oil like Aromadendrene, β-Selinene, Valencene, γ-Cadinene, δ-Cadinene, α-Cadinene and α-Eudesmol (Figure 3) (Vattekkatte *et al*. 2018).[16]

Many biological activities have been attributed to terpenoids and their derivatives as antioxidant, antimicrobial and anticancer properties (Bhatti *et al*., 2014).[17] These compounds are biotransformed in many ways by enzymes from metabolism of a variety of organisms including herbivorous larvae in which a complex chain of reactions involved secondary metabolites maybe resulting in a mixture of compounds (Vihakas et al., 2015).[18]

Figure 3. Biosynthesis pathway postulated to chemical constituents biotransformed in the gastrointestinal tract of predatory caterpillar *Automeris* Hubner



In fact, some observations have revealed that injury caused by feeding insects such as may result in a differentiated physiological post, in which the gene expression and/or formation of secondary metabolites were affected. Studies have been investigated the phenolic metabolism by different species of lepidopteran larvae. In general, some secondary metabolites suffer modifications by enzymes released at leaf-chewing and in the midgut insects. In this sense, secondary metabolites that passed through the larval alimentary system with relatively little metabolic change were simple to detected compared to the biotransformation molecules.[19-21]

The compounds *β*-Tujene, Sabinene, *β*-Pinene, Mircene, *α*-Fenlandrene, *β*-Cimene, Terpinolene, *γ*–Terpinene, Terpinen-4-ol, *α*-Terpineol, Espatulenol, Caryophyllene oxide and Viridiflorol may be absorbed and metabolized which did not allow their identification in the feces essential oil. [18] The presence of terpenoids in the feces of the caterpillar *Automeris* Hubner is related to the function of these compounds as semiochemicals. The literature reports that α-and β-pinene are already known as semiochemicals in the selection of the oviposition sites and in attracting and eliciting oviposition. In contrast, limonene is found to be repellent.[22]

4 CONCLUSION

The analysis of biosynthetic route of most terpenoids hydrocarbons showed that constituents like *α*-Pinene, D-Limonene and Germacrene D presentes at leaves essential oil were biotransformed by caterpillar *Automeris* Hubner in to monoterpenes Myrtenol, Perillal and *trans*-Shisool and sesquiterpenes aromadendrene, *β*-Selinene, Valencene, γ-Cadinene, *δ*-Cadinene, *α*-Cadinene and *α*-Eudesmol. The enzymatic action of biotransformation is selective to some monoterpenes and sesquiterpenes.

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